THERMOPHILIC MICROORGANISMS IN THE HOT SPRINGS OF TENGCHONG GEOTHERMAL AREA, WEST YUNNAN, CHINA

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Abstract—The distribution of thermophilic microorganisms in the hot springs of the volcanic geothermal area of Tengchong County, Yunnan Province was examined and the following observations made:

1. Large numbers of filamentous and rod-shaped non-photosynthetic bacteria grow in Big Boiling Pot, which is a siliceous, alkaline boiling spring with temperatures ranging from 86 to 91 °C. Macroscopic bacteria masses colonized on the surface of the geyserites in the spring pool. The bacteria show distinct ultramicrostructure of the cell wall, which has a striated appearance possibly caused by a number of penetrating micro-channels. Some sections of the bacteria cells also show an unusually thick wall.

2. Typical Synechococcus-Chloroflexus microbial mats grow and cover the substrata of the spring water flows with temperatures ranging from 68 to 72°C.

3. When the spring water cools, the number of species increases progressively. The microbial mats growing at temperatures below 60°C consist mainly of filamentous blue-green algae.

The significance of studies on thermophilic microorganisms and volcanic geothermal environments is discussed.

INTRODUCTION

In recent years biologists have been attaching great importance to thermal environments and thermophilic microorganisms. Although a wide variety of natural and man-made habitats with high temperatures exist, hot springs in geothermal regions are of particular interest to biologists, paleobiologists and geologists.

Geothermal activity often associated with volcanic and tectonic activities may create environments in which temperatures are extreme, and other environmental factors, such as hydrochemistry, pH and nutrients are also abnormal. Such environments with high temperature, low or high pH, and high contents of minerals and gases are very close to the environmental conditions of the early Precambrian. It is now known that primitive microorganisms with simple structures can grow under higher temperature better than advanced organisms with complicated structures (Brock, 1978), and most thermophiles adapt to their habitats very well. On the basis of Precambrian paleontological records, it would appear that thermophilic microorganisms may be as ancient as their habitats and have a very long evolutionary history that can be traced back to early Archean (see Table 1). Studies of the hot spring environments and thermophilic microorganisms may provide us with some valuable information on the early evolution of life on earth.

The microflora of the Yellowstone hot springs in the U.S.A. have been studied in detail (Brock, 1967a,b, 1978; Bott and Brock, 1969; Brock and Brock, 1971; Brock et al., 1971). Biological field surveys and laboratory studies of thermophilic microorganisms have also been carried out in North and South America, New Zealand, Iceland, Italy, Japan and other geothermal regions of the world (Brock, 1978; Stetter, 1984). Bacteria that grow in boiling springs at temperatures above 90°C have been found in some continental geothermal regions (Bott and Brock, 1969; Brock et al., 1971; Brock, 1978), and recently extremely thermophilic bacteria from submarine hydrothermal vents have been isolated along tectonic rises in the eastern Pacific at depths of about 2500 m and temperatures as high as 380 ± 30°C. They can
Table 1. Upper temperature limits and ages of earliest fossil records of various groups of organisms

<table>
<thead>
<tr>
<th>Group</th>
<th>Approximate upper temperatures for growth* (°C)</th>
<th>Approximate age of earliest fossil records† (Ma)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish and other aquatic vertebrates</td>
<td>38</td>
<td>350 – 400</td>
</tr>
<tr>
<td>Insects</td>
<td>45 – 50</td>
<td>400</td>
</tr>
<tr>
<td>Ostracods (crustacean)</td>
<td>49 – 50</td>
<td>500</td>
</tr>
<tr>
<td><strong>Plants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular plants</td>
<td>45</td>
<td>400</td>
</tr>
<tr>
<td>Mosses</td>
<td>50</td>
<td>?</td>
</tr>
<tr>
<td><strong>Eucaryotic microorganisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protozoa</td>
<td>56</td>
<td>700</td>
</tr>
<tr>
<td>Algae</td>
<td>55 – 60</td>
<td>2000 – 1500</td>
</tr>
<tr>
<td>Fungi</td>
<td>60 – 62</td>
<td>2000?</td>
</tr>
<tr>
<td><strong>Procaryotic microorganisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue-grean algae (Cyano-bacteria)</td>
<td>70 – 73</td>
<td>2000</td>
</tr>
<tr>
<td>Photosynthetic bacteria</td>
<td>70 – 73</td>
<td>3500</td>
</tr>
<tr>
<td>Chemolithotrophic bacteria</td>
<td>90</td>
<td>3500</td>
</tr>
<tr>
<td>Heterotrophic bacteria</td>
<td>90</td>
<td>?</td>
</tr>
</tbody>
</table>

*From Brock (1978).
†From paleontological references.

continue to grow in a titanium growth chamber at temperatures of at least 250°C (Baross and Deming, 1983). This has evoked new discussions about the upper limit of temperature for life (Walsby, 1983; Fisher et al., 1983; Stetter, 1984).

The geobiological action of thermophilic microorganisms has also attracted the attention of both geologists and economic geologists. It is possible that thermophilic bacteria communities in deep sea produce CH₄, H₂, and CO (Baross et al., 1982); redox of many metals and nonmetals has been found to be related to thermophilic bacteria activities (Brock, 1978).

Although laboratory studies have been conducted on thermophilic microorganisms in some other hot springs of China (Zhong et al., 1982), their distribution and ecological properties in the Tengchong geothermal region had not been studied previously.

In the spring of 1984, the author carried out a field investigation in the Hot Sea geothermal region of Tengchong County, Yunnan Province, followed by a TEM study on the ultramicro-structures of the thermophilic microorganisms. This paper contains the primary results of the studies during and following this expedition.

LOCALITIES AND ENVIRONMENT

The field work was conducted in Sulfur Ponds of the Hot Sea geothermal region, which is about 11 km southwest of the town of Tengchong. Sulfur Ponds is situated in the central part of the Hot Sea geothermal field, on the southern slope of Bange Hill along the northern side of Bath stream. In the Sulfur Ponds valley, which is about 200 m long, there are numerous hot springs and fumaroles. Big Boiling Pot and Spectacles Springs are among the largest and were chosen as the working localities. A location map is given in Fig. 1.

Big Boiling Pot is a rounded boiling spring pool with a diameter of about 3.5 m and a depth of 1.5 m. Three hydrothermal vents near the center of the spring pool spurt boiling water and steam, so that the pool resembles a big pot filled with boiling water. The water is clear and
colorless, pH = 8.5, with temperatures ranging from 88 to 89°C near the edge of the pool and to 91°C in the central part. The highest temperature, 96.6°C, was measured near the vents (unpublished data); this slightly exceeds the local boiling point (the elevation here is about 1450 m above sea-level). Repeated chemical analyses of the spring water showed that the major positive ions are Na⁺ and K⁺. Ca²⁺ and Mg²⁺ are in very low content and silica is abundant. A small amount of H₂S was detected. During the field work a small earthquake occurred. It was observed that before and after the quake, the water in Big Boiling Pot turned black, possibly due to large amounts of H₂S or sulfide which spouted from the vents. Thick geyserites are composed of amorphous opals with dispersed, brown-colored organic matter.

Spectacles Springs consists of a pair of spring pools, each with a diameter of about 1 m, resembling a pair of glasses. The temperature near the source is 90–93°C, pH = 8.5. Geyserites can be found along the edge of the pools and abnormal radiation was detected. The hot spring water flows out of the pools in a small channel along the slope and then turns into a fan-shaped stream which later falls into Bath stream. As the water flows away from the source its temperature gradually decreases, forming a temperature gradient along the water flow. A V-shaped temperature pattern in a cross-section of the water flow (Brock, 1978) is also present. A variety of color zones indicates different microbial communities distributed along the temperature gradient.

SAMPLING METHODS

A simply constructed laboratory equipped with a photomicroscope and other necessary instruments was set up about 50 m from Big Boiling Pot and 70 m from Spectacles Springs. Microorganisms were found in water with various temperature ranges: 88 – 91°C, 86 – 88°C (in the pool and the exit from Big Boiling Pot), 60 – 72°C and below 60°C (in the water flow of Spectacles Springs). Microorganism samples were taken by two methods.

The slide-immersion method described by Bott and Brock (1969, 1970) was used. Microscopic slides were inserted into the hot spring water. After 7 – 9 days’ immersion, the
slides were then examined directly under a microscope to check the bacteria growing on the surface. Then the bacteria was scraped off for study.

Natural bacteria mass samples and microbial mats were also collected from the walls and bottom of the spring pools, as well as the substrata of the water flows. Macroscopically visible bacteria masses growing on the surface of the geyserites in Big Boiling Pot were scraped off with a razor blade and microbial mats growing at different temperature ranges in the water flow were cut off with a knife. Both microscopic and electron-microscopic studies were conducted using these samples.

The samples for the transmitted electron microscope (TEM) study were fixed by 2.5 - 5% glutaraldehyde in a phosphatic buffer solution followed by osmium, and finally doubly stained with uranyl acetate and lead citrate. They were then embedded in resin (Epon-812) and sliced into ultra-thin sections of 300 - 500 Å using the LKB-III microtome.

A TEM (Transmitted Electron Microscope) study was carried out in the summer of 1984 at Peking University.

**COMPOSITION AND DISTRIBUTION OF THE MICROBIAL COMMUNITIES**

Microorganisms in the hot springs of this region often grow and develop into macroscopically visible mats or masses. Colorful microbial mats consisting of photosynthetic bacteria and blue-green algae (cyanobacteria) grow on the surface of substrata* immersed in hot spring water with temperatures below 72°C. In boiling spring pools no colored mats grow, but black or colorless bacteria masses attached to the surface of geyserites were observed. Distribution of the microorganisms along a temperature gradient was examined.

1. **Microorganisms growing in a temperature range of 88 - 91°C**
   
The slides immersed in the center of Big Boiling Pot indicate the presence of living bacteria at temperatures between 90 and 91°C; macroscopic bacteria masses grow at temperatures between 88 and 89°C on the geyserites in the pool wall. Microscopic observations of the bacteria masses showed dense accumulations at filamentous, rod-shaped bacteria [Fig. 2(A) and (B)]; slender filaments and rods were dominant and a few spheres were seen. The filamentous and rod-shaped bacteria were similar in cellular ultramicrostructures [Fig. 3(A) - (E)] and possibly belong to the same taxon. The 30 measured cells are 0.09 to 0.40 μm wide with an average width of 0.24 μm, and up to tens of μm long. Under the TEM, distinct cellular plasma membrane could be seen in some of the ultra-thin sections of the bacteria [Fig. 3(E)], and the cell wall has a striated appearance probably caused by numerous micro-channels crossing the wall [Fig. 3(C)]. The filaments are unbranched, non-septate, and sometimes partially constricted [Fig. 3(B)] with swelled ends [Fig. 3(K)]. The cells contain irregular light areas with low electron density, which may represent nucleoplasm [Fig. 3(E)]. Some dumb-bell- and amoeba-shaped cells were seen [Fig. 3(I) and (J)]; this could represent the cells in division or budding.

Because of low oxygen solubility, poor organic nutrients in the spring water and black mineral deposits often associated with bacteria masses, the bacteria in Big Boiling Pot are probably anaerobic and chemotrophic.

2. **Microorganisms existing in a temperature range of 86 - 88°C**

Slide-immersion shows bacteria growing near a small fumarole at temperatures between 86 and 88°C. The fumarole is situated at the exit from Big Boiling Pot and is surrounded by black geyserites. Microscopic examination of the bacteria masses scraped from the surface of the geyserites showed densely gathered filamentous, rod-shaped bacteria which are morphologically different from those growing at temperatures between 88 and 91°C in the Big Boiling Pot [Fig. 2(C) - (G)]. Most of the filaments and rods are opaque under a light microscope, the 20 cells measured are 0.26 - 0.80 μm wide with an average width of 0.6 μm,

*Substrata are the rock and the geyserite which form the bed of the spring's water flow.
Fig. 2. Photomicrographs of the living bacteria from Big Boiling Pot spring in the temperature range of 86 – 91°C. The bacteria were sampled from the surface of the geyserites and the slides immersed in hot spring water. The bacteria were photographed immediately without fixation and staining. All photos are equal in magnification; scale bar = 10 μm. (A) and (B). Bacteria from Big Boiling Pot in the temperature range of 88 – 91°C. (C) – (G). Bacteria growing near the exit from Big Boiling Pot in the temperature range of 86 – 88°C. The arrows in (E) and (G) indicate the swelled ends of the bacteria filaments.
Fig. 3. Electron microscope photographs of ultra-thin sections of the thermophilic bacteria from Big Boiling Pot in the temperature range of 86–91°C. The bacteria materials were fixed by glutaraldehyde followed by osmium, and doubly stained with uranyl acetate and lead citrate. (A)–(E). Vertical and cross-sections through the bacteria from 88 to 91°C spring water of Big Boiling Pot. The arrows in (C) indicate the micro-channels penetrating the inner layer of the cell wall. PM = plasma membrane; W = cell wall; N = possible nucleoplasm. (F)–(H). Oblique sections (F) and cross-sections (G and H) of the bacteria from the spring water of Big Boiling Pot in the temperature range of 86–88°C. The cell walls, consisting of a diffused outer layer and a dense inner layer, are unusually thick. (I) and (J). Dumb-bell shaped bacteria from spring water of 88–91°C. The cells seem to be in division or budding. (K). A filamentous bacteria with a swelled end (from spring water of 88–91°C).
and up to several tens of μm long. Occasionally swelling ends were seen (Fig. 2(E) and (G)). Electron microscopic views of the bacteria showed unusually thick walls in two layers: an outer diffused layer with low electron density and irregular outline and an inner layer with high electron density (Fig. 3(F)–(H)). The outer and inner layers of the cell wall had a total thickness ranging from 0.1 to 0.16 μm.

(3) *Microbial mats growing in a temperature range of 68–72°C*

The lower reach of the water flow in Spectacles Springs is about 30 m long and 2 m wide; the spring water slowly cools in this section. In a temperature range of 68–72°C colorful microbial mats, consisting of blue–green algae *Synechococcus* and photosynthetic bacteria *Chloroflexus* sp. grow and cover the substrata immersed in the spring water.

The mats are differentiated vertically and three layers can be distinguished (Fig. 4). The upper layer is glutinous, dark-green and 1–2 mm thick. It is composed of two taxa of coccoid blue–green algae: *Synechococcus lividus* and *Synechococcus* sp. The middle layer is yellow–orange to pink–red, 2–3 mm thick and consists of pure filamentous photosynthetic bacteria *Chloroflexus* sp. The lower layer is pink–whitish, 10–50 mm thick and consists of geyserites and decomposed microbial mats.

*Synechococcus* sp. are large-sized, rod-shaped blue–green algae (Fig. 5(A)–(F)). The 50 cells measured are 2.9–4.3 μm wide and 5.7–10.7 μm long, averaging 3.5 μm in width and 8.1 μm in length; they are often connected end to end and embedded in gelatinous matter. The gelatinous, dark-green matrix in the upper layer of the mats might give protection against solar radiation and thus provide a microenvironment favorable to the photosynthetic bacteria *Chloroflexus* growing below.

*Synechococcus lividus* Copeland is a widespread taxon of thermophilic blue–green algae. Since its first discovery by Copeland (1936) in Yellowstone National Park, U.S.A., it has been found in numerous hot springs in other areas of the world and studied in detail (Dyer and Gafford, 1961; Castenholz, 1969; Meeks and Castenholz, 1971; Brock, 1978). The cells of *Synechococcus lividus* from the mats in Spectacles Springs live at temperatures of 68–72°C and are slender rods. The 50 cells measured are 0.6–1.2 μm wide, 3.2–7.9 μm long, with average width of 0.9 μm and average length of 5.3 μm; they are often connected end to end (Fig. 5(G)) and assembled into colonies living over the *Chloroflexus* layer (Fig. 5(H) and (I)). Electron microscope analysis showed that the cells contain distinct photosynthetic membranes, which are roughly parallel to the cell wall (Fig. 6(A)–(E)).

The filaments of *Chloroflexus* sp. from the mats in Spectacles Springs are 0.3–0.6 μm wide, up to a hundred μm long, and often assembled in bunches; the individual filament is enclosed by a sheath (Figs 5(H)–(K) and 6(F) and (G)). Morphologically these filamentous bacteria appear similar to those of *Chloroflexus* described by Pierson and Castenholz (1974).

*Chloroflexus* is widespread in hot spring environments and commonly associated with blue–green algae *Synechococcus* to form a *Synechococcus–Chloroflexus* mat-community. This widespread distribution of *Synechococcus–Chloroflexus* mats in hot springs all over the world suggests that a constant, interbeneficial and interdependent relationship may exist between the two groups of microorganisms within the mat-community.

(4) *Microorganisms living below 60°C*

As the spring water cools the color and microbial composition of the mat-communities change distinctly. Blue–green algae increase progressively in number of taxa. In temperatures below 60°C, the microbial mats turn to dark-blue or dark-green and filamentous blue–green algae of Oscillatoriales and Nostocales dominate. Partially silicified stromatolitic mats consisting mainly of *Calothrix fusca* Born et Flash and *Phormidium* sp. were seen on the edges
Fig. 4. Vertical section of the *Synechococcus*—*Chloroflexus* sp. mats growing in the water flow of Spectacles Springs in the temperature range of 68 – 72°C. U, The upper layer, consisting of *Synechococcus* sp. and *S. lividus*. M, The middle layer, consisting of pure *Chloroflexus* sp. L, The lower layer, consisting of geyserites and decomposed mats. S, *Synechococcus* sp.; S₃, *Synechococcus lividus*. C, *Chloroflexus* sp.

of the water flow with temperatures ranging between 40 and 50°C [Fig. 6(H) and (I)]. In the vertical section view of the stromatolitic mats [see Fig. 6(H)], a vertical growth of the filamentous algae is followed by a horizontal growth. This alternating growth pattern gives the stromatolitic mats a microstructure similar to that of Precambrian stromatolites.

**DISCUSSION**

It is possible that Big Boiling Pot's weak alkaline pH and dark mineral deposits, which could be sulfide, are advantageous to the bacteria growing at high temperatures. In Sulfur Ponds, tests of slide-immersion did not show any bacteria growing in the acid boiling springs. The situation in Yellowstone is similar. Brock (1978) pointed out that bacteria did not live in boiling acid springs because high acidity may add an additional environmental stress that makes microbial growth at very high temperatures impossible. In Big Boiling Pot the bacteria are attached to black mineral deposits (possibly sulfide), whereas the white or light colored geyserites showed no bacteria attachment. This demonstrates that the metabolisms of these bacteria may be related to the minerals.

Macroscopic bacteria masses were found in both the boiling spring pool and the spring water flow. This indicates that macroscopic development of bacteria does not necessarily require a flowing water environment as Brock assumed (Brock, 1978).

In the Sulfur Ponds area *Synechococcus*—*Chloroflexus* mats were not found at temperatures above 72°C, which may represent the upper temperature limit for growing photosynthetic microorganisms in this area. *Synechococcus*—*Chloroflexus* mats were best developed at a temperature of 68°C.

*Eosynechococcus*, a possible Precambrian counterpart of *Synechococcus*, was found in Canada and in China (Hofmann, 1976; Zhang Yun, 1981). Some Precambrian filamentous microfossils are morphologically comparable to some modern photosynthetic bacteria (Zhang Yun, 1984) and the growth patterns of *Synechococcus*—*Chloroflexus* mats and other microbial mats resemble those of Precambrian stromatolitic mats. Studies of modern microbial mat-communities in the hot springs of geothermal regions may, therefore, contribute greatly to understanding Precambrian life and its environment.

Thermophilic bacteria living in boiling or superheated water belong to the archaeobacteria (Fox *et al.*, 1980; Fisher *et al.*, 1983; Stetter, 1984), and exhibit similar ultramicrostructural features of their cell walls; for example, the walls are unusually thick and a number of micro-channels or tubes penetrate the walls giving them a striated appearance (see Fig. 3(C), (F) – (H); Brock, 1978; Baross and Deming, 1983; Stetter, 1984). The special structures in the cell wall may help make the thermophilic bacteria capable of living in extremely high temperatures, but the mechanics of the wall structures are not yet clear.

The prevalent viewpoint regarding upper temperatures for life was questioned by the
Fig. 5. Photomicrographs of the thermophilic microorganisms that build the microbial mats in the water flow of Spectacles Springs in the temperature range of 68–72°C. The scale bar in B (10μm) is equal for all figures. (A)–(F). *Synechococcus* sp. (G). *Synechococcus lividus*. (H) and (I). The middle–upper part of the mats. The filaments of *Chloroflexus* sp. gathered into bunches, which are covered by slender rod-shaped cells of *Synechococcus lividus*. (J). The middle layer of the mats, consisting of pure filaments of *Chloroflexus* sp. (K). The lower layer of the mats, consisting of geyserites and decomposed filaments of *Chloroflexus* sp.
FIG. 6. Electron microscope and microscope photographs of the thermophilic microorganisms from Spectacles Springs.
(A) – (E). Electron microscope views of the cells of *Synechococcus lividus* in ultra-thin sections. Distinct photosynthetic membranes of the cells (PhM) can be seen in (D). (F) and (G). Electron microscope photographs of the filaments of *Chloroflexus* sp. in ultra-thin sections. (H) and (I). Microscope photographs of the stromatolites (in a vertical section) from the water flow of Spectacles Springs in the temperature range of 40–50°C. The main builder of the stromatolites is the filamentous blue–green algae (cyanobacteria) *Calothrix fusca* (I).
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discoveries of thermophilic bacteria in the superheated hydrothermal vents of the eastern Pacific deep sea and other places (Baross and Deming, 1983; Stetter, 1984); in fact, Brock predicted long ago that microorganisms may be able to live in any boiling habitat that contains liquid water (Brock, 1967a; 1978).

Evidence from Archean sedimentary talc, trace-elements and oxygen isotope analyses support the inference that surface earth temperatures may have been very high in the Archean, possibly about 80°C at 3800 Ma, and could have exceeded boiling point prior to 4000 Ma (Costa et al., 1980; Knauth and Lowe, 1978). If this is true, and because some Precambrian microfossils and modern thermophilic microorganisms are morphologically similar (Awramik et al., 1983; Zhang Yun, 1984), it is possible that thermophilic microorganisms associated with hydrothermal environments may have been dominant in the early Precambrian.

It is interesting that an array of upper growth temperature limits of various organism groups is roughly compatible with the emerging sequence of their fossil records in geological time (see Table 1). It seems that the origin of life and the evolution of organisms may be related to progressively decreasing surface earth temperatures (Costa et al., 1980).

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