CHAPTER 1

Ancient Hyperthermophiles and Thermophilic Green Nonsulfurs

It was hot when life originated on Earth about 4 billion years ago. Only half a billion years before this, the planet had begun as a sphere of molten rock. Gradually, temperatures dropped, a surface crust solidified, and rain began to fall from the cooling skies, filling shallow seas. Active volcanoes and thermal springs became abundant. Collisions with meteorites and fragments of asteroids and comets were also probably frequent. The atmosphere must have been filled with debris from these volcanoes and impacts, resulting in a rather hazy, reddish, or even dark sky, and possibly a greenhouse effect that maintained the warmth in the atmosphere. The first land masses may have been volcanoes rising up from the seas.

THE FIRST 500 MILLION YEARS OF LIFE

Life is hypothesized by some scientists to have originated and begun to evolve in boiling or near-boiling springs. No oxygen was present in the early atmosphere—which is fortunate, since the first chemical building blocks of life would never have held together in the presence of this highly reactive, destructive gas that without special processing is toxic to all organisms. Although humans, along with many other organisms, are breathing oxygen and would die without it, our relationship with that gas is a delicate one. Respiration seems to have evolved about 2½ billion years ago from mechanisms that detoxified oxygen. Because oxygen respiration is also a marvelous way of assimilating energy, oxygen-respiring organisms have been quite successful in terms of both distribution and number of species. However, all oxygen-respiring, or aerobic, organisms require numerous other mechanisms to contain and control oxygen. Many good hypotheses that explain why we age and die revolve around the cumulative effects of oxygen damage to our DNA and cells. Certain diseases may be attributed to oxygen damage as well. Ultimately, oxygen is a poisonous gas. Properly used, it is a slow poison, and the benefits of oxygen respiration seem to outweigh oxygen damage.

One theory posits that the earliest organisms (bacteria or bacteria-like cells) were thermophiles (literally, heat lovers)—and extreme ones at that, thriving at boiling or near-boiling temperatures. They were of necessity strict anaerobes, requiring no oxygen and perhaps even lacking any natural defenses against oxygen. This theory fits well with classifications of modern bacteria, which suggest that present-day anaerobic hyperthermophiles (extreme heat lovers) have retained more characteristics of ancient bacteria than have other bacterial groups.

Exactly how life originated is a subject of great speculation. In brief, there seems to have been a transition stage in which small building blocks of life, such as amino acids and nucleotides, formed into larger molecules—proteins and RNA—with the help of energy sources such as the heat of thermal springs. This much can actually be accomplished in laboratroy experiments in which hot spring conditions are simulated. The next step by which proteins, RNAs, and other large molecules "self-assembled" into replicating, energy-using, enclosed systems (that is, *life*!) has not been accomplished in any laboratory, although various aspects of these processes have been replicated. There remains considerable room for experimentation before that remarkable transition from chemistry to biology is well understood and can be demonstrated in a laboratory.

It is clear, however, that life indeed originated, and it is interesting to hypothesize about what those first organisms were like. Their immediate environment may have been strangely abundant in food molecules—amino acids, nucleotides, proteins, and RNAs. Any molecules that had not somehow assembled into organisms would likely have been among the most directly available and accessible sources of energy and materials (food). It was an unusual period of Earth's history and one not likely to be repeated again; in today's environments, anything resembling a food molecule is quickly scavenged by hordes of microbes. Waiting around for food to appear in the environment is not a good modern strategy for nutrition, unless the waiting period is spent in a dormant form. However, when life was still a rarity and edible molecules were in some abundance, that might have been the simplest and most effective strategy. Therefore, the earliest life forms—the anaerobic hyperthermophiles—may also have been consumers of food molecules from their environments, that is, "heterotrophs." Certain types of autotrophy (the synthesis of food) may also have been important at this time.

HYPERTHERMOPHILES
THE MOST ANCIENT BACTERIA

Several groups of bacteria that thrive in hot water (over 45°C [113°F]) are called "thermophiles" (heat lovers). At temperatures greater than 60°C (140°F), eukaryotes do not grow. Therefore, any activity at temperatures over 60°C may be considered a good indicator of bacterial life, especially if scums, slimes, or flocs are found. Exactly which bacterial groups are present may be indicated by specific smells, colors, and textures and by a further refinement of temperature range. Between 60 and 80°C (140 to 176°F) may be found colorful mats of thermophilic green nonsulfurs (described in this chapter) and cyanobacteria (chapter 13). Bacteria that thrive at temperatures above 80°C (176°F) are called "hyperthermophiles." This group includes the ancient hyperthermophiles (genera *Thermatoga*, *Hydrogenobacter*, and *Aquifex*; this chapter), the hyperthermophilic archaea (chapter 3), and species in the genus *Thermus* (chapter 17).

This chapter focuses on two groups that seem to have taken fewer detours during their evolutionary path from the first bacteria and to have retained more of their ancestral genes than other groups: the ancient hyperthermophiles and the thermophilic green nonsulfurs. Both live in the types of extreme environments that were typical of the early Earth, and to detect their field marks, it is necessary to journey to environments considered intemperate by humans. Ordinary warm springs will not do; the temperatures and conditions must be extreme.

WHERE TO LOOK FOR ANCIENT HYPERTHERMOPHILES

Many of the ancient hyperthermophiles tend to live in marine hot springs that are usually much too deep to be accessed except by research submarines. However, you can visit a hot spring environment on land, and as long as you have chosen one hot enough (over 80°C [176°F]), you can imagine that you are in the vicinity of something like the most ancient microbial communities. Yellowstone National Park, in Wyoming, is the best place to find this sort of environment. Three other major thermal areas, in Iceland, New Zealand, and eastern Russia, have been altered to gain thermal power or are less well exposed and therefore exhibit a less diverse display of heat-loving microbes. Other locations for hot springs of this temperature in the United States include sites in California, Utah, Oregon, Colorado, New Mexico, and Idaho. Hot springs (actually steam vents) greater than 80°C (176°F) may also be found in Washington and Hawaii, although these are often inaccessible because they are associated with active volcanoes. At hot springs cooler than 80°C, the microbial community may include bacteria of more recent lineages; their colors will tend to obscure your "view" of the ancient hyperthermophiles.

Yellowstone National Park

Yellowstone National Park should be considered a primary destination for any field microbiologist, amateur or professional. The park encloses and protects the largest pristine assemblage of hot springs, geysers, fumaroles (steam vents), and boiling mud pots in the world (table 1.1). A visit to Yellowstone is the best way to view a diversity

TABLE 1.1. Where to Look for Field Marks of Hyperthermophiles in Yellowstone Park, Wyoming

- Lower Geyser Basin
  - Octopus Spring (84–91°C [184–196°F]). This is a well-studied research area (see chap. 17).
- Midway Geyser Basin
  - Grand Prismatic Spring (64–87°C [147–188°F])
- Black Sand Geyser Basin

Source: Carl Schreier, *A Field Guide to Yellowstone's Geysers, Hot Springs, and Fumaroles* (Moose, Wyo.: Homestead, 1992).

Note: These features are likely to have a range of temperatures, and it may not be possible to get close enough to take samples. At Yellowstone, water boils at 93°C (199°F) because the park is 7,500 feet above sea level.

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of bacteria in thermal and hyperthermal environments. The U.S. Park Service has built miles of boardwalks so that many of the thousands of thermal features may be viewed up close safely and without disturbing the delicate balance of microbiology and geology.

Most of the area of the park consists of the collapsed center of a huge volcano, or caldera, that first erupted about 2 million years ago, again 1.2 million years ago, and most recently 600,000 years ago. It is still considered active and will erupt again. The myriad thermal features in and around the caldera are testament to the ongoing turmoil just beneath the thin surface crust.

Yellowstone Park is one of the few places in the world where one may look out at a vast panorama of bubbling, hissing, erupting, oozing fluids and minerals. This scene is probably as close as we can come to a view of what the Earth's surface was like 4 billion years ago. Such a view is possible from the Visitors' Center overlooking the Porcelain Basin in Norris Geyser Basin, one of the hottest areas of the park (plate 1).

Planetary geologists have hypothesized that the surface crust of the early Earth was much thinner than it is now, and therefore more active with earthquakes and thermal features of all kinds, including volcanoes. The crust at Yellowstone may be only about 40 miles thick rather than the more typical 90 miles for the rest of the Earth. Furthermore, Yellowstone sits atop a slowly moving hot spot (inching northeast) from which magma deep within the mantle has extended upward and has broken through parts of the Earth's crust. As a result, molten rock may be just 2 to 3 miles below the surface in some places. The whole park may be considered a rare window on the geothermal world below the Earth's surface, and as such is an equally rare window on what the Earth might have looked like when life originated.

FIELD MARKS AND HABITATS OF ANCIENT HYPERTHERMOPHILES

In general, temperatures above 80°C (176°F) and neutral to alkaline conditions are reliable field marks for the few bacterial genera considered to be ancient hyperthermophiles: *Thermatoga*, *Aquifex*, and *Hydrogenobacter*. (See chapter 3 for another ancient branch, the hyperthermophilic archaea, which are found in *acidic* hot springs, and chapter 17 for *Thermus*, another heat lover.) All other organisms are excluded at these extreme temperatures. Any slimes and some of the colors such as pinks and yellows that you observe above 80°C are fairly reliable bacterial field marks. Concentrate on hot springs that are stable enough—neither eruptive nor churning—to allow visible bacterial assemblages to form. Be aware, however, that colorful sediments at temperatures over 80°C may also be due to mineral precipitates such as red iron and yellow sulfur. The following are good rules of thumb for distinguishing minerals from bacteria:

1. Some minerals grow in from the edge of a feature, while bacteria spread out from the source of the heat.
2. Minerals tend to be crusty or crystalline and rigid, whereas bacterial assemblages may appear to shimmer or "wave in the breeze" in flowing water. In general, bacterial growths are softer than mineral deposits.

Determining the temperature of a hot spring likely to have hyperthermophiles is the next challenge in identification. Usually, you should not get close enough to a boiling spring or geyser to be able to insert a thermometer. The surrounding ground may be thin and unstable, and a fall into boiling water may be lethal. Instead, take advantage of any signage indicating temperatures, as well as the knowledge of the park rangers. Carl Schreier's *A Field Guide to Yellowstone's Geysers, Hot Springs, and Fumaroles* also provides temperature information, although this is highly subject to change according to the season and because of the instability of most features. For taking temperatures at a distance, consider an infrared temperature sensing "gun." If you use a regular thermometer, do not use one containing mercury, as breakage will release this dangerous pollutant. Make sure that the thermometer registers boiling temperatures.

One of the most interesting ways of determining temperature is by "colorimetrics"—that is, using certain-colored bacteria or a lack of them as indicators of particular temperatures (table 1.2). Below 60°C (140°F) eukaryotic organisms may be found. For example, *Cyanidium*, a bright grass-green alga, lives at temperatures

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Table 1.2. Colorimetric Determination of Temperatures in Neutral to Alkaline Hot Springs

Approximate temperature range	Color of water or sediment	Confounding factors
93°C (199°F)*	Bright-blue steaming water, often with dissolved silica and steam or vapor. Also white sediment in steamy water, due to silica minerals such as geyserite or sinter. (In Mammoth Terraces area, the white is travertine, or calcium carbonate.)	Bubbling may or may not indicate boiling; sometimes it is just escaping gas.
75°C (167°F)	Gradient of yellow-orange-brown-green (going from hot to warm).	In iron-rich or sulfur-rich areas, the colors could be due to minerals rather than bacteria; see text for hints on distinguishing the source.

* At the elevation of Yellowstone, this is boiling.

up to 55°C (130°F) in *acidic* waters, along with diatoms, a type of protist.

An especially effective use of table 1.2 is to locate boiling features (e.g., erupting geysers) in which a gradient of concentric colors may be observed in pools or in ribbons of color where hot water is flowing away from the feature (plates 2–4).

The focus for detection of ancient hyperthermophiles should be any white area of sediment flanked by boiling or near-boiling water on one side and colorful yellow-orange bacteria on the other. The thermal feature should be alkaline to neutral in pH. Some pink-colored filaments in such areas have been determined to be a species

or relative of *Aquifex*. Be aware that in the same areas you may also find pinkish-yellow filaments, which are likely field marks of *Thermus* bacteria (see chapter 17). An acidic hot spring is likely to have a different assemblage of field marks and is more appropriate for detecting hyperthermophilic archaea (see chapter 3; full interpretation of a hot spring environment should include information from chapters 1, 3, and 17).

The ancient hyperthermophiles, as well as hyperthermophilic archaea and *Thermus* species, may be found at temperatures lower than the narrow range recommended for observing field marks. At these lower temperatures, they may be present and even thriving but obscured by other bacteria, especially brightly pigmented, photosynthetic ones.

VIEWING ANCIENT HYPERTHERMOPHILES UNDER A MICROSCOPE

In most cases it will not be possible or advisable for you to get close enough to the boiling or near-boiling waters of thermal springs to collect a sample. One exception might be tiny, unmarked features away from the usual tourist areas. For example, on a roadside near West Thumb Geyser Basin, small flows of hot water can be seen bubbling up on the shore of Lake Yellowstone (fig. 1.1). In such an area, you could determine the temperature and collect a small drop of water or speck of sediment for viewing under a microscope (fig. 1.2). To preserve your sample for later viewing, add formalin to make a total concentration of about 3% (3 parts formalin to 97 parts sample water). Many ancient hyperthermophiles are coccoids, rods, or filaments, and they are often large. Keep in mind that at lower temperatures, eukaryotes such as green algae and diatoms will obscure (or distract you from) the bacteria. Very small thermal features will probably not maintain a large temperature gradient, so your sampling area may be just a few millimeters.

If you have found a tiny, unmarked thermal feature and have about a week to spend in the area, consider submerging a glass microscope slide in the hot water or moist sediment and leaving it for a week. (Realize, of course, that the U.S. Park Service could

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FIGURE 1.1. The author and her daughter at a tiny thermal feature on the shore of Lake Yellowstone near West Thumb. A sample (the size of a speck) is being prepared for the field microscope.

rightly interpret this as the leaving of trash; consider asking permission or being very discreet, taking care to remove your experimental slide when done.) Microbiologists have had good success getting hyperthermophiles to actually grow on the glass, which may be wiped clean on one side and viewed beneath a coverslip on the other.

CULTURING ANCIENT HYPERTHERMOPHILES

Culturing hyperthermophiles involves maintaining boiling or near-boiling conditions in a complex medium, devoid of oxygen. This is not a simple amateur activity.

THERMOPHILIC GREEN NONSULFURS
A FORK IN THE EVOLUTIONARY TREE

Green nonsulfur bacteria are descendants of the first photosynthetic bacteria. Their branch on the family tree appeared soon after that of the ancient hyperthermophiles. Green nonsulfurs love hot water too, although they are not true extremophiles (lovers of extreme conditions). Look for them in hot springs between 60 and 80°C (140–176°F). While some grow at cooler temperatures, so too do many eukaryotes that obscure your view of the bacteria. To make a reliable identification of green nonsulfurs, look for hot springs in the right temperature range (table 1.2).

FIGURE 1.2. Taking the temperature of the feature described in figure 1.1. The water bubbling up was boiling, and by standing too long right next to it, I let the soles of my sneakers melt a little! The sampled (orange) area shown here was at 65°C (149°F), a good habitat for green nonsulfurs. A little farther away, at 55°C (131°F), a green sample was full of eukaryotes such as diatoms.

The somewhat cumbersome name *green nonsulfurs* is derived from two major aspects of their metabolism. These bacteria are photosynthesizers that use a version of the green pigment chlorophyll to capture light energy. Unlike other groups of photosynthetic bacteria that appeared early on the tree of life, green nonsulfurs do not require sulfur compounds such as hydrogen sulfide (H_2S) as a source of hydrogen in their metabolism, nor do they use water (H_2O) as a

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source of hydrogen, as more advanced photosynthesizers such as the cyanobacteria do. Instead they use organic molecules.

THE EARLIEST PHOTOSYNTHESIS

Chloroflexus is the green nonsulfur most commonly observed in hot spring environments. These bacteria are used here as an example of some of the peculiarities of ancient bacterial photosynthesis.

All photosynthesizers—whether trees, eukaryotic green algae (pond scum), cyanobacteria, or green nonsulfurs—capture light energy using some version of the green pigment chlorophyll. They use that light energy to make food molecules (often sugars) from decidedly non-foodlike molecules, most commonly carbon dioxide (CO_2). Some form of hydrogen is also needed because food molecules invariably turn out to be some type of *hydro*carbon. For example, trees take water (H_2O) as a source of hydrogen to make the sugar glucose ($C_6H_{12}O_6$) from carbon dioxide:

$$CO_2 + H_2O + \text{light} \rightarrow \text{food molecules}$$

Most variations on photosynthesis center around those starting compounds. Water is a convenient, nontoxic source of hydrogen that is used along with carbon dioxide by all eukaryotic photosynthesizers and most cyanobacteria. The other photosynthetic bacteria as well as some cyanobacteria use other sources of hydrogen with carbon dioxide. Hydrogen sulfide is a favorite, although users of this compound are limited to sulfur-rich environments, which are fairly rare on present-day Earth.

The starting compounds used by *Chloroflexus* for photosynthesis make it seem rather strange, even by comparison with other obscure bacterial photosynthesizers. For *Chloroflexus*, the optimal source of carbon and hydrogen (including free hydrogen) is some small, available food molecule such as acetate or pyruvate. These molecules are presumably waste products of other bacteria, such as certain anaerobic heterotrophs that only partially digest their food. If oxygen is present, *Chloroflexus* acts as a heterotroph and consumes whatever small food molecules are available, through an unusual type of respiration. However, if oxygen is not present (as was the case on the early Earth), *Chloroflexus* can use light energy to make larger food molecules from smaller ones.

CHLOROFLEXUS AND THE EVOLUTION OF METABOLIC PATHWAYS

Evolutionists studying the early photosynthesizers are reluctant to form conclusions about the exact order in which different types of metabolism evolved. After all, we do not have the original organisms in hand. We can only look at their descendants, which have undergone almost 4 billion years of evolution. Surely there have been some changes in that time period! Nevertheless, the metabolic versatility of *Chloroflexus* and its position on the bacterial family tree—right before the divergence of most of the other bacteria—give clues to how bacterial evolution might have occurred.

Chloroflexus needs quite an accumulation of genes to accomplish its diverse metabolism, which includes photosynthesis under anaerobic conditions and respiration (heterotrophy) under aerobic conditions. According to one hypothesis, heterotrophy evolved before photosynthesis, and the ancestor of *Chloroflexus* was a fermenter (consumer of food). Metabolic processes tend to be complex, coded for by many genes. While individual genes might be lost or modified by mutation, it is difficult to lose an entire set of genes that codes for a complex function. Therefore, if the ancestor of *Chloroflexus* was a fermenter, then *Chloroflexus* might be expected to still contain genes for fermentation. According to this scenario, it is as if during evolution, *Chloroflexus* kept sets of old genes (like old furniture) in its attic or basement, rather than getting rid of them. However, this collection was not idle. Sometimes items were restored to use, combined in some new way, or even modified through mutation. This is how two new metabolic processes—photosynthesis and aerobic respiration—might have evolved in *Chloroflexus*. Indeed, some aspects of photosynthesis in *Chloroflexus* look like parts of fermentation run backward. By running such a reaction backward and applying lots of free energy from the sun, an anaerobic heterotroph could become an autotroph. (Yes, biological reactions can be run backward, with interesting consequences, as long as there is enough energy to force the reaction into reverse.) Similarly, aerobic respiration can be

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viewed as an elaboration of fermentation. Now imagine the ancestral *Chloroflexus* with bulging attic and basement maintaining a diversity of metabolic pathways, perched on the evolutionary tree just before the divergence of most bacterial groups. With various modifications and combinations of the *Chloroflexus* genes, almost all subsequent types of bacterial metabolism (featured in chapters 5 to 18) could have evolved. The peculiar heterotrophy of present-day *Chloroflexus* may have evolved following this divergence, after significant oxygen had become available in the atmosphere.

CHLOROFLEXUS IN THE FOSSIL RECORD

The most ancient microfossils and stromatolites, which are fossilized microbial communities dating from 3 to 3½ billion years ago, are usually interpreted to be cyanobacteria (see chapter 13). An alternative explanation, however, is that these are fossilized bacteria of the *Chloroflexus* type. This is a reasonable idea, given the fact that *Chloroflexus* bacteria seem to predate the cyanobacteria, but firm evidence as to the exact identity of early fossilized bacteria is not available.

FIELD MARKS AND HABITATS OF THERMOPHILIC GREEN NONSULFURS

The cells of green nonsulfurs are long, thin filaments capable of a gliding type of motility. They are most visible when forming colorful gelatinous mats in alkaline to neutral hot springs between 60 and 80°C (140–176°F) (table 1.3). The mats range in color from yellow or beige to orange or orange-red as a result of carotenoid accessory pigments; in some cases they are green due to the presence of chlorophyll. Green nonsulfurs are fairly easy to see as long as they are any color but green (plate 5). Green mats of green nonsulfurs are difficult to distinguish from greenish or blue-green mats of cyanobacteria, which are often present in the same environment. Generally a mat of yellow- to beige-colored green nonsulfurs will be positioned below a thin, green to blue-green mat of cyanobacteria. If the hot spring is quite sulfury smelling, the mats may be inverted such that the cyanobacterial layer lies beneath and in more direct contact with sulfur compounds.

Be cautious in making macroscopic identifications. Sometimes cyanobacterial mats may become bleached to an orange color, revealing their orange carotenoid accessory pigments. Green nonsulfurs can also form greenish or orange mats independent of visible cyanobacterial mats. The presence of obvious cyanobacteria (green to blue-green mats) with underlying or overlying gelatinous beige-orange mats in a temperature hot enough (60–80°C [140–176°F]) to omit eukaryotes may be the best combination of field marks for the green nonsulfurs (plate 6). Green nonsulfurs have also been observed in low-temperature marine and hypersaline microbial mat communities, although they are likely to be present in lower numbers and are difficult to distinguish from cyanobacteria.

TABLE 1.3. Where to Look for Field Marks of Green Nonsulfurs in Yellowstone Park, Wyoming

- Mammoth Hot Spring Terrace
  - Opal Terrace, 160°F (71°C)
  - Minerva Spring, 161°F (72°C)
  - Orange Spring Mound, 157°F (69°C)
  - New Highland Spring, 160°F (71°C)
  - Canary Spring, 160°F (71°C)
- Midway Geyser Basin
  - Grand Prismatic Spring, 147–188°F (64–87°C)
  - Turquoise Pool, 142–160°F (61–71°C)
- Black Sand Geyser Basin
  - Opalescent Pool, 144°F (62°C)
  - Emerald Pool, 154.6°F (68°C)
  - Rainbow Pool, 161°F (72°C)
- Upper Geyser Basin
  - Morning Glory Pool, 171.6°F (77°C)
  - Beauty Pool, 164–175°F (73–79°C)
- West Thumb Basin
  - Abyss Pool, 172°F (78°C)
  - Blue Funnel Spring, 172–182°F (78–83°C)

Source: Carl Schreier, *A Field Guide to Yellowstone's Geysers, Hot Springs, and Fumaroles* (Moose, Wyo.: Homestead, 1992).
Note: Look for green-blue to green cyanobacterial mats with tan-orange bacteria directly above or below green nonsulfurs. Temperatures may vary, with edges and runoff areas likely to be cooler. You may not be able to take samples or insert a thermometer. Also check cooler runoff areas of features listed in table 1.1.

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VIEWING THERMOPHILIC GREEN NONSULFURS UNDER A MICROSCOPE

Do not touch or collect any colorful mat samples in any areas at or near the boardwalks in Yellowstone Park. This is not only dangerous but it is also a form of vandalism that can leave semipermanent marks. Rather, seek out tiny transient features (e.g., as shown in fig. 1.1) and take only a speck of sample, which is all you need for microscopy. If you wish to preserve the speck, place it in 3% formalin (3 parts formalin to 97 parts sample water). Make sure that the sample is from the correct layer in a neutral to alkaline thermal spring (60–80°C [140–176°F]). Compare samples from different layers to be sure. Look for long filaments capable of a gliding type of motility. The filaments may or may not be green, depending on the color of the layer you sample.

CULTURING GREEN NONSULFURS

Maintaining the right temperature and other conditions to grow cultures of predominantly green nonsulfurs is not a simple amateur activity. Green nonsulfurs may grow at more temperate conditions in a Winogradsky column (appendix A). However, it will be difficult to distinguish them from the other photosynthesizers that will proliferate.

SUMMARY: FIELD MARKS AND HABITATS OF ANCIENT HYPERTHERMOPHILES AND THERMOPHILIC GREEN NONSULFURS

Ancient Hyperthermophiles
- thermal features with temperatures greater than 80°C (176°F), alkaline to neutral pH (indicated by white geyserite [sinters]), and stable enough conditions to allow large assemblages of bacteria to develop
- soft, colorful assemblages (yellows-reds-pinks) that appear to flow or shimmer, radiating from a heat source (minerals, which may have similar colors, are harder and crustier and are usually deposited from the edge of a spring)

- long, hairlike strands, possibly pigmented (with pinks and yellows but not greens)
- white sediment flanked by near-boiling water on one side and yellow-orange bacteria on the other; cooler runoff waters may have orange or blue-green cyanobacteria

Thermophilic Green Nonsulfurs
- thermal features with temperatures of 60–80°C (140–176°F) (at cooler temperatures, green nonsulfurs may be confused with other organisms) and alkaline to neutral pH
- beige to orange gelatinous mats (sometimes greenish, but then difficult to distinguish from other photosynthesizers)
- presence of obvious cyanobacterial mats (green to green-blue) either just above or just below (if the cyanobacteria are orange or bleached, the green nonsulfurs will be difficult to distinguish from them)

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