

Extreme Environments

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Extreme environments are important to environmental microbiologists because there is much speculation that such environments harbor unique microorganisms with activities and metabolic strategies that are not only of scientific interest, but also have commercial potential. The interest in extreme environments has engendered support for large research efforts focused on such sites. Scientific agencies such as the European Science Foundation (ESF) and the U.S. National Science Foundation (NSF) are supporting long-term projects that study specific ecological systems which focus on extreme environments. The purpose of these projects is to help provide knowledge that can be used to conserve, protect and manage unique global ecosystems and the biodiversity they sustain, and to help evaluate the potential global significance of poorly understood ecosystems. The **ecosystem services** (Information Box 7.1) they provide, and the discovery of natural products and processes that can be harnessed for societal benefit in the areas of biotechnology, medicine and remediation, are of immense value. The concept of ecosystem services is becoming more important as the world population grows and we place increasing stress on these fragile environments that cycle critical nutrients and sustain our Earth and aquatic environments.

Microbial communities in extreme environments have adapted to amazing levels of stress. These adaptations are of interest for development of remediation approaches for some contaminated sites including acid

mine drainage sites and radioactive waste sites. They also are of interest for applications of novel enzymes adapted to temperature or pH extremes. Finally, they are of interest for understanding evolutionary history and possible impacts of future climate change. Here we describe six different extreme environments: including environments characterized by either low or high temperature; a desert environmental characterized by aridity and UV stress; and three environments where low levels of organic matter and the absence of photosynthesis have resulted in chemoautotrophy playing an important latter role. These are: the marine hydrothermal vents; an acid mine drainage system; and a carbonate cave located in the Sonoran Desert. This is by no means an exhaustive list of extreme environments, but will give an idea of how unique microbial communities develop in such environments, and how novel discoveries are made through the study of such environments.

7.1 LOW TEMPERATURE ENVIRONMENTS

7.1.1 McMurdo Dry Valleys, Antarctica

The McMurdo Dry Valleys in Antarctica represent one of the driest and coldest ecosystems on Earth. The average mean annual surface air temperature is -27.6°C and the average surface soil temperature is -26.1°C . This



FIGURE 7.1 (A) Permanently ice-covered Lake Vanda in the McMurdo Dry Valleys, Antarctica. (B) A sediment core taken by the McMurdo Dry Valley Microbial Observatory researchers from Lake Vanda. Photos courtesy (A) Vladamir Samarkin, (B) A. Chiuchiolo.

Information Box 7.1 Ecosystem Services

Natural ecosystems provide resources, or ecosystem services, that benefit society including provision of clean drinking water and healthy soils for growing crops, regulation of climate, biogeochemical cycling activities, and species diversity. It has been estimated that ecosystems, underpinned by microbial activity, provide at least \$33 trillion per year in global services (e.g., climate regulation, nutrient cycling, waste treatment, water supply and regulation) (Costanza *et al.*, 1997). The World Bank 2006 estimate of the gross world product (the value of all final goods and services produced globally) was \$48 trillion, a very similar figure. As a global society we have thus far considered ecosystem services as free. However, as Earth's human population continues to grow and place increasing stress on the environment, it is increasingly recognized that we must place a value on ecosystem services that is factored into the gross world products.

ecosystem has the only permanently ice-covered lakes on Earth, varying in ice-cover thickness from 3 to 5 meters (Figure 7.1). The permanent ice cover greatly impacts several aspects of normal lake characteristics (see Chapter 6) including:

- reduced wind-driven mixing resulting in vertical transport that is decreased to the level of molecular diffusion
- reduced direct gas exchange between liquid water and the atmosphere
- reduced light penetration
- reduced sediment deposition into the water column

The long mixing times mean that some chemical gradients can exist in the water column for at least 20,000 years before they are dissipated by diffusion. Ecosystem properties in the water columns of the lakes are also controlled by the seasonal uncoupling of photoautotrophic and heterotrophic processes resulting from the unusual solar cycle: 4 months of darkness followed by 4 months of continuous light with twilight in between (MDV, 2007).

The McMurdo Dry Valleys are part of the NSF Long Term Ecological Research network and also serve as an NSF Microbial Observatory. One site that has been studied extensively from a microbial perspective is Lake Fryxell, a freshwater lake that is one of the most productive lakes in the region. Geochemical analysis of the water column shows the presence of oxygen to a depth of 10 meters. There is a sulfide gradient ranging from 0 at the ice–water interface to >1 mM sulfide (S^{2-}) at the sediment surface (Figure 7.2). A complementary sulfate gradient occurs with low concentrations at the sediment surface building to concentrations >1.5 mM just below the chemocline (Information Box 7.2). These gradients suggested the existence of sulfur cycling (see Section 16.4). In fact, the researchers studying the site have found a diverse community of phototrophic purple bacteria (Karr *et al.*, 2003), sulfur chemoautotrophs and heterotrophic sulfate reducers. For example, cell numbers of sulfur-oxidizing bacteria were found to peak at 200 cells per ml at a depth of 9.5 m (Sattley and Madigan, 2006). As shown in Figure 7.2, this is precisely where both dissolved oxygen and sulfide coexist in the water column. Three sulfur oxidizers were cultured from lake water samples, all most closely related to *Thiobacillus*

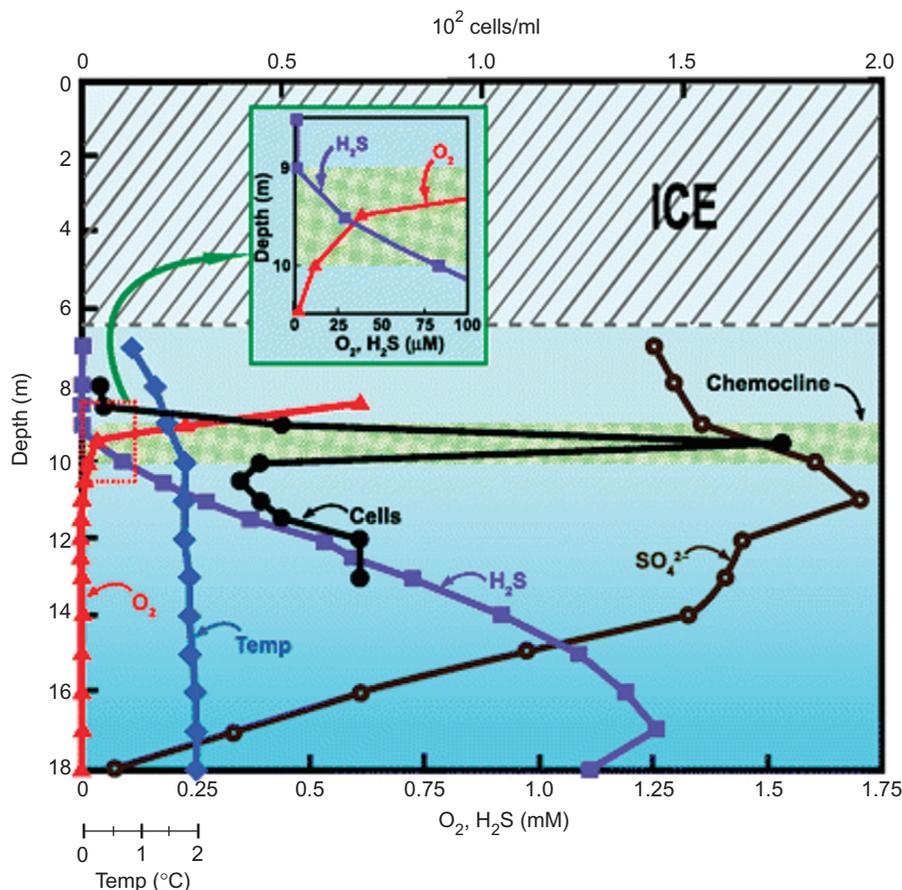


FIGURE 7.2 Diagram showing relevant physicochemical parameters related to the sulfur cycle in Lake Fryxell including cell numbers of sulfur-oxidizing bacteria based on most probable number (see Section 10.3.2) analyses. The expanded scale (inset) shows concentration of sulfide and dissolved oxygen at the chemocline. Diagram from Sattley and Madigan (2006). Reproduced with permission from the American Society for Microbiology Journals Department.

Information Box 7.2 Chemocline in Lake Fryxell

The chemocline is defined as the interface between two different chemistries in a body of water. In Lake Fryxell, the chemocline is the interface between oxygen-rich and anoxic water (Figure 7.2). In general, both nutrients and bacteria tend to accumulate at a chemocline or a thermocline (see Section 6.2.1 and Figure 6.2). Indeed, in Lake Fryxell, the chemocline harbors the largest numbers of sulfur-oxidizing bacteria and also harbors photosynthetic purple sulfur bacteria that consume the hydrogen sulfide, completing a sulfur minicycle in the chemocline.

thioparus, a known sulfur oxidizer, but the Lake Fryxell strains were classified as **psychrotolerant**. The isolates were able to grow in temperatures as low as -2°C with a temperature optimum of 18°C and an upper limit of 31°C . Sulfate-reducing bacteria were also found throughout the water column as determined by analysis for the dissimilatory sulfite reductase gene. Interestingly, several clone groups were highly localized with respect to lake depth suggesting that there are depth-specific population niches (Karr *et al.*, 2005).

In addition to the sulfur gradient, a methane gradient begins at a depth of 12 meters and increases to nearly 1 mM at the sediment surface. This gradient implies the presence of a methane cycle as well as the presence of both methanogenic and methanotrophic microorganisms (Karr *et al.*, 2006). In fact, of 13 archaeal clones found in the site, the majority represent methanogens and anoxic methanotrophs.

What is interesting about cold-adapted microorganisms? Their ability to survive and grow in the cold requires specialized adaptations that have the potential to be harnessed for the benefit of human society. For example, these microorganisms synthesize cold-adapted enzymes which have had to evolve specific structural features that make them highly flexible in comparison to their warm temperature equivalents (Siddiqui and Cavicchioli, 2006). This flexibility, particularly around the active site of the enzyme (the site where the enzyme interacts with its substrate), means that the enzyme can operate efficiently at low temperatures. This also means that at high temperature, the enzyme becomes unstable. In fact, it is these two properties of cold-active enzymes that make them suitable for biotechnological application; their high activity at low temperature and their low stability at elevated temperatures. The ability to carry out a

reaction at low temperature can have several advantages. Sometimes, low temperature is more suitable for labile reactants, but also reactions carried out at low temperature require less energy input, which is a direct cost saving. The fact that these enzymes have low stability at elevated temperatures means that the reaction can be stopped quite easily by simply raising the temperature. Low temperature enzymes that have been examined or used in industry include; α -amylase (bread making, textiles, brewing and detergents); cellulase (textiles and the pulp and paper industries); β -galactosidase (removes lactose from milk), lipase (detergents and flavorings), proteases (detergents and meat tenderizers); and xylanase (bread making) (Cavicchioli *et al.*, 2002).

7.2 HIGH TEMPERATURE ENVIRONMENTS

7.2.1 Geothermal Hot Springs

Yellowstone National Park, Montana, U.S.A., has over 10,000 unique geothermal features that contain a wide and varied range of temperature, pH and geochemical profiles (Figure 7.3). This site has been a focus of research interest ever since it was realized that the thermostable DNA polymerase enzyme from *Thermus aquaticus*, which was isolated from Yellowstone, could be used for polymerase chain reaction (PCR) (Saiki *et al.*, 1988) (Chapter 13). *Thermus aquaticus* is an extreme thermophile that is able to grow between 40 and 79°C with an optimum temperature of 70°C (Brock and Freeze, 1969). Hot springs, such as those found in Yellowstone, have

temperatures of up to 100°C. Similarly, deep-sea hydrothermal vents can harbor hyperthermophiles (in fact, one of the record holders for high temperature tolerance is a deep-sea vent organism, *Pyrolobus fumarii*, an Archaea that can tolerate up to 113°C). Genera commonly found in these environments include *Thermus*, *Methanobacterium*, *Sulfolobus*, *Pyrodictium* and *Pyrococcus*.

The study of the thermophilic microorganisms in Yellowstone hot springs has been the subject of another NSF Microbial Observatory. In this case, researchers have examined the microbial community associated with the large amounts of sulfur that are fed into hot springs by geothermal fluids. One study site is called Dragon Spring. The source waters for Dragon Spring are acidic (pH 3.1 at the source); range in temperature from 66 to 73°C; and contain up to 80 μ M of dissolved organic carbon. As these fluids reach the surface of the springs, the elemental sulfur in the incoming geothermal fluids forms flocs that are a distinctive feature of the entire outflow channel of the spring (Figure 7.3). This flocculent is the basis for a sulfur-cycling microbial community. Although the flocculent is formed abiotically, it is quickly colonized by two groups of organisms.

The first group is composed of two sulfur-respiring archaeal populations, *Caldisphaera draconis* and *Acidilobus sulfurireducens*. Quantitative PCR analysis (Chapter 13) shows that these two populations represent a major portion, 17 to 37%, of the floc-associated DNA (Boyd *et al.*, 2007) (Information Box 7.3). These isolates face an intriguing problem as sulfur reducers. It is speculated that these microorganisms do not reduce elemental sulfur (S^0) as it exists in the flocculent, but rather, reduce



FIGURE 7.3 The source of Dragon Spring in the Hundred Springs Plain of Norris Geyser Basin, Yellowstone National Park, Wyoming, U.S.A. The whitish-yellow material is flocculent elemental sulfur that becomes quickly colonized by sulfur-respiring Crenarchaea in the domain of Archaea and hydrogen sulfide-oxidizing bacterium *Hydrogenobaculum* spp. Photo courtesy Gill G. Geesey, Montana State University.

Information Box 7.3 Isolation of Novel Microorganisms

Analysis of DNA sequences from the environment can reveal the existence of novel and potentially intriguing microorganisms. But to actually study the physiology of these microbes, they must be cultured. Culturing an unknown microorganism is a major challenge and has been approached in two ways. First, by sequencing the 16S rRNA gene of a novel microbe, one can phylogenetically analyze the organism in relation to its nearest neighbors. This can provide clues to possible metabolic preferences, which can be tested by constructing specialized culture media. The second approach was used by researchers at Dragon Spring in Yellowstone National Park (Boyd *et al.*, 2007). In this case the 16S rRNA genes of the two novel sulfur-respiring microbes recovered from Dragon Spring did not closely match any known sulfur-reducers. Therefore, the scientists analyzed the geochemistry of Dragon Spring and mimicked these conditions in the laboratory. Using this approach they successfully cultured the archaeal sulfur-reducers *Caldisphaera draconis* and *Acidilobus sulfurireducens*.

sulfur from polysulfide (S-S-S-S-S-S-S^-). Interestingly, polysulfide is not stable at the acidic pH conditions found in Dragon Spring, and it disproportionates almost completely into S^{2-} and S^0 (Rickard and Morse, 2005). Thus these microbes have some, as yet undescribed, mechanism to obtain polysulfide.

The second group that colonizes the flocculent is composed largely of the chemoautotrophic sulfide-oxidizing bacteria from the genus *Hydrogenobaculum*. This thermophile oxidizes the sulfide produced by the sulfur-respiring Archaea back to elemental sulfur (S^0). Thus, a very dynamic and tightly coupled sulfur cycle occurs at the very source of Dragon Spring. Interestingly, as the water flows away from the spring over a distance of a very few meters, there are drastic changes in the biogeochemistry of the system. These include pH increases, temperature decreases, the introduction of oxygen and iron, and arsenic becoming important in the energy flow in the system (Inskeep and McDermott, 2005).

Many mechanisms allow microorganisms to survive at temperatures that would normally denature proteins, cell membranes and even genetic material (Bouzas *et al.*, 2006). The key to enzyme function, whether in cold or hot environments, is the maintenance of an appropriate balance between molecular stability and structural flexibility. One general adaptive mechanism exhibited by thermophilic microorganisms is the production of **chaperonins**, which are specialized thermostable proteins that help refold and restore other proteins to their functional form following thermal denaturation. In addition, there are microbe-specific adaptations to increase protein stability at high temperature including:

- an increased number of disulfide bridges
- increased interactions among aromatic peptides
- increased hydrogen bonding among peptides

In terms of cell membranes, most hyperthermophiles belong to the Archaea. The archaeal cell membrane differs in structure from the bacterial cell membrane, as shown in Figure 7.4. The archaeal membrane is more thermostable than bacterial membranes (although it should be noted that the membrane structure is the same whether or not an archaean is thermophilic). Finally, in terms of nucleic acids, all hyperthermophiles produce a unique enzyme called DNA gyrase. This gyrase acts to induce positive supercoils in DNA, theoretically providing considerable heat stability (Kikuchi and Asai, 1984; Bouthier de la Tour *et al.*, 1990).

What has been learned about microbial adaptation to life as an extreme thermophile? One example is a recent report of *Thermus thermophilus* HB27, which was isolated from a hot spring in Japan. This organism has a DNA translocator system that allows it to take up DNA very broadly from various members of all three domains of life, Bacteria, Archaea and Eukarya (Schwarzenlander

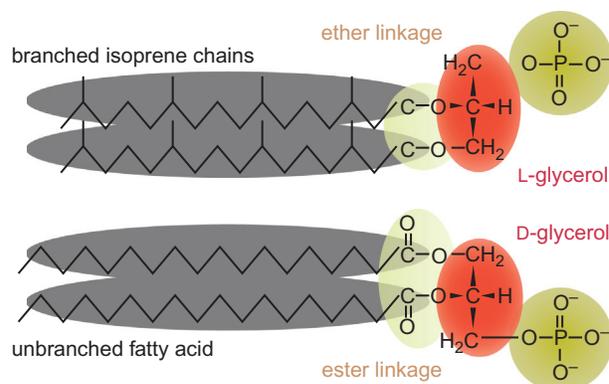


FIGURE 7.4 A comparison of membrane phospholipids from Archaea (top) and Bacteria (bottom). Note that in the Archaea the lipids are linked to glycerol through an ether linkage while for Bacteria an ester linkage is used. Further, archaeal lipids are based on a repeating 5-carbon unit isoprene usually 20 carbons in length. Bacterial lipids are straight chain fatty acids of 16 to 18 carbons in length.

and Averhoff, 2006). Further, this translocator system exhibits extremely high rates of DNA uptake. In fact, this organism has a DNA uptake velocity of 40 kilobase pairs per second (i.e., 40,000 base pairs). This can be compared to some known mesophilic organisms which have uptake velocities that are 2.5 to 10 times lower including *Bacillus subtilis* (4 kbp/sec), *Haemophilus influenzae* (16 kbp/sec) and *Streptococcus pneumoniae* (4 kbp/sec). The authors of this report suggest that the DNA translocator system has been of benefit to this organism by allowing it to adapt quickly to life in extreme environments. Moreover, this DNA translocator might be one of the most powerful tools for interdomain transfer of thermophilic and physiological traits between microorganisms thriving in extreme environments.

What do thermophiles have to offer biotechnology? There are numerous biotechnological applications for enzymes isolated from thermophilic microorganisms and the number of applications is growing rapidly, especially in commercial industry. The prime example is the thermostable DNA polymerase used in PCR. Other examples include proteases, lipases, amylases and xylanases that are used in the agricultural, paper, pharmaceutical, water purification, bioremediation, mining and petroleum recovery industries.

7.3 DESICCATION AND UV STRESS

7.3.1 The Atacama Desert, Chile

The deserts of the world represent both hot and cold semiarid to hyperarid environments where extreme conditions severely limit primary productivity and, thus, the diversity of life. Factors limiting microbial life in the arid deserts include water availability, temperature and the



FIGURE 7.5 The hyperarid core region of the Atacama Desert, Chile. Photo was taken at 974 m elevation (24.073°S, 70.204°W) southeast of Antofagasta. Courtesy Julia W. Neilson.

intensity of UV radiation. Arid and semiarid deserts are characterized by mean annual rainfall levels of 25–200 mm while hyperarid deserts have a mean annual rainfall of <25 mm. Water availability in a desert is not only determined by mean annual rainfall, but also by the combined effects of precipitation (P) and potential evapotranspiration (PET). Hyperarid areas are defined as those with a P/PET ratio less than 0.05 (Houston and Hartley, 2003). These moisture-deficient environments are of global relevance because they occupy 37% of the terrestrial surface (Middleton *et al.*, 1997), and little is known about the arid and hyperarid ecosystems that occupy 12% and 7.5%, respectively, of the global land area.

One of the driest deserts on Earth is the Atacama Desert, Chile, where the hyperarid core of the desert experiences intervals of years to decades with no rain (Betancourt *et al.*, 2000). Due to the lack of available moisture, plants are sparse or completely absent, creating soil conditions with extremely low soil organic carbon levels that further limit the potential diversity of microbial life (Figure 7.5). Examples of total organic carbon levels in a range of desert soil samples include: 0.7% in the Mojave (Eureka Valley, California, U.S.A.); 0.17% in the Sahara (near Abu Simbel, Egypt) (Lester *et al.*, 2007); and 0.02–0.09% in samples taken along an elevational transect through the hyperarid core region of the Atacama Desert (Drees *et al.*, 2006). For comparison, organic carbon in temperate soils ranges from 1 to 5% (see Section 4.2.2.6).

A commonly studied refuge for life in arid environments is the **lithobiontic** microbial communities that inhabit rock surfaces and subsurface rock pores. These communities contain a surprising diversity of heterotrophic bacteria that are sustained by photoautotrophic nitrogen-fixing microorganisms. These include cyanobacteria and green algae, and are capable of colonizing a

diverse group of minerals including dolomite, granite, gypsum, halite, limestone, quartz and sandstone. These communities have been found in a range of hot and cold deserts including the Atacama, the Mojave, the al-Jafr Basin (Jordan), the deserts of northwestern China and the McMurdo Dry Valleys of Antarctica (Figure 7.6). These **hypolithic** (inhabit the underside of rock surfaces) and **endolithic** (inhabit pore spaces within the rocks) communities are believed to exploit the protection offered by mineral surfaces that scatter UV radiation and trap limited water supplies while still allowing enough light penetration for photosynthesis (Dong *et al.*, 2007).

One dominant photoautotroph found in many of these communities is the desiccation and radiation tolerant cyanobacterium *Chroococcidiopsis* (DiRuggiero *et al.*, 2013). Cyanobacteria, for example *Nostoc* sp., have been shown to remain in a state of desiccation for months or years at a time (Potts, 1999). This has been measured at a level of 2–5% cell water content, which is one order of magnitude lower than that of eubacterial spores (Gao and Ye, 2007)! Adaptation to desiccation is unique among the extremes experienced by bacteria (i.e., temperature, pH, pressure) because the cells do not grow while desiccated, and the greater portion of their viable lifetime may be spent in the dehydrated state. Thus, cycles of desiccation appear to induce survival strategies for the cells rather than the ability to function under extreme conditions. The survival strategies identified include:

- the ability to protect and repair DNA exposed to UV radiation
- maintenance of protein stability in the dehydrated state
- maintenance of membrane integrity

The primary adaptive mechanism of the Cyanobacteria is the production of an extracellular polysaccharide sheath (EPS). This sheath regulates the uptake and loss of water, serves as a matrix for immobilization of cellular components produced by the cell in response to desiccation, and may protect cell walls during shrinking and swelling (Potts, 1999). Several molecules are produced by the cell in response to desiccation and UV exposure. These are often found in the EPS sheath, and include UV absorbing compounds such as mycosporine-like amino acids and scytonemin, carotenoids and detoxifying enzymes or radical quenchers that provide protection from harmful radicals and oxygen species, and water stress proteins (Gao and Ye, 2007). Water stress proteins are extremely stable and have been found to comprise up to 70% of the soluble proteins in environmental samples of *Nostoc commune*. In addition, *N. commune* cells contain trehalose and sucrose, which have the ability to stabilize proteins and protect the integrity of the membrane during desiccation. Electron microscopy indicates that the nucleoplasm of *N. commune* cells requires 5 days of rehydration before

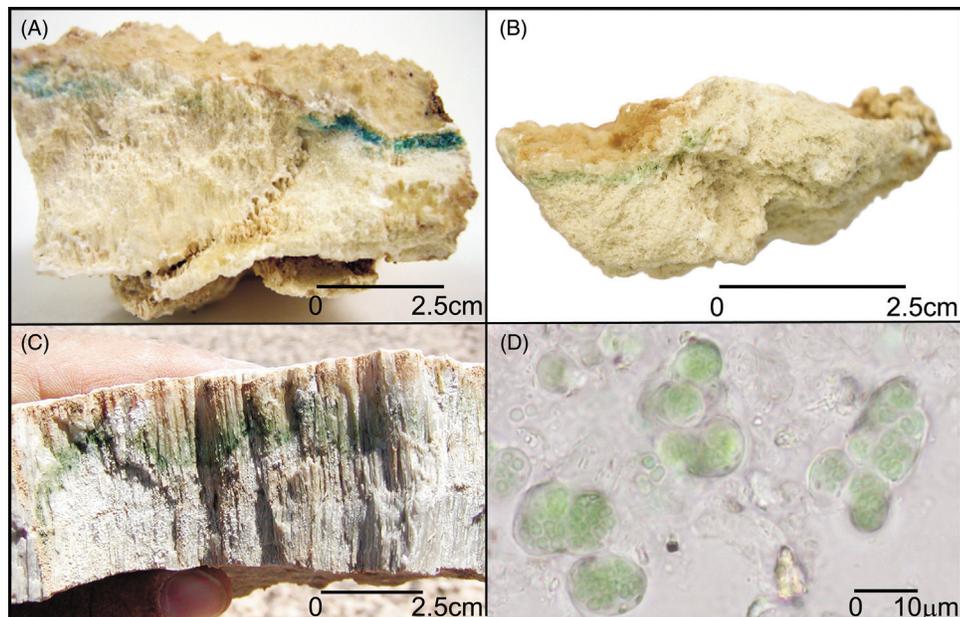


FIGURE 7.6 Samples of soil gypsum with cyanobacteria: (A) Soil gypsic crust sample AT326b from the Atacama Desert; (B) soil gypsic crust sample DG from the Mojave Desert; (C) fibrous gypsum sample JB1 from a secondary vein exposed by erosion at the surface from Al-Jafr Basin, Jordan; (D) light micrograph of cyanobacterium *Chroococcidiopsis* in an enrichment culture from sample AT326b from the Atacama Desert. From Dong *et al.* (2007). Reproduced with permission of AUG.

appearing similar to fully hydrated cells. In addition, a very ordered recovery of gene expression has been documented during rehydration beginning with respiration, followed by photosynthesis and finally nitrogen fixation. Although desiccation and UV tolerance are widespread among cyanobacteria, the interactions are complex and considerable work is still needed to fully understand the mechanisms involved.

Due to the presence of these photoautotrophic nitrogen-fixing primary producers, the lithic communities in arid environments are not dependent on exogenous carbon or nitrogen supplies. This has allowed the investigation of other physical factors that may limit microbial life in hyperarid environments. Two studies have examined such lithic systems to determine the physical factors most limiting to life in hyperarid regions. In the first study, the microbial diversity was characterized in hypolithic crusts on quartz substrates from three hyperarid desert locations in northwestern China (Turpan Depression, Taklimakan Desert and Qaidam Basin). Regression analysis revealed a positive correlation between the availability of liquid water and two diversity indices: the Species Richness ($R^2 = 0.738$) and the Shannon Diversity Index ($R^2 = 0.650$). The availability of liquid water was calculated from the interaction of temperature and rainfall (Pointing *et al.*, 2007). A similar study was conducted by Warren-Rhodes *et al.* (2006) in which translucent quartz and quartzite stones were collected from four locations along an aridity gradient in the Atacama Desert where rainfall declined from 21 to ≤ 2 mm year⁻¹. The percentage of rocks collected that were colonized by hypolithic cyanobacteria declined from 28 to $< 0.1\%$, from the less arid to the most hyperarid region, reinforcing the

conclusion that the absence of available water severely limits microbial biomass in the extreme hyperarid deserts.

Less is known about the energy dynamics of microbial communities that inhabit soils of these expansive hyperarid and arid regions. Intriguing data suggest that the soils of these barren, unvegetated regions contain numerous radiotolerant and halotolerant species, as well as bacteria with phylogenetic associations to chemolithoautotrophic taxa that obtain energy through the oxidation of nitrite, carbon monoxide, iron or sulfur. This suggests a genetic potential for nonphototrophic primary production and geochemical cycling in these arid ecosystems (Neilson *et al.*, 2012).

What knowledge can be gained from the study of organisms capable of survival in extreme hyperarid regions? First, an understanding of where life can occur in the hyperarid regions on Earth helps to narrow and focus the search for life beyond our planet. For example, it has been suggested that life would have been forced into endolithic habitats on Mars as liquid water slowly disappeared from the planet. Second, research suggests that expanded knowledge of microbial diversity in hyperarid regions could be used to evaluate precipitation history. A recent study of soil bacterial diversity in the Atacama Desert was performed along a west/east elevational transect (400–4500 m) through the driest region of the core absolute desert (Drees *et al.*, 2006). Mean annual temperature and precipitation along the transect ranges from 17°C to 7°C and 0.6 to 35.7 mm, respectively. In 2002, soil samples were collected at a depth of 25 cm to obtain bacteria unaffected by surface radiation or eolian (windborne) dispersion. Community DNA was extracted from the samples, amplified by PCR using universal

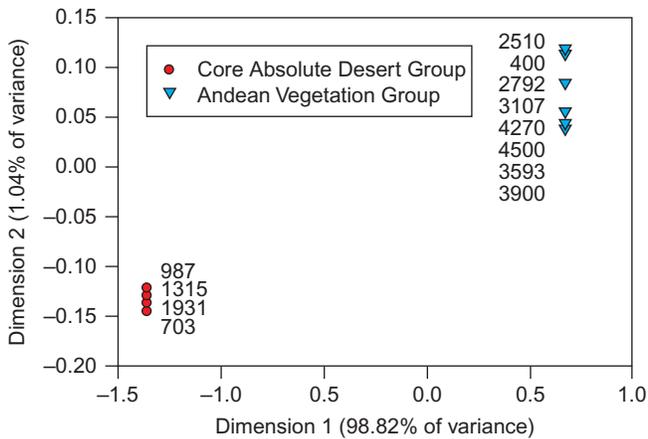


FIGURE 7.7 Kruskal's multidimensional scaling analysis of the DGGE profiles from the Atacama Desert elevational transect analyzed in three dimensions with 99.5% of the variance explained. Data labels represent sample elevations (meters) along transect. The microbial communities in samples along this transect clustered into one of two groups; the core absolute desert group and the Andean group (Drees *et al.*, 2006). Reproduced with permission from the American Society for Microbiology Journals Department.

bacterial 16 S rDNA primers, and the diversity analyzed by denaturing gradient gel electrophoresis (DGGE) (see Section 13.5.2.2). The results revealed the presence of two distinct microbial community types, one found in samples taken from the central hyperarid core and the other occurring in samples taken at elevations above and below the hyperarid central plateau, where sample sites showed evidence of exposure to either onsite precipitation or moisture runoff from higher elevations (Figure 7.7). The two microbial community groups did not correlate exactly with any other specific factors such as temperature, precipitation, TOC or percent plant cover gradients. A second sampling 2 years later, in 2004, indicated that the observed microbial communities were stable over time. Thus, these results suggest that microbial diversity patterns in hyperarid and arid environments can reveal information concerning the frequency of precipitation events or even historical climactic variations. Finally, research groups hope to identify heat and desiccation-tolerant mineral-oxidizing microbes from among the novel populations present in arid deserts that can be exploited for the microbially driven heap-leaching strategy used by mining companies to extract minerals such as copper from low-grade ore (Soto *et al.*, 2013).

7.4 APHOTIC ENVIRONMENTS BASED ON CHEMOLITHOAUTOTROPHY

Photosynthesis is the primary driver of energy dynamics in surface terrestrial and marine ecosystems, where light energy is harnessed for the conversion of atmospheric

carbon dioxide to reduced carbon. Despite the abundance of life on the terrestrial surface where photosynthesis dominates, most biomass is actually located beneath the surface and the majority of this biomass is microbial (Whitman *et al.*, 1998). Thus, avenues for understanding alternate nonphotosynthetic primary production strategies can be found in subterranean or deep-sea ecosystems that function in the absence of sunlight. As will be explained in the following sections, a significant amount of research has been devoted to characterizing primary production in subsurface ecosystems to discover and understand the unique metabolic strategies employed for survival (see also Chapter 6).

7.4.1 Deep-sea Hydrothermal Vents

In 1977, geologists first described deep-sea hydrothermal vents (Figure 7.8). These are areas on the ocean floor where, driven by magma-derived hydrothermal convection, hot water laced with minerals flows up through cracks and fissures. The cracks, which are known as **hydrothermal vents**, often have a buildup of chemical precipitates that resemble chimneys surrounding them (Figure 7.9). Water, reaching temperatures of up to 400°C, is emitted from these vents at rates of 1 to 5 m/sec. In addition, most vent fluids are anoxic, highly reduced, acidic (pH from 2 to 4), and enriched in CO₂, H₂S, CH₄, H₂, Fe²⁺, Zn²⁺, Cu²⁺ and other transition metals. As the hot mineral-rich, hydrothermal water emerges from the vent, it quickly mixes with cold seawater forming a dark cloud of mineral precipitates. The appearance of this dark cloud has given the name “black smoker” to these vent chimneys. It was surprising to find whole self-contained ecosystems consisting of microscopic and macroscopic life in this environment, which has no light and extremely high temperature and pressure.

It is the confluence of the superheated hydrothermal vent water, which contains reduced minerals, that can act as electron donor, and the oxidized seawater, which contains a variety of electron acceptors, that serves as the basis for a chemoautotrophic community of microorganisms that sustains the entire heterotrophic component of the vent community ranging from microorganisms to animals (Fisher *et al.*, 2007). Thus, the entire food web in a hydrothermal vent community is based on chemoautotrophy, not photoautotrophy as in surface environments (see Sections 6.3.1.1 and 16.2.2). Examples of electron donor/acceptor pairs supporting autotrophy that have been identified in hydrothermal vents include: H₂ (donor) with O₂, Fe³⁺, NO³⁻, CO₂, SO₄²⁻, S⁰ (acceptors); H₂S, S⁰, S₂O₃²⁻ (donors) with O₂, NO³⁻ (acceptors); Fe²⁺ (donor) with O₂ (acceptor). In addition, the presence of CH₄ in vent fluids can support heterotrophic growth of methanotrophic

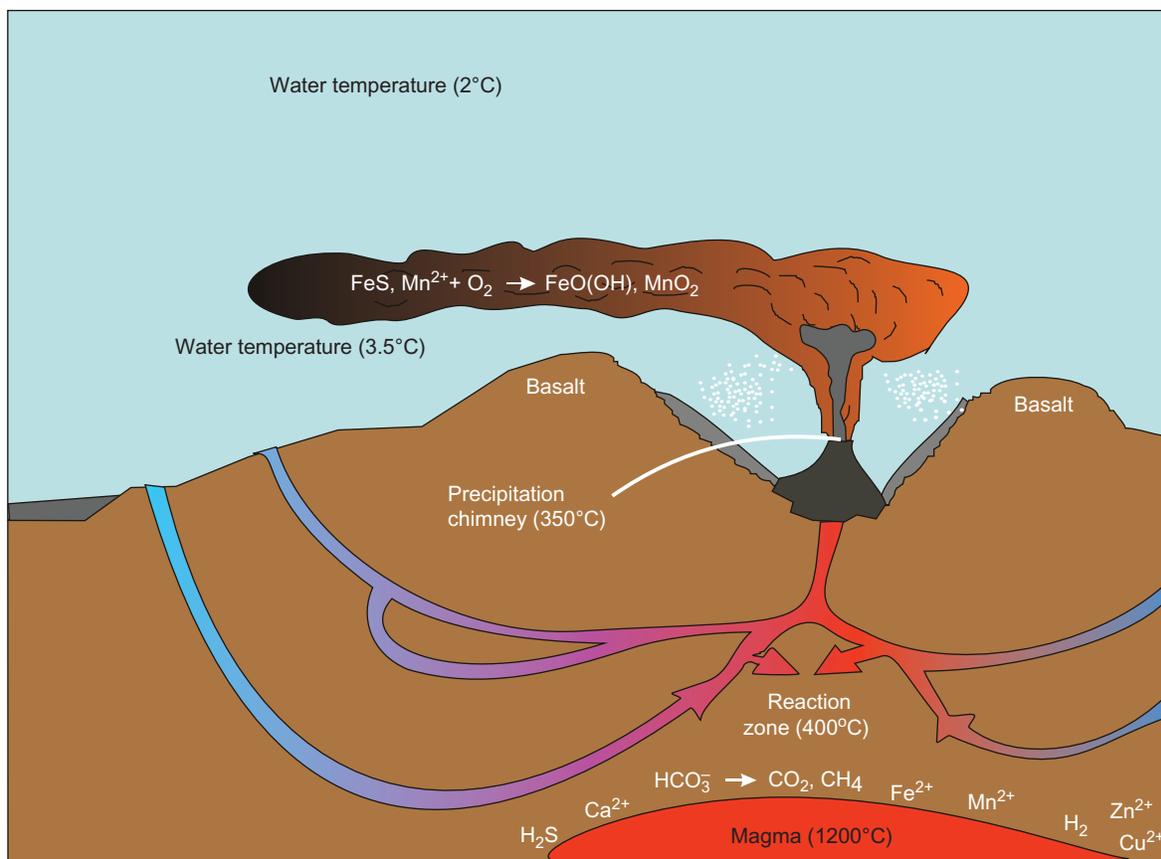


FIGURE 7.8 Schematic representation of a hydrothermal vent depicting a black smoker rising from the ocean floor creating a plume of chemical-rich superheated water around it.

microorganisms in the presence of either O_2 or SO_4^{2-} as terminal electron acceptors.

The vent communities support macrofauna that rely on the chemoautotrophic bacterial populations as a source of organic carbon. There are at least three major mechanisms for transfer of this bacterial carbon and energy to the next trophic level. The first is an endosymbiotic relationship between vent bacteria and an invertebrate, *Riftia pachyptila*, which has been dubbed “tube worm” (Markert *et al.*, 2007). *Riftia pachyptila* are large tube-shaped creatures that grow from the seafloor (Figure 7.10). These worms have no mouth, gut or any other digestive system, and depend completely on bacteria for their nutrition. Instead of consuming the bacteria, the worms have interior surfaces that are colonized by massive quantities (3×10^{11} bacteria per ounce of tissue) of sulfur-oxidizing chemoautotrophs (Karl, 1995) (see Section 16.4.3.1). Chemoautotrophy is sustained by the presence of H_2S originating from vent fluids and oxygen in the seawater. The worm’s body is filled with blood containing large amounts of hemoglobin that binds H_2S . The blood transports the H_2S to the bacteria, which oxidize it and fix CO_2 into organic compounds that nourish the worm. The symbionts benefit from high nutrient

concentrations within the worm’s body, which results in high microbial metabolic activity. In turn, the microbially fixed organic carbon is transferred to the host, making *R. pachyptila* one of the fastest growing marine invertebrates that has been studied (Markert *et al.*, 2007).

The second mechanism by which microbially produced carbon and energy is transferred to the next trophic level is termed microbial gardening. In this case, bacterial cultures are maintained by mussels and other invertebrates on specialized appendages such as tentacles and gills. These invertebrates periodically harvest and consume the bacteria, retaining small inocula to initiate the next crop. The third mechanism for carbon transfer to higher trophic levels is direct consumption of free-living bacterial cells, filaments or mats. Crabs, amphipods, predatory fish and even other microorganisms, including bacteria, have been observed to feed directly on the chemoautotrophic or chemoheterotrophic primary producers (Karl, 1995).

What new insights are hydrothermal vent communities offering to environmental microbiologists? It has recently been hypothesized, through comparison of genomic DNA of vent bacteria and closely related pathogenic bacteria, that there are evolutionary links between the

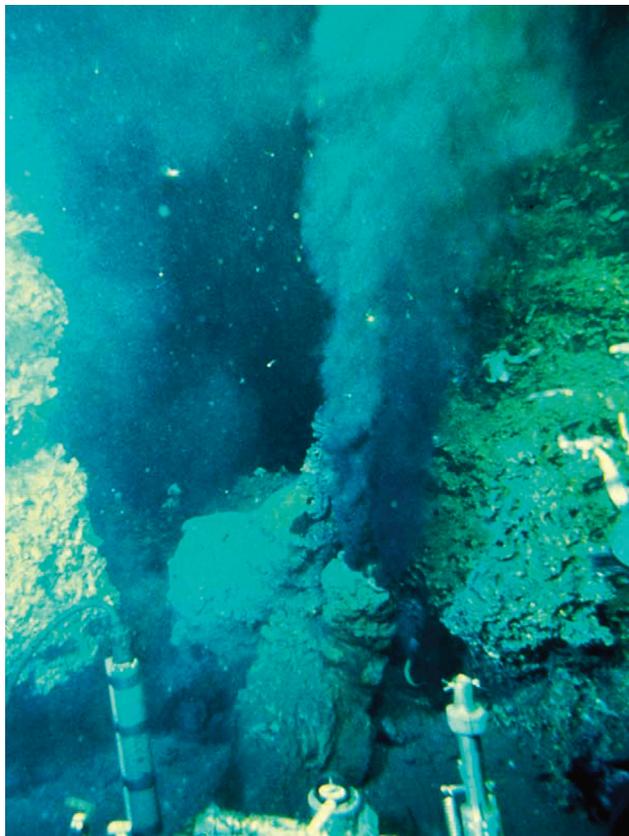


FIGURE 7.9 A photograph of a black smoker vent. This was first published on the cover of *Science* magazine. The vent water is exploding out of the vent at 1–5 meters per second and is 380°C. From Spiess *et al.* (1980). Reprinted with permission from AAAS.



FIGURE 7.10 An adult *Riftia pachyptila* tubeworm community *in situ*. Note the clams surrounding one of the worms in the community. Photo courtesy Andrea D. Nussbaumer, Charles R. Fisher and Monika Bright.

chemolithoautotrophic vent bacteria (which operate as symbionts to vent animals) and important closely related human pathogens (Nakagawa *et al.*, 2007). In this study, the genomes of two deep-sea vent ϵ -Proteobacteria strains, *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp.

SB155-2, were compared to their pathogenic relatives, *Helicobacter* and *Campylobacter*. Although they are not pathogenic, these two deep-sea vent bacteria share many virulence genes with the pathogens. These traits provide an ecological advantage for the hydrothermal vent bacteria which need to form symbiotic relationships with vent animals. However, in their pathogenic relatives, these traits are used for efficient colonization and persistent infection of the host.

7.4.2 An Acid Mine Drainage System

Although there are naturally occurring acidic environments such as Dragon Spring discussed in Section 7.2, acid mine drainage is an excellent example of an anthropogenically caused extreme environment. Mineral mining often focuses on pyrite (FeS_2) deposits that contain metals such as silver (Au), gold (Ag), copper (Cu), zinc (Zn) and lead (Pb), usually as impurities in the pyrite ore. In some cases these metals are part of the sulfide minerals such as chalcopyrite (CuFeS_2), chalcocite (Cu_2S), sphalerite (ZnS) and galena (PbS). In either case, mining exposes these sulfide minerals to both air and water, resulting in biologically mediated acid generation (Section 16.4.3). In sites where excess water is generated, acidic leachates form ($\text{pH} < 2$), which are sometimes also high in toxic metals. These leachates are called **acid mine drainage (AMD)**. Despite extremes of acidity and heat and high concentrations of sulfate and toxic metals, a range of specialized microorganisms populate AMD environments.

Given the difficulty of culturing the majority of environmental microorganisms (and especially those found in extreme environments), there has been great interest in the study of the community based on its DNA (Chapter 13). The term **metagenome** was introduced in 1998 to describe the entire DNA that is represented by the microbial community (see also Chapter 21) (Handelsman *et al.*, 1998). At the time, the focus of **metagenomic analysis** was on the discovery of new natural products and biochemical pathways. However, the metagenome can also be explored to better understand ecological and evolutionary processes that drive community development (Allen and Banfield, 2005). In fact, this approach was used to study a subsurface AMD community from the Richmond Mine Site, Iron Mountain, California, U.S.A. (Tyson *et al.*, 2004). Like the hydrothermal vent community discussed in Section 7.4, the AMD community, which was taken beneath the surface of this site, is self-sustaining and based on chemoautotrophy rather than photoautotrophy. The pH and temperature of the biofilm sample taken from this site were 0.83 and 42°C, respectively. Solution concentrations of metals in the AMD were 317 mM iron, 14 mM zinc, 4 mM copper and 2 mM arsenic.



FIGURE 7.11 Fluorescent *in situ* hybridization (FISH) image of the AMD biofilm taken from a subsurface site at the Richmond Mine at Iron Mountain, California, U.S.A. Three different FISH probes were used. The first was specific for bacteria and used a fluorescein isothiocyanate probe which fluoresces green. The second problem was specific for archaeans and used the Cy5 fluor which appears blue. This probe detected the *Ferroplasma* genus. The third probe was one that targeted the *Leptospirillum* genus and used the Cy3 fluor appearing red. Note that overlap of red and green appears yellow and indicates *Leptospirillum* cells. The dominance of yellow in this image shows the dominance of *Leptospirillum*. From Tyson *et al.* (2004).

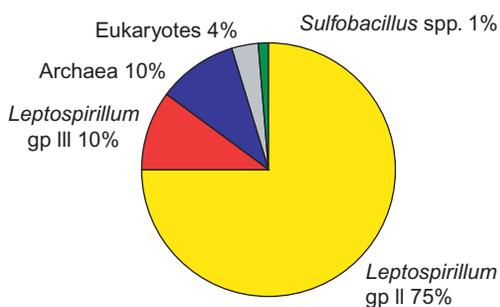


FIGURE 7.12 Relative microbial abundances in AMD determined using quantitative FISH counts. From Tyson *et al.* (2004).

In the metagenomic analysis, a total of 76.2 million base pairs of usable DNA sequence were obtained. These sequences were reconstructed to give nearly complete genomes for two iron-oxidizing bacteria, *Leptospirillum* group II and *Ferroplasma* type II, as well as partial recovery of three other genomes. Fluorescent *in situ* hybridization (Chapter 9) analysis of an AMD sample confirmed the presence of these organisms, and suggested that the *Leptospirillum* group II is dominant (75%) with the *Ferroplasma* type II representing approximately 10% of the community (Figures 7.11 and 7.12).

What did the researchers learn in terms of evolution and ecology at this site? In terms of evolution, analysis of the *Leptospirillum* group II genome indicated very few nucleotide polymorphisms (changes in DNA sequence), implying that only a single strain of this isolate dominates the community (Tyson *et al.*, 2004). In contrast, for *Ferroplasma* type II, the researchers observed between one and three distinct patterns of nucleotide polymorphisms in the assembled genome. These data suggest that *Ferroplasma* type II has undergone evolutionarily recent homologous recombination resulting in three distinct strains of the organism.

In terms of ecology, analysis of the genomes of these two microbes gave these scientists intriguing insights into how each bacterium processes carbon, fixes nitrogen and generates energy. For example, several CO₂ fixation pathways were identified for *Leptospirillum* group II, indicating that it is definitely chemoautotrophic. In contrast, while the *Ferroplasma* type II has a mechanism to fix CO₂, it also contains a large number of sugar and amino acid transporters, suggesting that it may prefer to metabolize heterotrophically. Both genomes were also examined for nitrogen fixation genes which were found to be absent. However, they were found in one of the partially sequenced genomes for *Leptospirillum* group III indicating an important role for this organism in the AMD biofilm system, although it represents only about 10% of the community as analyzed by fluorescence *in situ* hybridization (Figure 7.12).

The researchers note that the metagenomic analysis of the AMD site was successful in part because the diversity in the biofilm was low, and the frequency of genomic rearrangements and gene insertions/deletions was relatively low (allowing reconstruction of each genome).

7.4.3 A Desert Carbonate Cave

Carbonate caves represent subterranean ecosystems that are largely devoid of phototrophic primary production. Caves are typical features of karst terrains that represent approximately 20% of Earth's dry ice-free surface (Ford and Williams, 2007). Though they are aphotic, caves can receive fixed organic carbon from the surface of Earth from drip water that feeds the growth of the beautiful carbonate formations known as speleothems (Figure 7.13) and from streams that flow through the cave either constantly or ephemerally. However, for desert caves, the allochthonous organic carbon inputs that normally enter with drip water or streams are minimal, resulting in unique highly oligotrophic conditions.

The NSF Kartchner Caverns Microbial Observatory was created to examine the microbial diversity and energy strategies present in a desert cave system (Kartchner, 2008). Kartchner Caverns are located in the Sonoran

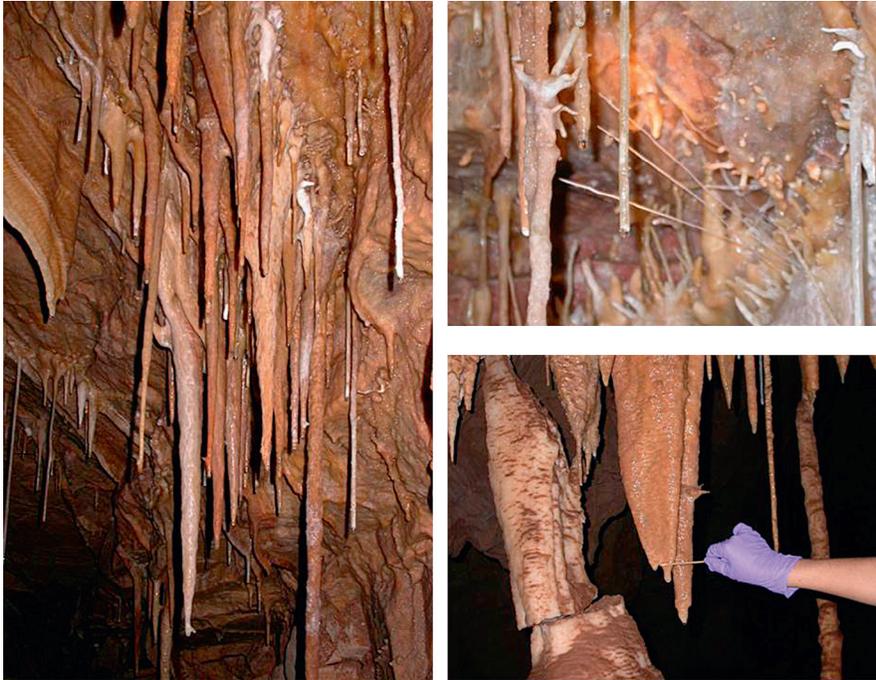


FIGURE 7.13 Speleothems in Kartchner Caverns. (Left) a variety of stalactites with a bacon formation on the left and a soda straw on the right. (Top right) a close-up of small formations including helectites, formations that grow while defying gravity. (Bottom right) sampling a speleothem for metagenome analysis. Photos courtesy Robert Casavant, Arizona State Parks.

Desert and developed within a Lower Carboniferous-age Escabrosa limestone formation in the Whetstone Mountains of southeastern Arizona, U.S.A. The only natural entrance is a very small blow-hole, largely limiting entrance to all but a bat colony, insects, small rodents, amphibians and reptiles. The cave has been developed for tourism but human access is tightly controlled and environmental conditions within the cave have been continuously monitored since 1989 (Tufts and Tenen, 1999).

Unexpectedly high bacterial diversity on Kartchner speleothem surfaces was revealed by a **pyrotag analysis** or **next generation sequencing** (Section 13.6.2) with approximately 2000 operational taxonomic units (OTUs) per speleothem. These OTUs were classified into 21 phyla and 12 candidate phyla, and appeared to be dominated by organisms associated with heterotrophic growth (Ortiz *et al.*, 2013a). Evidence for chemolithoautotrophy came from examination of a clone library with full-length 16S rRNA sequences where phylogenetic associations to *Nitrospira* and *Leptospirillum* were revealed, bacteria that obtain energy from nitrite and iron oxidation, respectively.

Metagenome analysis was performed to elucidate the community structure and search for clues to the potential energy dynamics sustaining the diverse speleothem microbial communities (Ortiz *et al.*, 2013b). One key insight discovered in this analysis was the potential contribution of archaeans to the energy dynamics in Kartchner. A recent 16S rRNA pyrotag analysis of soils from around the world found the average abundance of archaeans in soils to be 2%. Archaeal abundance was inversely correlated with C:N ratio, suggesting that archaeans can tolerate or even exploit

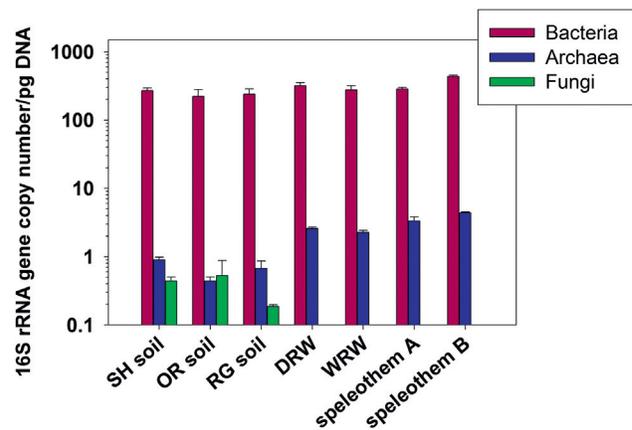


FIGURE 7.14 Quantitative PCR analysis of archaeans, bacteria and fungi in three soil samples taken from above Kartchner Caverns (SH soil, OR soil and RG soil), a dry (DRW) and wet (WRW) rock wall sample taken inside Kartchner, and two speleothem surfaces (A and B) in Kartchner. From Ortiz *et al.* (2013b)

low nutrient conditions such as those found in Kartchner (Bates *et al.*, 2011). In fact, the taxonomic analysis of the Kartchner speleothem metagenome found that unassembled archaeal sequences comprised 10% of the metagenome. This finding was confirmed using a quantitative PCR analysis which revealed that archaeal abundance in Kartchner speleothem communities is significantly higher than in soil communities from above the cave (Figure 7.14).

Thaumarchaeota dominated the speleothem Archaea and represented the third most abundant phylum in the cave. A significant percentage of the Thaumarchaeota

metagenome reads (18%) were associated with chemoautotrophic ammonia-oxidizing archaeans. This led the researchers to examine whether there were Archaea-associated CO₂-fixation genes in the metagenome. In fact, two key genes, including ATP-citrate lyase (required for the rTCA CO₂ fixation pathway), and 4-hydroxybutyryl-CoA dehydratase (required for the HP/HB and the DC/HB CO₂ fixation pathways), showed taxonomic associations to the Thaumarchaeota. Finally, examining a key gene in the ammonia oxidation pathway (*amoA*), the researchers found that half of the speleothem *amoA* genes were classified as archaeal. Taken together, these pieces of evidence suggest that archaeans make a significant contribution to the energy dynamics of this ecosystem by contributing to primary production through ammonia oxidation.

A recent metagenomic analysis of microbial slime in the subterranean aquatic Weebubbie cave below Australia's Nullarbor Plain also found a pattern of aphotic primary production driven by inorganic nitrogen metabolism (Tetu *et al.*, 2013). Like Kartchner, Weebubbie microbial communities included an abundance of ammonia-oxidizing Thaumarchaeota, although the Weebubbie community had a much higher relative abundance of Thaumarchaeota, and the ratio of ammonia oxidizing archaeans to ammonia oxidizing bacteria was more similar to that found in marine environments. Thus, it appears that the Kartchner Caverns ecosystem may represent an oligotrophic terrestrial counterpart to the energy dynamics observed in oligotrophic marine habitats such as Weebubbie cave.

A second aspect of this analysis helps to demonstrate how metagenomes can be used to reveal the functional potential of a community. The Kartchner metagenomes were analyzed in two ways. First, a full analysis of one speleothem was performed by mining multiple databases and employing various analysis strategies to compensate for the limited representation of cave microbes and oligotrophic metagenomes in current databases. Second, four cave speleothem metagenomes were compared to reported metagenomes from other environments including ocean, bulk soil and rhizosphere soil.

This two-pronged analysis was performed to examine potential carbon-fixation strategies in Kartchner. In-depth analysis of one speleothem metagenome detected genes for all six known CO₂-fixation pathways, and suggested a community genetically adapted to low-nutrient conditions. The RuBisCo genes representative of the Calvin–Benson–Bassham cycle were overrepresented in Kartchner speleothem metagenomes relative to bulk soil, rhizosphere soil and deep-ocean communities. The abundances of genes in two other pathways, ATP-citrate lyase (rTCA) and 4-hydroxybutyryl-CoA dehydratase (HP/HB and DC/HB), were comparable to the nutrient-limited ocean environments but greater than both soil and rhizosphere ecosystems. The abundance of HP/HB genes was of particular interest because enzymes in this cycle use

bicarbonate as the active inorganic carbon species, whereas bicarbonate is not a RuBisCo substrate. The HP/HB pathway is hypothesized to be advantageous for chemolithoautotrophic marine archaea because bicarbonate availability under slightly alkaliphilic conditions (e.g., ocean water) is significantly higher than dissolved CO₂ (Berg, 2011), conditions that may also apply to this carbonate cave ecosystem, where drip water pH averages 8.0.

The comparative metagenome analysis also suggested an intriguing survival strategy used by Kartchner speleothem communities. One gene category that was significantly overrepresented in the cave metagenomes was DNA repair enzyme genes belonging to the RAMP (Repair Associated Mysterious Proteins) superfamily. The RAMP family is a group of proteins for which no specific function is known, but that clearly associate with DNA repair mechanisms. The overabundance of DNA repair genes was surprising given the absence of typical DNA-damaging agents such as UV light. It was hypothesized that the exceedingly high calcium concentrations in cave drip water (Legatzki *et al.*, 2012) might be the source of stress. Previous work has suggested that cave bacteria precipitate calcium carbonate as a mechanism to overcome calcium toxicity (Banks *et al.*, 2010). Although little is known for bacteria, in eukaryotic cells calcium toxicity has been linked to DNA strand breakage (Cantoni *et al.*, 1989). Specifically, when cells are under oxidative stress, calcium homeostasis is disrupted, leading to an increase in intracellular calcium concentrations which in turn activates nucleases that damage DNA. Thus, the abundance of DNA repair enzymes may be an adaptation of cave microbes to the presence of extremely high calcium concentrations in the cave ecosystem where the primary mineral found in a speleothem is calcium carbonate.

What new insights does the study of an ecosystem like Kartchner Caverns offer? This oligotrophic subterranean environment supports a unique chemolithoautotrophic microbial community with unusual nutrient cycling strategies. This Microbial Observatory provides insight into the life survival strategies of other ecosystems dominated by oligotrophy including: subsurface soils or aquifers, arid deserts and even extraterrestrial ecosystems. For example, Kartchner provides a template for evaluating the role of archaeans in oligotrophic terrestrial ecosystems; Kartchner can be compared to Movile Cave in Romania (Chen *et al.*, 2009). Like Kartchner, Movile Cave is aphotic and sustained by chemolithoautotrophy; however, the atmosphere in Movile is rich in hydrogen sulfide and methane, and supports high microbial productivity and rich fauna. Microbial mats sustained by sulfur and ammonia-oxidizers contain a diversity of autotrophic bacterial phylotypes, but no archaeans. The absence of archaeans is in stark contrast to their abundance in the less energy-rich caves discussed, Kartchner and Weebubbie.

QUESTIONS AND PROBLEMS

1. Define the term ecosystem services.
2. Give two examples of extreme environments not discussed in this chapter.
3. Which of the extreme environments discussed in this chapter likely has the slowest growth rates?
4. Of the six extreme environments discussed in this chapter, which are based on chemoautotrophy?
5. If you were a microorganism, what type of extreme environment would you choose to live in?
6. Discuss adaptations for enzymes at low and high temperature.
7. Compare and contrast a thermocline (Chapter 6) and a chemocline.

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