INTRODUCTION TO CAVE MICROBIOLOGY:
A REVIEW FOR THE NON-SPECIALIST

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Within the past decade there has been an increasing interest in cave microbiota. Such interest has helped many speleologists both recognize and understand the importance of microbial species in caves, which has led to improvements in cave conservation practices to better conserve these unseen ecosystems. While much information on the metabolic properties and functions of such subterranean ecosystems has been published in the microbial ecology literature, is it sometimes unusable by the non-specialist due to technical "jargon" and unexplained background information. It is the aim of this review to provide such background information and to explain the current technologies available to study cave microbiota. In doing so, it is hoped that this material will make the microbiology literature more accessible to interested non-specialists, and open new areas of inquiry in the study of microbial-mineral interactions.

INTRODUCTION

The study of cave microbiology deals with the microscopic life that resides in caves. Microscopic life has historically been grouped into the Monera, one of the five kingdoms of life, and a pretty broad group that includes any microscopic organism that lacks a nucleus; also historically known as the prokaryotes (Whittaker, 1969). The protozoa, which are also microscopic and include such organisms as the amoeba, do contain a nucleus and are classed within the eukaryotes (Whittaker, 1969). While viruses are also of microscopic, they are not technically alive and, although fascinating, will not be discussed within the scope of this review. Animals, plants and fungi all constitute the forms of life that can be seen with the naked eye (macroscopic) and are nucleus-containing eukaryotes.

A BRIEF HISTORY OF MICROBIOLOGY

During the Dark Ages (c.400-700 AD) devastating diseases, such as the Black Death, ravaged humanity. At that time, there was only a vague inkling in medicine that these maladies were transmitted by an infectious agent. Rather, physicians believed in mystical spirits and lethal miasmas, which were responsible for such pestilence. Patients would attempt to ward off these evil spirits with charms and even flowers; the "pocket-full of posies" of the nursery rhyme. These attempts were somewhat beneficial, although not for immediately obvious reasons – breathing through containers of dried flowers effectively filtered out the bacteria that were responsible for the spread of pneumonic plague, perpetuating the myth of evil vapors.

The first awareness of a microscopic world occurred around 300 years ago, when Dutch linen merchant Antoni van Leeuwenhoek devised a rudimentary, hand-made microscope to look at linen fibers. Leeuwenhoek also used his microscope to look at other materials; in pond water he saw tardigrades (for slow-walker), small insect-like organisms he termed animalcules. Eventually, by refining his microscope lens, Leeuwenhoek went on to describe the first bacteria, which he saw in his own dental plaque. These bacteria were so small that it would take two thousand of them stacked end-to-end to traverse the head of a pin, which Leeuwenhoek accurately described in great detail.

Leeuwenhoek's animalcules were considered nothing more than a curiosity for almost 200 years, until the golden age of microbiology and the work of such luminaries as Pasteur and Koch. Louis Pasteur wanted to understand the then-accepted notion of spontaneous generation – that a vital force allowed life to arise spontaneously in food or wine. In attempting to define the existence of this vital force, in 1861 Pasteur successfully demonstrated that this "vital force" was comprised of microorganisms in the air. Pasteur subsequently went on to design ways of killing these organisms in food to prevent spoilage and developed the technique of pasteurization. Robert Koch took the principles of Pasteur and other investigators further, to show that the same microorganisms that could spoil food could also cause disease in humans. In 1876 Koch developed the techniques to identify the causative agent of anthrax. These techniques became an accepted scientific principle, termed Koch's postulates, that can be applied to any pathogenic microbe today, including as recently as in the case of severe acute respiratory syndrome (SARS) virus. Following the work of Pasteur, Koch and other eminent microbiologists, Leeuwenhoek's animalcules went from being simply a curiosity to the basis of the accepted science of microbiology.

With its founding in the medical sciences, the study of microorganisms has historically concentrated on the impact of microorganisms on humans, whether through disease or food spoilage. Nonetheless, in 1887 Sergei Winogradsky made a groundbreaking discovery; he identified microorganisms that did not obtain food through photosynthesis, but could use mineral energy to literally pull food out of thin air, a process termed chemolithotrophy (chemical energy – chemo, food from the environment – litho, to eat – trophy) (Winogradsky, 1887). Despite the identification of microorganisms that defied

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all the previously understood conventions of life (that it relies on plant-derived material), Winogradsky's discoveries were again considered a curiosity, and most microbiologists continued to concentrate on just a handful of microbes responsible for human disease. Of course, once again it was proved that an important microbial activity was overlooked: microbiologists have since determined that Winogradsky's chemical-eating organisms are among the most significant life forms on the planet (Madigan *et al.*, 2000).

Due to an ability to consume inorganic material for energy, it is not surprising that microorganisms interact directly with the geology of our planet. Their activities have helped shape the global environment in which we live, provided the oxygen that we breathe and even the oil that drives our economy (Gold, 1999; Madigan *et al.*, 2000). These microbes also turn over the essential nutrients, such as carbon (C), nitrogen (N), sulfur (S) and phosphorus (P), of our ecosystem. Indeed, without the activities of these microorganisms, all macroscopic life on the planet would cease, while these microbes would survive quite happily without us (Madigan *et al.*, 2000). Obviously, as the science of microbiology continues to evolve, our level of understanding continues to change. This has led to the rapid inception of extremophile research, which began in 1967 when Thomas Brock identified bacteria growing in the boiling hot springs of Yellowstone National Park (Brock, 1967). These hot conditions demonstrated the extreme conditions under which microorganisms can survive and the chemistries that can support their growth (Madigan *et al.*, 2000).

Extremophile research extended the physical and chemical limits of the environment in which microorganisms can survive, from the sterilizing temperatures (121° C) of volcanic vents miles below the surface of the Pacific Ocean, the battery-acid-like conditions of acid mine drainages, to surviving for millions of years within crystals of salt (Bond *et al.*, 2000; Jannasch *et al.*, 1992; Mormile *et al.*, 2003; Shivaji *et al.*, 2004; Thomas and Dieckmann, 2002). Today, almost every environment imaginable has been examined for the presence of microorganisms, including, of course, caves (Angert *et al.*, 1998; Barton and Luiszer, 2005; Barton *et al.*, 2004; Canaveras *et al.*, 2006; Canaveras *et al.*, 2001; Caumartin, 1963; Chelius and Moore, 2004; Cunningham *et al.*, 1995; Engel *et al.*, 2004; Groth and Saiz-Jimenez, 1999; Groth *et al.*, 1999; Holmes *et al.*, 2001; Hose *et al.*, 2000; Northup and Lavoie, 2001; Sarbu *et al.*, 1996)

STUDYING MICROORGANISMS IN EXTREME ENVIRONMENTS

In attempting to understand the difficulties in carrying out microbiology in cave environments, we must go back to the traditional methods developed by Koch and others in the 1880s (Koch, 1881). Traditional microbiology techniques rely on growing a microorganism in a Petri plate, and then identifying this organism based on the sum of its reactions to obtain food, respire and break down products (the sum of which is referred to as metabolism). This is why swabs are often taken when a

bacterial pathogen is thought to be responsible for an infection, which are used to grow the suspected organism and carry out metabolic tests for its identification. Unfortunately, there is a small problem when attempting to grow environmental microbes: the majority of microorganisms cannot be cultivated (or as microbiologists say 'cultured') in a Petri plate. This is a phenomenon that microbiologists have known about since the time of Winogradsky and is based on a simple observation: if you took a gram of soil, you would be able to see about 5,000 different bacterial species under the microscope, but you would be lucky if you could induce 50 different species, or 1% of those observed, to grow (Amann *et al.*, 1995; Amann *et al.*, 1996). This is even worse compared on a global scale: of the millions of species of bacteria thought to exist on Earth, we have only been able to cultivate approximately 16,000 (American Type Culture Collection, 2006).

The reason that so many microbes from the environment are non-culturable is still not completely understood (Bloomfield *et al.*, 1998; Bogosian *et al.*, 2000; Oliver, 1995; Whitesides and Oliver, 1997). One reason thought to be important comes from the founding of microbiology in medicine; historically all our cultivation strategies to grow microorganisms are based on the growth characteristics of a small number of microbes able to infect humans. As a result, the majority of microbial growth media (the contents of a Petri plate) are designed, to some extent, to be chemically similar to human tissues. Indeed, such media is often made from boiling down meat products with agar to make a nutrient gel. While these growth media work extremely well for human pathogens, for environmental microorganisms that grow using unusual chemical reactions, the 'food' in these Petri dishes is inappropriate. To grow these environmental microorganisms requires a certain amount of guesswork based on the environment from which they were isolated. It is like trying to decide which cave food to pack for someone else; everyone has their own preference and won't eat what they don't like (Amann *et al.*, 1995).

Such non-culturability is likely more pronounced in cave microorganisms. Without photosynthesis, caves are cut off from most energy that supports life on the surface, confining most nutrients to the entrance zone. As a result, cave microorganisms must turn to alternative sources of energy, such as those found in the atmosphere, or present in the very rock itself (Barton *et al.*, 2004; Chelius and Moore, 2004; Engel *et al.*, 2003; Northup *et al.*, 2003; Spilde *et al.*, 2005). In adapting to these extremely starved environments, microorganisms produce elaborate scavenging mechanisms to pull scarce nutrients into the cell (Koch, 1997). When these organisms are then exposed to the rich nutrients of a Petri plate, they cannot turn down these scavenging mechanisms and quickly gorge themselves to death (Koch, 1997, 2001). As a result, microorganisms from starved cave environments may have a hard time adapting to rapidly changing nutrient status, and simply die from osmotic stresses (Koch, 1997). Therefore, only a tiny minority of microorganisms from caves might grow on standard Petri plates by surviving the changing nutrient conditions,
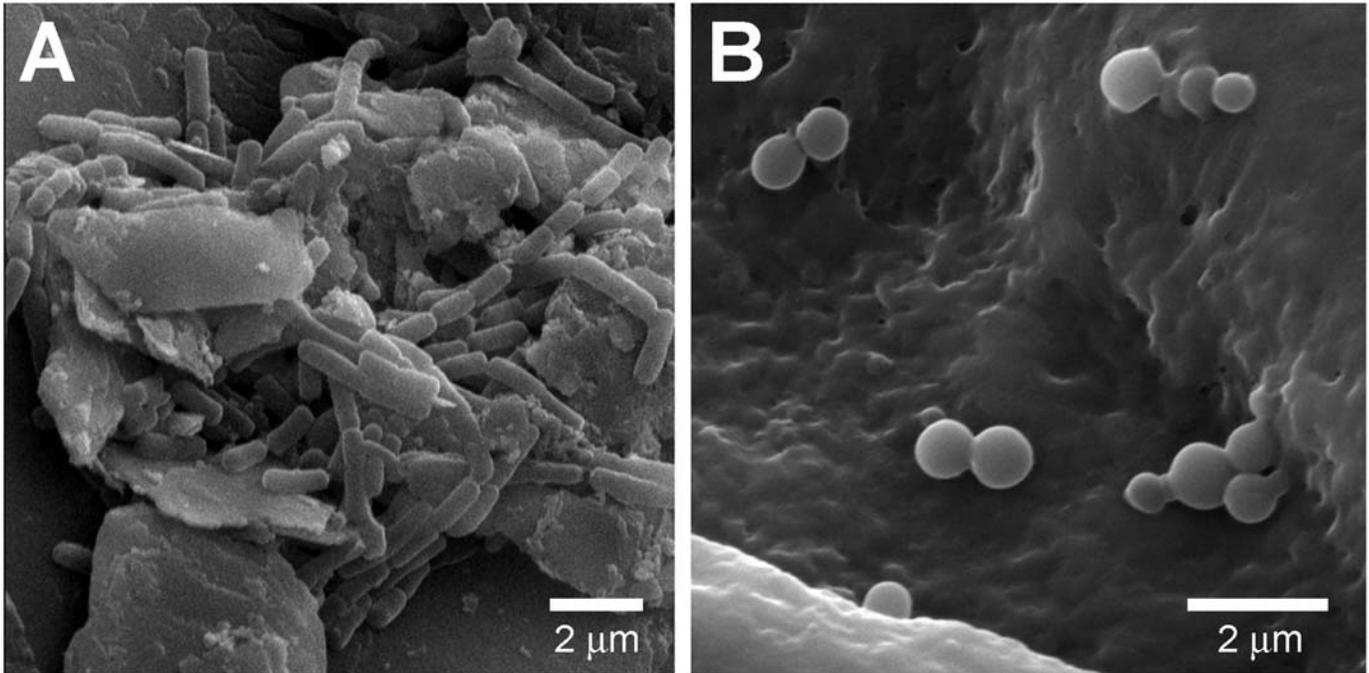
Figure 1. Scanning electron microscopy images of common themes in bacterial structure. The two most common structures are the A) bacilli (in chains, as streptococci) and B) cocci (in pairs, as diplococci).

including such species as *Pseudomonas* and *Bacillus* species (Pemberton *et al.*, 2005). Unfortunately these species are unable to tell us much about the unique microbial processes taking place in cave environments. In order to overcome these problems, cave microbiologists have therefore spent a great deal of time trying to developed improved techniques to grow cave microorganisms (Gonzalez *et al.*, 2006; Groth *et al.*, 1999; Laiz *et al.*, 1999). Many of these techniques are quite specialized, and it is unlikely that many microbiologists (such as those within diagnostic or hospital microbiology labs) have access to the materials necessary to cultivate rare cave species.

TO GROW OR NOT TO GROW

The non-culturability of microbial species, rather than simply being a curiosity, poses a significant road-block if you want to study microbes in natural cave environments. Fortunately, classical microbiology lost some of its dependence on cultivation in 1977, when Woese was attempting to understand the genetic differences between microbial species (Woese and Fox, 1977). Historically, bacteria had been identified by their growth characteristics and classified based on their shape and structure. This process of grouping life based on structure is called taxonomy, and is extremely useful in determining evolutionary relationships between plants, animals and insects, etc. (Whittaker, 1969). In bacteria, using structure to group species turns out to be pretty futile. Bacterial structures generally represent variations on a theme: either spheres (technically called cocci) or sausages (bacilli) (Figure 1). As a result, structural taxonomy tended to cluster the bac-

teria into simplified, but artificial groups. We therefore tended to believe that eukaryotic organisms were the most complex form of life on Earth, with their broad structural diversity; taxonomy identifies over 1,000,000 species of insects alone (May, 1988). Faced with this limitation, Woese decided on a different approach to resolve bacterial taxonomy. Rather than examining structure, he looked inside the cells at the DNA.

All of us inherit our genetic information from our parents, which creates a genetic blueprint of our family tree (blue-eyed children to blue-eyed parents, etc). By looking at the surnames in a traditional family tree, it is also quite easy to see who is related to whom, based on how the surnames change; the same surname indicates direct descendency, while a change in surname may indicate a marriage into the family. Similarly, Woese extracted the genetic surnames of a number of microorganisms to determine a family tree for microbial life (Woese and Fox, 1977). By examining this data, he discovered that bacteria are much more diverse than we previously suspected. This diversity, rather than being expressed through structure, was elaborated through phenomenal capabilities of physiology and metabolism (Woese, 1987). Woese also identified an entirely new kind of life using this technique, which was previously unknown to science. This new form of life looked just like the bacteria, but the internal genetic controls looked more like those found in eukaryotic organisms (such as plants and animals). These organisms were also primarily found in the harshest conditions; conditions similar to those that may have been found on early Earth. Woese therefore named this newly discovered form of life after its potentially ancient origins, calling them the Archaea (Woese and Fox, 1977). What
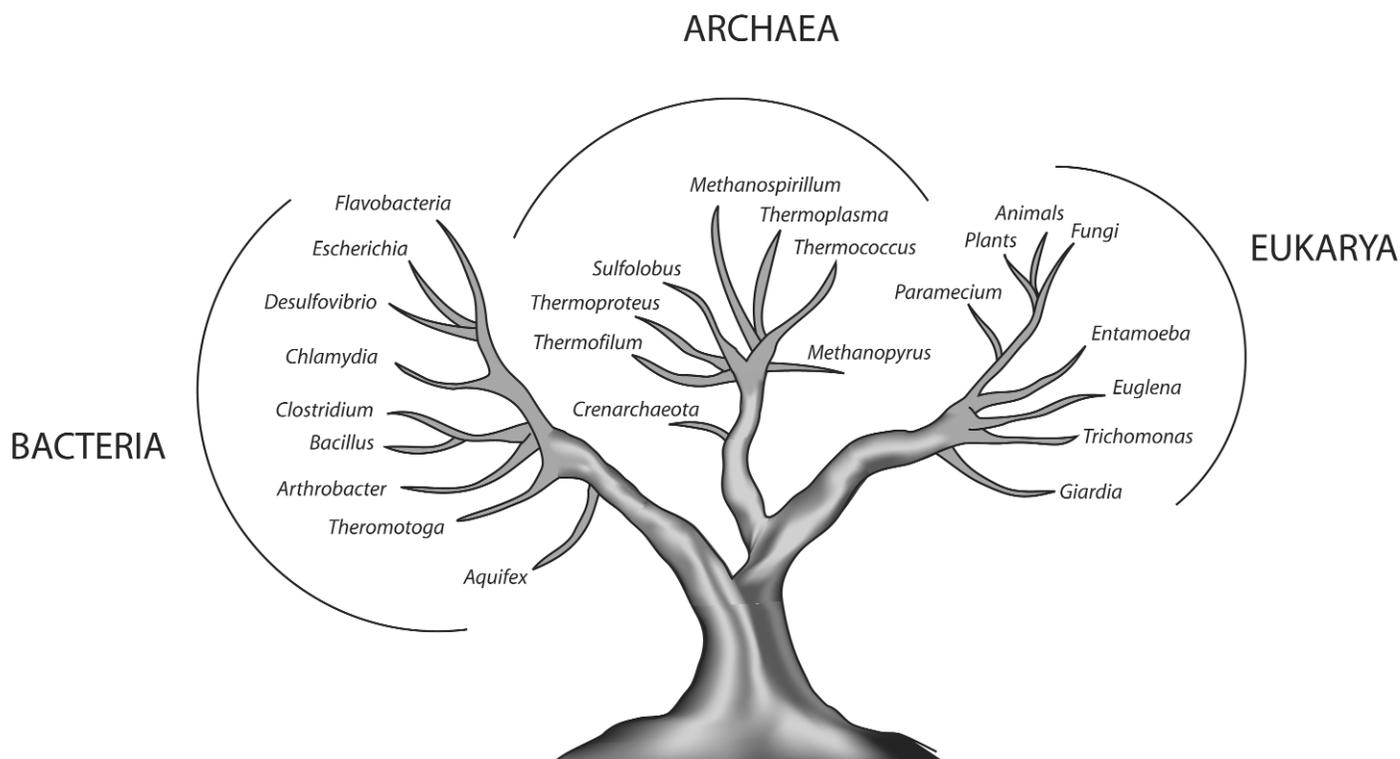
Figure 2. A universal tree-of-life based on the genetic comparisons of Woese and Fox (1977), with further refinement by Pace (1997). The base of the tree emerges from the 'root' or last common ancestor of life and splits into the three major domains of life; the Bacteria, Archaea and Eukarya. The branches represent the (simplified) major divisions (Kingdoms) of life within each domain.

emerged from this work was that, above and beyond the five-kingdom tree-of-life, there exist domains; the primary groups of life on Earth. These domains represent 1) the Bacteria (prokaryotes; lacking a nucleus), 2) the Archaea (also prokaryotic, but with structures that differentiate them from bacteria and a DNA structure more similar to a eukaryote), and 3) the Eukarya (eukaryotes; containing a nucleus) (Figure 2).

BEYOND THE GENETIC TREE

The work of Woese was groundbreaking and shifted our understanding of what is "life" on Earth. With further refinement by other investigators, it also showed a five-kingdom tree was an oversimplification, with a current estimate of almost 100 distinct kingdoms (divisions) of life on Earth (Hugenholtz *et al.*, 1998; Pace, 1997). The new tree also revealed that the most ancient, most complex and most abundant life on Earth was actually microscopic (Pace, 1997). This work excited a number of scientists, including Pace (a microbiologist and Indiana caver) who decided to look for Woese's genetic surnames in the environment without cultivation (Lane *et al.*, 1985; Stahl *et al.*, 1985). The technique uses the polymerase chain reaction, or PCR for short, and allows investigators to make millions of copies from a single piece of DNA. A similar technique is commonly used today to look for pieces of

genetic information in crime scene investigations, or to detect the presence of the HIV virus in a patient's blood. When Pace used the PCR reaction to look for bacterial genetic surnames within the environment, it altered our understanding of microbial ecology (Pace, 1997). Rather than generating a tree of lineage from a handful of organisms that could be grown in the laboratory, this new technique allowed entire microbial ecosystems to be examined at once without the limitations of cultivation. Soon, other investigators began using the same techniques to study environments, and the number of genetic surnames that were known to science rapidly went from 177 to the >120,000 that are available today (Maidak *et al.*, 2001). Such techniques have also improved our ability to estimate the true diversity of microorganisms (putting entomologists to shame) with an estimated worldwide population of 200,000,000 different species of bacteria alone (Curtis *et al.*, 2002; Dojka *et al.*, 2000; Torsvik *et al.*, 2002).

IDENTIFICATION WITHOUT CULTIVATION

Using genetic surnames to identify microorganisms (technically called molecular phylogenetics: molecular – using DNA molecules; phylogenetics – a reconstruction of the evolutionary history), it gave us tools to identify microorganisms within an environment without cultivation. Molecular phylo-
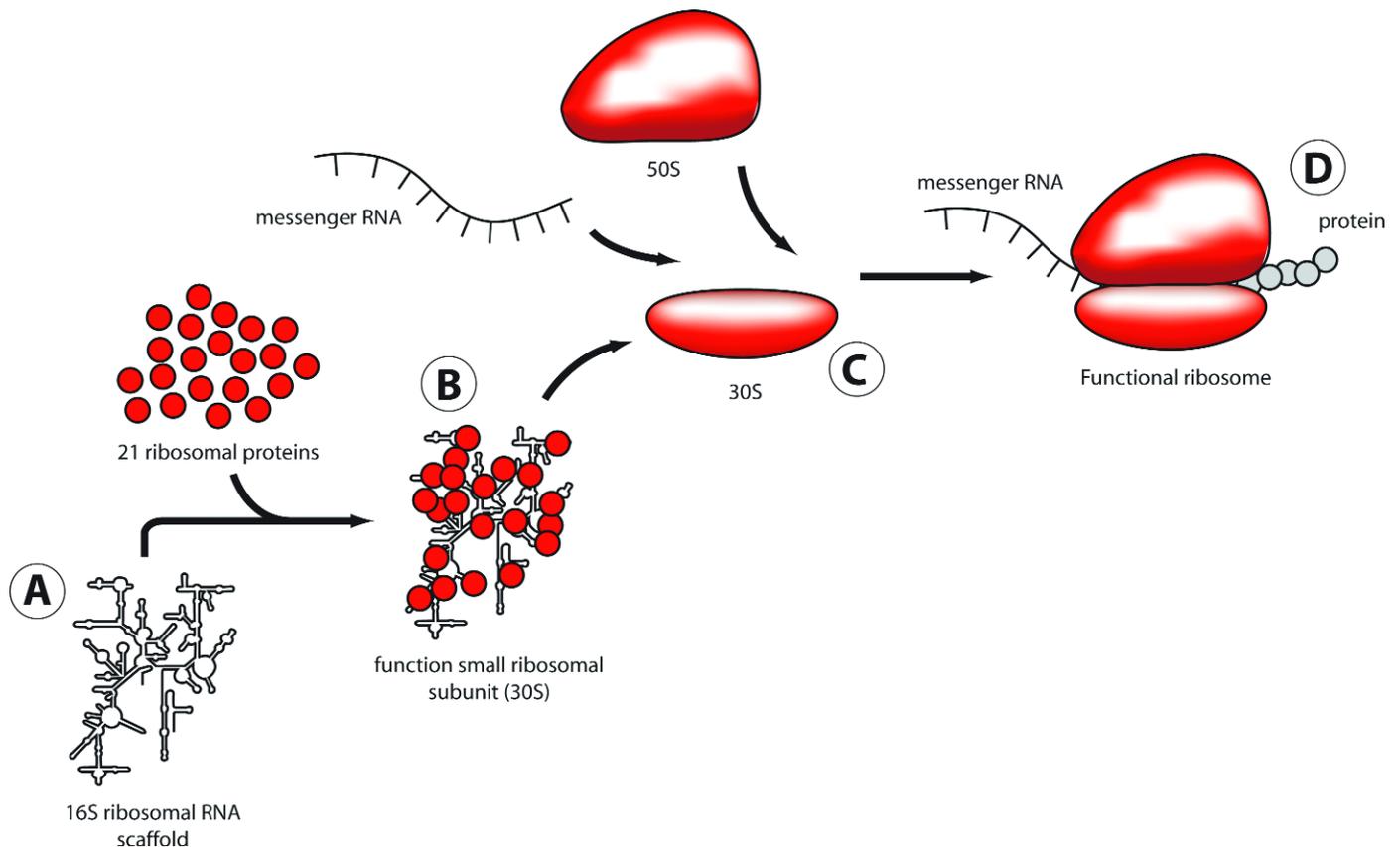
Figure 3. The function of 16S ribosomal RNA (rRNA) within assembly of the ribosome. The 16S rRNA is transcribed from genomic DNA, folding into a three-dimensional structure (A). This 'scaffold' allows the 21 ribosomal proteins to bind (B) and assemble into the 30S small subunit of the ribosome (C). In this functional structure, the 30S subunit assembles with the larger 50S subunit around protein encoding messenger RNA, allowing protein synthesis (D).

genetics have therefore allowed us to effectively examine microorganisms in extreme environments; conditions that would be almost impossible to replicate in the laboratory for growth using the approach of Koch. These genetic "surnames" are genes with a fairly long name: the 16S ribosomal RNA gene sequence, or 16S rRNA for short. This gene is critically important in the cell as it encodes a structural component of the ribosome, the cellular factory used to build proteins (Figure 3). The ribosome is a complicated structure, made from two functional domains, a large 50S and small 30S subunit. Each subunit is built from protein building blocks and due to its complexity, a piece of RNA acts as a scaffold upon which the ribosome can assemble, much like a scaffold can help the building of a house. The 30S subunit is assembled around the 16S rRNA. If the shape of the 16S rRNA was to change dramatically the ribosome would not be built correctly and the cell could no longer make proteins, leading to an almost instantaneous death. This need for an unaltered structure constrains the shape of the ribosomal RNA such that only tiny modifications can be made over time, without damage to protein synthesis – in biology this is known as an evolutionary constraint. Therefore, over the history of the microbes there have only been subtle changes in the structure of the rRNA molecules,

much like "Chinese Whispers" (where tiny mistakes in a sentence subtly change its context as it is whispered from person to person). These changes in the 16S rRNA allowed Woese to identify the primary lineages of life and trace descendency to predict the most ancient forms of life on Earth (Woese, 1987; Woese and Fox, 1977).

While molecular phylogenetics may appear to be an interesting exercise in evolutionary biology, these techniques allow us to determine how microbes are related by how similar their 16S rRNA sequences are. To explain how this works, we need to return to the analogy of the genetic surname and the family tree. In determining ancestry, if a surname is the same then there is a high likelihood that two people are closely related; however, what about if the surnames are similar, but not the same? In the case of microbes, such as *Escherichia coli*, we know based on structure and biochemistry that it is closely related to *Salmonella entrica*. Due to their being close relatives, we can assume that *Salmonella* has many of the same metabolic activities and lives in the same environment as *E. coli*. This is indeed correct, in that they both reside in mammalian gastrointestinal tracts. In the same way, we can compare the 16S rRNA sequences from *E. coli* and *Salmonella* and see that they are 99% identical. Therefore, if we had no other
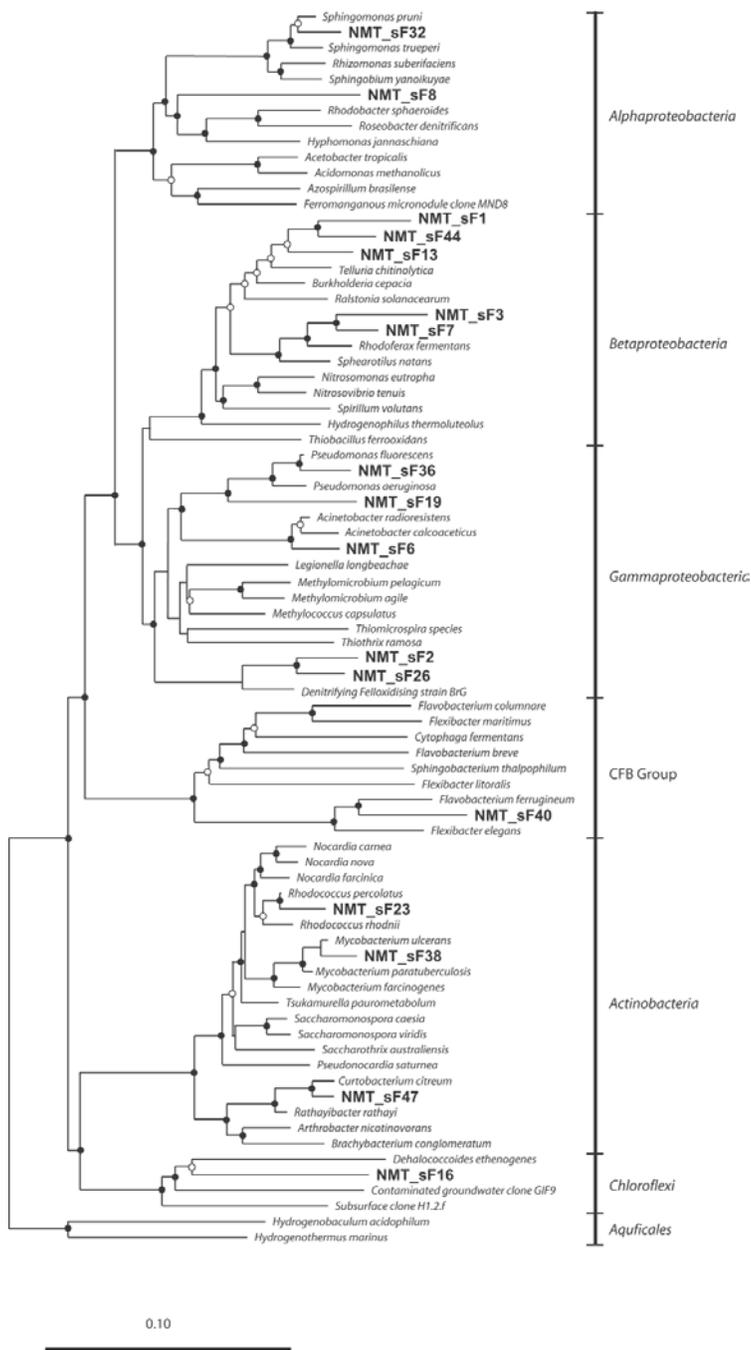
Figure 4. Dendogram of the 16S rRNA gene sequences identified within Carlsbad Cavern (NMT-sF). The bar indicates 10% divergence between the 16S rRNA sequences. Where the dendogram branches, those with branches supported by >70% of all alignments (bootstrap values >70% in neighbor-joining and a heuristic search) are indicated by closed circles. Marginal branch support (bootstrap values >50% but >70% in both analyses) are shown by an open circle.

information other than the 16S rRNA sequence, we can hazard a guess that *Salmonella* had a similar metabolic activity to *E. coli*. This is essentially the basis of the molecular phylogenetic approach, allowing analysis of microbial communities without cultivation. While the technique seems pretty straightforward, in practice it's actually quite a challenge due to the complexity of aligning thousands of DNA sequences to find the closest match (Maidak *et al.*, 2001; Maidak *et al.*, 2000; Nei *et al.*, 1998). Nonetheless, how closely species are related can be shown graphically as a dendrogram, which is a mathematical model of a family tree based on a 16S rRNA gene sequences (Figure 4). In scientific journals, molecular phylogenetic analyses of environments are often explained with dendrograms – with a scale at the bottom to indicate the relative distance between organisms. These dendrograms allow us to see how closely organisms are related to the cultivated representatives with known metabolic activity; the smaller the distance the more closely an organism is related. These dendograms also allow a visual representation of the structure of the community; much like a yellow pages allows us to determine what businesses drive the economy of a city, dendograms allow us to determine what activities drive the processes that support a microbial community within an environment. The tree in Figure 4 is from Carlsbad Caverns and suggests that there are a number of metabolic activities that are occurring to support growth in this extremely starved cave environment, such as fixing nitrogen gas from the atmosphere and obtaining energy from the rock itself (Barton *et al.*, 2005a). Molecular phylogenetic analyses can also give us information of what nutrients are available in an environment, providing clues as what to add to our Petri plates to improve the chances of growing those rare cave microbes.

BROADER IMPLICATIONS

As the original observations by Leeuwenhoek changed our understanding of the world around us, the recognition of microorganisms in geologic cave samples has altered our perception of cave ecosystems (Barton and Northup, 2006). The science of cave geomicrobiology has similarly mirrored the meteoric rise of microbiology as a science, with new insights suggesting that cave microorganisms may be involved in processes as varied as speleothem deposition to cavern enlargement (Canaveras *et al.*, 2006; Engel *et al.*, 2004). While the important role of cave microorganisms may be of interest to speleologists, the implications of this research go well beyond caves. For example, the work of Saiz-Jimenez and colleagues has led to the identification of microorganisms degrading the ancient, prehistoric paintings within Altamira Cave, Spain (Schabereiter-Gurtner *et al.*, 2002). Not only did this work contribute to understanding the
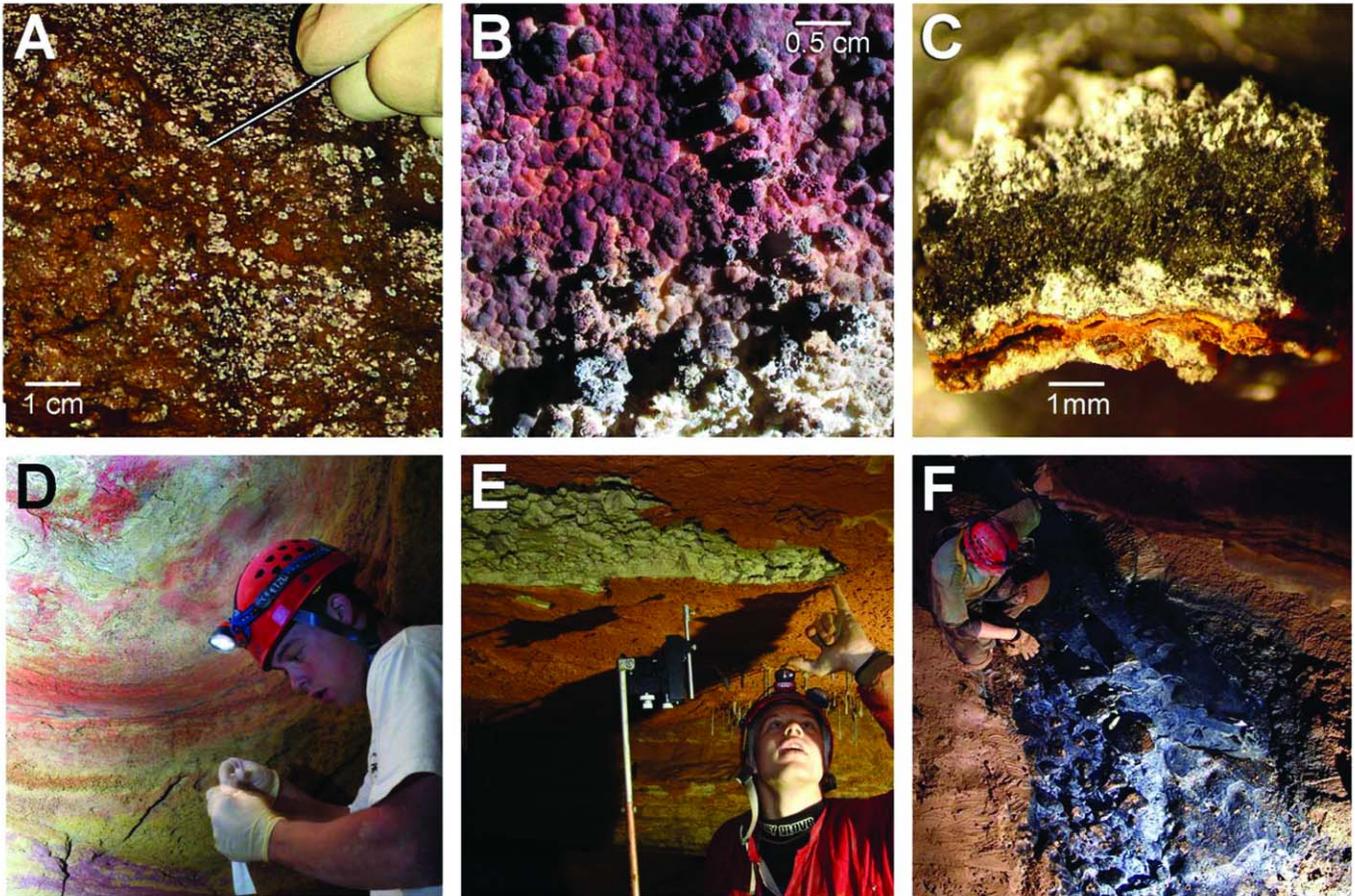
Figure 5. Microbiological activity in caves. A) Dots – microbial colonies on the surface of a rock being sampled using a hypodermic needle; B) Color – Microbial activity leading to the discoloration of a rock surface; C) Precipitation – Banded mineralization on a rock surface by microbial activity (the precipitate has peeled away revealing the deposit's banded formation); D) Corrosion residues – brightly-colored corrosion residue formed on a cave ceiling; E) Structural changes – using a microelectrode assembly to examine chemical gradients formed within a soft cave ceiling; F) Biofilms – a white biofilm coating in the streambed of a cave.

role of microorganisms in the degradation and subsequent conservation of these paintings, but it identified microbial species that could colonize carbonate surfaces, depositing calcite. Such work had significant implications in the preservation of ancient marble monuments and statues, where microorganisms could be used to deposit a veneer of calcite to protect ancient structures from continued erosion (Laiz *et al.*, 2003). Similarly, within Carlsbad Caverns we have identified a novel species of microorganism that can degrade complex aromatic compounds, such as benzothiazole and benezenesulfonic acid for growth, which are compounds involved in the manufacture of plastics and are dangerous environmental contaminants (Bennett and Barton, 2006). Such a capability could allow these species to be inoculated into contaminated environments, to rapidly degrade such pollutants and allow restoration of natural habitats in a process called bioremediation. Cave microorganisms, with their adaptation to extreme starvation, also have the potential to harbor other important biomolecules. Similar environments have yielded microbes with properties that allow

efficient ethanol production for fuel, enzymes for environmentally friendly paper processing and even the improved stonewashing of jeans. Cave microorganisms also have the potential to harbor unique antibiotics and cancer treatments (Onaga, 2001). Finally, one of the philosophical questions of humanity regards our place in the Universe: Are we alone? Is life on Earth unique? Cave microbiology can not only answer questions about the limits of life, but also help us to identify the geochemical signatures of life. Such signatures are capable of surviving geologic uplift, which allows them to be detected on the surface of planets, such as Mars (Boston *et al.*, 2001). While such ideas may seem an extraordinary application of cave geomicrobiology, NASA has recently undergone a dramatic refocus by gearing its activities to returning humans to the moon and exploration of Mars and world's beyond to find evidence of past life, activities in which cave geomicrobiology may play an important role (White House Press Release, 2004).

CAVE MICROBIOLOGY

While this extensive preamble has explained how difficult cave microbiology can be and the technical solutions possible, it does not preclude the average caver from being involved in cave microbiology; however, it does hopefully explain why running out and collecting "goo" in plastic bags is not going to advance the field. In addition to the traditional leave-no-trace conservation ethics of cave exploration, there are two significant roles that cavers can play in cave microbiology; 1) is the identification of new cave biota and ecosystems through continued exploration and survey, and 2) taking steps to limit human contamination and conserving the microbial habitats of caves.

CAVE SURVEYOR AS MICROBIOLOGIST

It may be a stretch to suggest that by exploring and surveying a cave you are contributing to the field of cave geomicrobiology, but it remains almost impossible to predict the location of microbial activity without its active discovery. Microbial activity in caves range from the obvious slimy goop to the more subtle deposition of calcite or alteration of the rock surface. There are a number of features that can be identified within caves as evidence of microbial activity (Figure 5):

Dots on surfaces: The appearance of dots on surfaces may not necessarily be due to abiotic mineral deposition. Often, where conditions are appropriate, microorganisms can grow up as a colony large enough to be seen with the naked eye. These colonies are similar in shape to those that might be seen on a Petri plate and each represents the growth of millions of bacteria. Sometimes these colonies may be more apparent through the concurrent deposition of minerals (such as calcite), creating a contrast against the host bedrock. Such microbial colonies are particularly obvious in areas of seeping water.

Unusual coloration: When microorganisms grow on surfaces, they can alter the surface chemistry, leading to a subtle change in coloration of the rock. Classic examples of this are the black/red residues seen within the Lunch Room of Carlsbad Caverns. The exact mechanism of this coloration may vary from site to site with local chemistry and microbial activity.

Precipitates: Microorganisms use chemical gradients to generate energy, much as we use the chemical gradient between food and oxygen to generate energy for life. In using these gradients microorganisms may often change the surrounding conditions of their environment, leading to a change in the chemical properties of minerals. In the case of minerals such as iron and manganese oxides, this will lead to a change in the solubility of these minerals and lead to their precipitation. Such microbially-mediated precipitation often forms banded layers of minerals on surfaces.

Corrosion residues: A significant amount of work has been carried out in caves of the Guadalupe Mountains on the corrosion residues that form as a result of microbial interactions with the minerals of limestone and dolomite (Barton *et al.*,

2005a; Cunningham *et al.*, 1995; Northup *et al.*, 2003). While researchers are still trying to elucidate the exact mechanism of their formation, it appears that microbial metabolic activity is involved in dissolving the host rock, while energy-generating chemical gradients cause continued mineral transformation and precipitation. Whatever the mechanism, these soft and powdery corrosion residues are an exciting component of geomicrobial activity in cave environments and may provide useful insights to the mechanisms of energy generation in such extremely starved environments.

Structural changes: When microorganisms interact with the rock on which they live they can cause different chemical changes in the rock. These changes may be distinct from the bright color changes seen with corrosion residues, resulting in subtle structural changes, such as decreased density or softening of the host rock. In Grayson-Gunnar Cave in Kentucky, areas of the ceiling have been reduced to a wet, toothpaste-like consistency that allows unique microbial energy acquisition strategies.

Biofilms: One of the most obvious signs of microbiological activity in a cave environment is the presence of biofilms. These coatings are comprised of microbial communities, held together with gel-like polymers that produce a range of structures including: wads of snot-like goo, floating dumplings, slippery sub-aqueous coatings and hair-like tendrils. These structures tend to form a sticky polymer that clumps bacterial species together, with the environmental conditions and mechanical action of water dictating the structure the community will form. These biofilms often form where energy enters the cave environment and have proven important insights into the sources of energy supporting cave life (Angert *et al.*, 1998).

A rule of thumb as a caver is that if you see anything unusual that cannot be easily explained by geologic phenomena, it may be microbiological. There are a number of active caver/microbiology researchers in the United States who can help you determine its relative significance (Barton *et al.*, 2005b; Boston *et al.*, 2001; Chelius and Moore, 2004; Engel *et al.*, 2004; Northup *et al.*, 2003; Spear *et al.*, 2005). In addition to the identification of unique cave ecosystems, one of the most important tools of our research is a cave line-plot/survey and map. Such maps allow researchers to determine how far below the surface the microbial activity is found, its relative position to geologic faults and other factors that might bring energy in to the system for microbial growth.

MICROBIAL CONSERVATION

While it can be difficult to remember to protect things that you cannot see, to minimize your impact on microbes, minimum impact caving techniques are appropriate. These include keeping to the established trail, removing all waste, avoid touching anything unnecessarily and generally minimizing your impact on the environment. While we often caution novices against touching formations, it is important to think similarly about placing limits on unnecessarily touching walls

(particularly where moisture is obvious), undisturbed sediments and corrosion residues. Some cave systems can withstand more human impact than others, and the same is true for microbial systems. If a cave floods, it is likely that the microbes are adapted to sudden influxes of material and energy, so a level of anxiety is not called for and less care need be taken. Other systems with perennially dry passages are more easily impacted. In some extreme instances, such as Lechuguilla Cave, flagged trails limit all travelers to the same area, to avoid walls and other surfaces unnecessarily.

In addition to conservation activities within the cave, also think about activities that you are carrying out in preparing for the cave. The biggest impact people can have on microbial ecosystems in caves is not washing caving gear and seeding one cave with another cave's microbes. I've heard of some cavers who will go down and smell their coveralls between caving trips, to get their cave fix from that earthy-smell of the dirt in their coveralls. That smell is a mixture of organic products being produced when microbial species, known as *Actinomycetes*, decompose organic material (Jachymova *et al.*, 2002; Scholler *et al.*, 2002). While you may get your kicks from smelling microbial excrement, it also means that if unwashed, those *Actinomycetes* will be traveling in to the next cave system with you.

In limiting your impact on such environments think about the potentially delicate nature of microbial ecosystems and, while I wouldn't recommend bathing in disinfectant (showers are counter productive as it dries skin out, increasing skin cell shedding) or wearing environmental suits, common sense should prevail. Think about your personal hygiene when entering a delicate cave system: Have you brushed your hair recently? Is long hair tied back so it won't be shedding in the cave? Think about the food you take with you into a cave. Avoid anything that generates crumbs and try to eat over a plastic bag, even when there is little chance of spilling, because one crumb is enough to feed a million microbes for many months. In addition to following leave-no-trace ethics to maintain the aesthetic beauty and visible ecosystems of the cave environment, similar care can go a long way to conserving microbial ecosystem health.

CONCLUSION

The science of geomicrobiology is still in its infancy, and as with other fields within microbiology, it is continuing to evolve. As it does, significant discoveries are updating our understanding of microbe-rock interactions and how microorganisms have helped shape our global environment (Balkwill *et al.*, 1997; Banfield and Nealson, 1997; Barghoorn and Schopf, 1966; Ben-Ari, 2002; Colwell *et al.*, 1997; Schopf, 1983). Similarly, cave microbiology is continually changing; from the preliminary description of cave microbiota at the turn of the last century to the sophisticated techniques employed today (Caumartin, 1963; Sarbu *et al.*, 1996). As cavers continue to explore caves in search of the unknown, they can help geomicrobiologists identify unique microbial ecosystems and help us to preserve this important resource.

REFERENCES

Amann, R.I., Ludwig, W., and Schleifer, K.H., 1995, Phylogenetic identification and in situ detection of individual microbial cells without cultivation: Microbiological Reviews, v. 59, p. 143–169.

Amann, R.I., Snaidr, J., Wagner, M., Ludwig, W., and Schleifer, K.H., 1996, *In situ* visualization of high genetic diversity in a natural community: Journal Bacteriology, v. 178, p. 3496–3500.

Angert, E.R., Northup, D.E., Reysenbach, A.-L., Peek, A.S., Goebel, B.M., and Pace, N.R., 1998, Molecular phylogenetic analysis of a bacterial community in Sulphur River, Parker Cave, Kentucky: American Mineralogist., v. 83, p. 1583–1592.

Balkwill, D.L., Drake, G.R., Reeves, R.H., Fredrickson, J.K., White, D.C., Ringelberg, D.B., Chandler, D.P., Romine, M.F., Kennedy, D.W., and Spadoni, C.M., 1997, Taxanomic study of aromatic-degrading Bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov: International Journal of Systematic Bacteriology, v. 47, p. 191–201.

Banfield, J.F., and Nealson, K.H., eds., 1997, Geomicrobiology: Interactions between microbes and minerals: Reviews in Mineralogy, v. 35: Washington, D.C., Mineralogical Society of American, 448 p.

Barghoorn, E.S., and Schopf, J.W., 1966, Microorganisms three billion years old from the Precambrian of South Africa: Science, v. 152, p. 758–763.

Barton, H.A. and Luiszer, F., 2005, Microbial Metabolic Structure in a Sulfidic Cave Hot Spring: Potential Mechanisms of Biospeleogenesis: Journal of Cave and Karst Studies, v. 67, p. 28–38.

Barton, H.A. and Northup, D.E., 2006, Geomicrobiology in cave environments: Past, current and future perspectives: Journal of Cave and Karst Studies, submitted.

Barton, H.A., Taylor, M.R., and Pace, N.R., 2004, Molecular phylogenetic analysis of a bacterial community in an oligotrophic cave environment: Geomicrobiology Journal, v. 21, p. 11–20.

Barton, H.A., Taylor, N.M., Kreate, M., Bertog, J., and Oehrle, S., 2005a, The impact of organic load on geomicrobial transformation in oligotrophic cave environments: Applied and Environmental Microbiology, submitted.

Barton, H.A., Taylor, N.M., Lubbers, B.R., and Pemberton, A.C., 2005b, DNA extraction from low biomass carbonate rock: an improved method with reduced contamination and the Low-Biomass Contaminant (LBC) database: Journal of Microbiological Methods, in press.

Ben-Ari, E.T., 2002, Microbiology and Geology: Solid marriage made on Earth: ASM News, v. 68, p. 13–18.

Bennett, A., and Barton, H.A., 2006, Description of *Polaromonas subterraneae* sp. nov., a new representative of the genus *Polaromonas* identified within oligotrophic cave and Karst environments: International Journal of Systematic and Evolutionary Microbiology, v., in preparation.

Bloomfield, S.F., Stewart, G.S., Dodd, C.E.R., Booth, I.R., and Power, E.G.M., 1998, The viable but non-culturable phenomenon explained?: Microbiology, v. 144, p. 1–3.

Bogosian, G., Aardema, N.D., Bourneuf, E.V., Morris, P.J.L., and O'Neil, J.P., 2000, Recovery of hydrogen peroxide-sensitive culturable cells of *Vibrio vulnificus* gives the appearance of resuscitation from a viable but nonculturable state: Journal of Bacteriology, v. 182, p. 5070–5075.

Bond, P.L., Smriga, S.P., and Banfield, J.F., 2000, Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site: Applied and Environmental Microbiology, v. 66, p. 3842–3849.

Boston, P.J., Spilde, M.N., Northup, D.E., Melim, L.A., Soroka, D.S., Kleina, L.G., Lavoie, K.H., Hose, L.D., Mallory, L.M., Dahm, C.N., Crossey, L.J., and Schelble, R.T., 2001, Cave Biosignature Suite: Microbes, Minerals and Mars: Astrobiology, v. 1, p. 25–55.

Brock, T.D., 1967, Micro-organisms adapted to high temperatures: Nature, v. 214, p. 882–885.

Canaveras, J.C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J.M., and Saiz-Jimenez, C., 2006, On the origin of fiber calcite crystals in moonmilk deposits: Naturwissenschaften, v. 93, p. 27–32.

Canaveras, J.C., Sanchez-Moral, S., Soler, V., and Saiz-Jimenez, C., 2001, Microorganisms and microbially induced fabrics in cave walls: Geomicrobiology Journal, v. 18, p. 223–240.

Caumartin, V., 1963, Review of the microbiology of underground environments: National Speleological Society Bulletin, v. 25, p. 1–14.

Chelius, M.K., and Moore, J.C., 2004, Molecular phylogenetic analysis of Archaea and Bacteria in Wind Cave, South Dakota: Geomicrobiology Journal, v. 21, p. 123–134.

Colwell, F.S., Onstott, T.C., Delwiche, M.E., Chandler, D., Fredrickson, J.K., Yao, Q.-J., McKinley, J.P., Boone, D.R., Griffiths, R., Phelps, T.J., Ringelberg, D., White, D.C., LaFreniere, L., Balkwill, D., Lehman, R.M., Konisky, J., and Long, P.E., 1997, Microorganisms from deep, high temperature sandstones: Constraints of microbial colonization: FEMS Microbiology Reviews, v. 20, p. 425–435.

Cunningham, K.I., Northup, D.E., Pollastro, R.M., Wright, W.G., and LaRock, E.J., 1995, Bacteria, fungi and biokarst in Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico: Environmental Geology, v. 25, p. 2–8.

Curtis, T.P., Sloan, W.T., and Scannell, J.W., 2002, Estimating prokaryotic diversity and its limits: Proceedings of the National Academy of Science USA, v. 99, p. 10494–10499.

Dojka, M.A., Harris, J.K., and Pace, N.R., 2000, Expanding the known diversity and environmental distribution of an uncultivated phylogenetic division of bacteria: Applied and Environmental Microbiology, v. 66, p. 1617–1621.

Engel, A.S., Stern, L.A., and Bennett, A., 2003, Condensation on cave walls: Implications for cave enlargement and sulfuric acid speleogenesis: Geochemica et Cosmochimica Acta, v. 67, p. A455.

Engel, A.S., Stern, L.A., and Bennett, P.C., 2004, Microbial contributions to cave formation: New insights into sulfuric acid speleogenesis: Geology, v. 32, p. 369–372.

Gold, T., 1999, The Deep Hot Biosphere: New York, Springer-Verlag, 235 p.

Gonzalez, J.M., Portillo, M.C., and Saiz-Jimenez, C., 2006, Metabolically active Crenarchaeota in Altamira Cave: Naturwissenschaften, v. 93, p. 42–45.

Groth, I., and Saiz-Jimenez, C., 1999, Actinomycetes in hypogean environments: Geomicrobiology Journal, v. 16, p. 1–8.

Groth, I., Vettermann, R., Schuetze, B., Schumann, P., and Saiz-Jimenez, C., 1999, Actinomycetes in karstic caves of Northern Spain (Altamira and Tito Bustillo). Journal of Microbiological Methods, v. 36, p. 115–122.

Holmes, A.J., Tujula, N.A., Holley, M., Contos, A., James, J.M., Rogers, P., and Gillings, M.R., 2001, Phylogenetic structure of unusual aquatic microbial formations in Nullarbor Caves, Australia: Environmental Microbiology, v. 3, p. 256–264.

Hose, L.D., Palmer, A.N., Palmer, M.V., Northup, D.E., Boston, P.J., and DuChene, H.R., 2000, Microbiology and geochemistry in a hydrogen-sulfide-rich karst environment: Chemical Geology, v. 169, p. 399–423.

Hugenholtz, P., Goebel, B.M., and Pace, N.R., 1998, Impact of culture-independent studies on the emerging phylogenetic view of Bacterial diversity: Journal of Bacteriology, v. 180, p. 4765–4774.

Jachymova, J., Votruba, J., Viden, I., and Rezanka, T., 2002, Identification of Streptomyces odor spectrum: Folia Microbiology (Praha), v. 47, p. 37–41.

Jannasch, H.W., Wirsen, C.O., Molyneaux, S.J., and Langworthy, T.A., 1992, Comparative physiological stud-

ies on hyperthermophilic archaea isolated from deep-sea hot vents with emphasis on Pyrococcus strain GB-D: Applied and Environmental Microbiology, v. 58, p. 3472–3481.

Koch, A.L., 1997, Microbial physiology and ecology of slow growth: Microbiology and Molecular Biology Reviews, v. 61, p. 305–318.

Koch, A.L., 2001, Oligotrophs versus copiotrophs: BioEssays, v. 23, p. 657–661.

Koch, R., 1881, Zur Untersuchung von pathogenen Organismen: Mitth. a. d. Kaiserl. Gesundheitsampte 1: 1–48. *In* Milestones in Microbiology: 1556 to 1940, translated and edited by Thomas D. Brock, ASM Press. 1998, p. 101.

Laiz, L., Groth, I., Gonzalez, I., and Saiz-Jimenez, C., 1999, Microbiological study of the dripping water in Altamira Cave (Santillana del Mar, Spain): Journal of Microbiological Methods, v. 36, p. 129–138.

Laiz, L., Pinar, G., Lubitz, W., and Saiz-Jimenez, C., 2003, Monitoring the colonization of monuments by bacteria: Cultivation versus molecular methods: Environmental Microbiology, v. 5, p. 72–74.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., and Pace, N.R., 1985, Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses: Proceedings of the National Academy of Sciences USA, v. 82, p. 6955–6959.

Madigan, M.T., Matinko, J.M., and Parker, J., 2000, Brock: Biology of Microorganisms: Upper Saddle River, N. J., Prentice-Hall Inc.

Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, J., Saxman, P.R., Farris, R.J., Garrity, G.M., Olsen, G.J., Schmidt, T.M., and Tiedje, J.M., 2001, The RDP-II (Ribosome Database Project): Nucleic Acids Research, v. 29, p. 173–174.

Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T., Saxman, P.R., Stredwick, J.M., Garrity, G.M., Li, B., Olsen, G.J., Schmidt, T.M., and Tiedje, J.M., 2000, The RDP (Ribosomal Database Project) continues: Nucleic Acids Research, v. 28, p. 173–174.

May, R.M., 1988, How many species are there on Earth?: Science, v. 241, p. 1441–1449.

Mormile, M.R., Biesen, M.A., Gutierrez, M.C., Ventosa, A., Pavlovich, J.B., Onstott, T.C., and Fredrickson, J.K., 2003, Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions: Environmental Microbiology, v. 5, p. 1094–1102.

Nei, M., Kumar, S., and Takahashi, K., 1998, The optimization principle in phylogenetic analysis tends to give incorrect topologies when the number of nucleotide or amino acids used is small: Proceedings of the National Academy of Sciences USA, v. 95, p. 12390–12397.

Northup, D.E., Barnes, S.M., Yu, L.E., Spilde, M.N., Schelble, R.T., Dano, K.E., Crossey, L.J., Connolly, C.A., Boston, P.J., Natvig, D.O., and Dahm, C.N., 2003, Diverse microbial communities inhabiting ferromanganese deposits in Lechuguilla and Spider Caves: Environmental Microbiology, v. 5, p. 1071-1086.

Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of Caves: A Review: Geomicrobiology Journal, v. 18, p. 199–222.

Oliver, J.D., 1995, The viable but non-culturable state in the human pathogen *Vibrio vulnificus*: FEMS Microbiology Letters, v. 133, p. 203–208.

Onaga, L., 2001, Cashing in on nature's pharmacy: EMBO Reports, v. 2, p. 263–265.

Pace, N.R., 1997, A molecular view of microbial diversity and the biosphere: Science, v. 276.

Pemberton, A., Millette, J., and Barton, H.A., 2005, Comparative Study Of Oligotrophic Bacterial Species Cultivated From Jack Bradley Cave, Kentucky: 14th International Congress of Speleology.

Sarbu, S.M., Kane, T.C., and Kinkle, B.K., 1996, A chemoautotrophically based cave ecosystem: Science, v. 272, p. 1953–1955.

Schabereiter-Gurtner, C., Saiz-Jimenez, C., Pinar, G., Lubitz, W., and Rolleke, S., 2002, Cave paleolithic paintings harbour complex and partly unknown microbial communities: FEMS Microbiology Letters, v. 211, p. 7–11.

Scholler, C.E., Gurtler, H., Pedersen, R., Molin, S., and Wilkins, K., 2002, Volatile metabolites from Actinomycetes: Journal of Agricultural Food Chemistry, v. 50, p. 2615–2621.

Schopf, J.W., ed., 1983, Earth's earliest biosphere: its origin and evolution: Princeton, N.J., Princeton University Press, 531 p.

Shivaji, S., Reddy, G.S., Raghavan, P.U., Sarita, N.B., and Delille, D., 2004, *Psychrobacter salsus* sp. nov. and *Psychrobacter adeliensis* sp. nov. isolated from fast ice from Adelie Land, Antarctica: Systematic and Applied Microbiology, v. 27, p. 628–635.

Spear, J.R., Walker, G.C., McCollom, T.M., and Pace, N.R., 2005, Hydrogen and bioenergetics in a Yellowstone geothermal ecosystem: Proceedings of the National Academy of Sciences USA, v. 102, p. 2555–2560.

Spilde, M.N., Northup, D.E., Boston, P.J., Schelble, R.T., Dano, K.E., Crossey, L.J., and Dahm, C.N., 2005, Geomicrobiology of cave ferromanganese deposits: A field and laboratory investigation: Geomicrobiology Journal, v. 22, p. 99–116.

Stahl, D.A., Lane, D.J., Olsen, G.J., and Pace, N.R., 1985, Characterization of a Yellowstone hot spring microbial community by 5S rRNA sequences: Applied and Environmental Microbiology, v. 49, p. 1379–1384.

Thomas, D.N., and Dieckmann, G.S., 2002, Antarctic sea ice - a habitat for extremophiles: Science, v. 295, p. 641–644.

Torsvik, V., Ovreas, L., and Thingstad, T.F., 2002, Prokaryotic diversity - magnitude, dynamics, and controlling factors: Science, v. 296, p. 1064–1066.

White House Press Release, 2004, President Bush Announces New Vision for Space Exploration Program, Washington, D.C., Office of the White House Press Secretary, p. 1–3.

Whitesides, M.D., and Oliver, J.D., 1997, Resuscitation of *Vibrio vulnificus* from the viable but nonculturable state: Applied and Environmental Microbiology, v. 63, p. 1002–1005.

Whittaker, R.H., 1969, New concepts of kingdoms of organisms. Evolutionary relations are better represented by new classications than by the traditional two kingdoms: Science, v. 163, p. 150–160.

Winogradsky, S., 1887, Uber Schwefelbacterien: Botanische Zeitung, v. 40.

Woese, C.R., 1987, Bacterial evolution: Microbiological Reviews, v. 51, p. 221–271.

Woese, C.R., and Fox, G.E., 1977, Phylogenetic structure of the prokaryotic domain: the primary kingdoms: Proceedings National Academy Sciences, v. 74, p. 5088–5090.