INTRODUCTION

WHAT ARE THE LIMITS OF LIFE ON EARTH?

Currently we do not have a complete picture of how life originated on this planet. We do have pieces of the puzzle, but the entire picture has not yet been assembled. Only within the last couple of decades have we been able to place constraints on the limits of life on the Earth, but much still needs explanation as to why those limits exist. These gaps in our understanding of the origin and extent of life on Earth make it especially challenging to explore other planetary bodies in our solar system for life. This book is about the exploration of the limits of life in the rocky crust deep beneath the surface of our planet and how what we have discovered over the past twenty-five years informs our exploration for life in the solar system, and in particular, the planet Mars.

We do not normally think of rock as harboring life. We quarry granite for building stone, volcanic rock for road gravel, and marble for table tops and great works of art. I would wager that the last thought on your mind as you gaze upon Michelangelo's *David* towering above you in the Accademia Gallery in Florence is the fact that within microscopic pores buried inches beneath the smooth surface of Carrara marble are living bacteria. These bacteria may have been trapped inside the marble for a million years and are slowly reproducing, releasing carbon dioxide (CO_2) and making nanocrystalline calcite, the same mineral phase that forms the marble. I can personally guarantee that as miners tunnel into the Earth to remove the coal from beneath the Appalachian Mountains or extract metal-rich veins from miles beneath the surface of South Africa, they do not typically think of rocks as crawling with life. I have never encountered any roughnecks on a drill rig who have taken the time to stop and ponder whether the oil or gas reservoir into which they were drilling a mile or more beneath their feet happens to be something's domicile.

I am a geologist by training, and like most geologists, I too have viewed rocks as inanimate entities. We examine the microscopic details of rocks primarily to infer the most probable explanation of how and when they came to be what they are. We try to infer the underlying physical processes that formed them and how those processes relate to geological history at the global scale. Paleontologists focus on those rocks that contain fossilized life forms that existed on the surface of the Earth at the time the materials forming the rock were being deposited. Some paleontologists who study rocks from the Precambrian, a time prior to the rise of multicellular organisms, look for traces of microorganisms that once existed on the surface. They do this by analyzing the composition of relic organic molecules, their stable isotopic composition, such as the ratio of carbon-13 to carbon-12 ($^{13}C/^{12}C$), or some physical structures in the rock that may represent a fossilized surface biofilm. Their goal is to understand the evolution of surface life and how that evolution relates to the evolution of Earth's oceans and atmosphere over time. Even from their perspective, those ancient microorganisms supposedly quickly died as the rock was formed from the sand or mud that contained them. Geologists used to view rock as being dead.

Of course, scientifically speaking, microbiologists are the antithesis of geologists, even though microbiologists travel to the same regions of the world as geologists in order to carry out fieldwork. Microbiologists gather samples, take them to the lab, isolate microorganisms from them, and then perform experiments on the microbes to deduce what they are doing in their environment and how they are doing it. During the past one hundred years, a subset of these scientists has been steadfastly isolating microorganisms from environments that at first glance would not be what we would consider likely places for life to exist. They have isolated bacteria from the Arctic that can grow at temperatures below freezing (psychrophiles), and in the hot springs of Yellowstone National Park, they have discovered bacteria that can thrive at temperatures close to the boiling point of water (thermophiles and hyperthermophiles). Also in Yellowstone, microbiologists have found microbes that can live in acid (acidophiles). In the hypersaline, soda volcanic lakes of East Africa, they have isolated bacteria that can flourish at pH 10 (alkaphiles). One of the pioneers of microbiology, Antonie van Leeuwenhoek, performed experiments in the seventeenth century that led to the discovery of microbes that can live in the absence of oxygen (anaerobes). Microbiologists have even found bacteria, known as gram-positive bacteria, that show a remarkable tenacity to survive by remaining dormant as spores. Deprived of any sustenance for decades, the spores germinate as soon as they are provided with some nutrients in water. In fact, microbiologists have been hard-pressed to find any corner on the surface of the Earth, no matter how harsh the environment, that has not been colonized by some form of microbe that could be grown in the lab.

Geologists first joined forces with microbiologists in the 1920s to explore the one realm into which microbiologists had not yet ventured: the subsurface. Edson Sunderland Bastin, an economic geologist from the University of Chicago, teamed up with a bacteriologist, Frank E. Greer, from the Department of Hygiene and Bacteriology at the University of Chicago to test a straightforward hypothesis. They proposed that sour oil (oil containing hydrogen sulfide, or H_2S, the gas that exudes from rotting eggs) was created by anaerobic sulfate-reducing bacteria (SRBs). When Greer grew SRBs at high temperatures from the oil-field water collected from geological formations dating from the Pennsylvanian Epoch provided by Bastin, they were able to demonstrate that their proposed answer was definitively correct! But it raised a second, more interesting question. Had those SRBs been living in the same formation beneath the surface for the 300 million years since they were first deposited in the oceans? This was a question the bacteriologist could not answer by deduction. Nor could the answer be inferred by the geologist.

Bastin and Greer were not the only ones to speculate about the existence of subsurface ancient life. Before World War I, German microbiologists had begun exploring their deepest coal mines. They were looking for bacteria inhabiting the coal, hypothesizing that they could be living fossils of the bacteria that had inhabited the 300-

million-year-old peat bogs from which the coal had formed. Why did microbiologists at that time believe in the longevity of bacteria? One of those microbiologists, Charles Bernard Lipman, of the University of California Berkeley, explained in a 1931 article in the *Journal of Bacteriology*: "I have been asked frequently since the inception of the studies under consideration to state what led me to make such an investigation when *a priori* one would not expect anything but negative results. My answer to this question is that for twenty years prior to the initiation of these experiments I had been accumulating more and more evidence on the persistence of the life of bacteria and bacterial spores for periods of forty years, as a maximum in the latter case, as authentic facts. The unabated virulence of pathogenic organisms grown from very old spores and the remarkable viability of bacteria from very old and very dry soils preserved in unopened bottles for about forty years have furnished me with much food for thought for well nigh a quarter of a century."[1]

But the many bacteria grown from coal and oil reservoir samples from the 1920s to the 1930s, including Lipman's remarkable spore-forming bacteria, were largely dismissed as modern bacterial contaminants from the surface. After all, a drill rig or a mining operation cannot be sterilized. Undeterred, microbiologists reported finding bacteria that produced alkanes and began speculating on the bacterial genesis of petroleum deposits. Geologists, however, were not receptive to this idea, primarily because they had a geological model based upon thermal alteration of dead organic matter that explained the origin of oil quite nicely. This geological model was solidly built upon observations of when and where in the geological record that oil formed and migrated, and upon inductive reasoning about the common steps required to produce all petroleum and natural gas deposits around the world and over a wide span of geological ages. Subsurface bacteria were simply not required to explain oil or gas. Sporadically over the subsequent decades, microbiologists would report the discovery of bacteria from deep within our planet, only to have the discovery summarily repudiated and dismissed as surface contamination. During the 1950s Soviet microbiologists remained the last holdouts for deep-dwelling life and the central role of bacteria in forming many economic ore deposits. They introduced the term "geological microbiology" in the early 1960s, the practitioners of which were geomicrobiologists.[2] However, there was not an enormous waiting list of scientists signing up to become geomicrobiologists. By the 1970s even soil microbiologists were certain that "depth is another secondary ecological variable that affects the bacteria. In temperate zones, these organisms are almost all in the top meter, largely in the upper few centimeters."[3] The perception was widespread in both the geological and microbial communities that even a lowly bacterium would have to struggle to live within the tiny cracks and pores of rocks forever separated from sunlight and survive on the occasional dribs and drabs of organic matter that might descend downward past the soil layer.

At this same time, the first communities of organisms were discovered at black-smoker vents at the bottom of the Pacific Ocean not far from the Galápagos Islands, the birthplace of Darwin's theory of evolution by natural selection. Before *Riftia pachyptila* tube worms were discovered at black smokers, no one had imagined that such complex organisms or ecosystems could survive on chemical gradients in the utter darkness of the seafloor. But miles beneath the surface of the ocean, there was at least plenty of room to grow and evolve complex ecosystems. It seemed highly improbable that complex ecosystems could exist in the tiny pores a thousand feet beneath the rocky surfaces of Yosemite, the forest floor of the Amazon jungle, the desert dunes of the Sahara, the ice sheets of Antarctica, or the wheat fields of Kansas. By the 1970s, it was accepted that, with the exception of some shallow aquifers, the deep subsurface of our planet was generally a sterile environment. There was simply not enough energy or room down there to support life for long.

In many respects this belief was reassuring. After all, the Atomic Energy Commission (AEC), the predecessor to the U.S. Department of Energy (DOE), had contaminated the subsurface beneath the laboratory complexes where they had been concentrating enormous amounts of radioactive material for nuclear warheads. These contaminants were a witch's brew of carcinogenic organics, toxic metals, and radionuclides. Modeling their geochemical behavior and hydrological transport was inherently simpler in the age of punch-card, mainframe computers if biology was not part of the equations. The AEC had also been exploding these warheads underground in Nevada since the early 1950s and then solely underground after John F. Kennedy, Nikita Khrushchev, and Harold Macmillan agreed to the Limited Nuclear Test Ban Treaty in 1963. The AEC had become so adept at subsurface atomic detonations that they began to detonate A-bombs beneath the surface of New Mexico and Colorado to help release natural gas from tight formations as part of the Plowshare Program. In today's parlance, we might call this practice fissionogenic-fracking and be grateful that it was abandoned. The U.S. DOE was also busily working on burying high-level radioactive waste in the "sterile" subsurface salt, granite, and volcanic formations, as were other countries. These formations had to retain the radioactive waste for a hundred thousand years. The possibility that subsurface life forms might interact with the radioactive waste and, hence, complicate their calculations seemed a very remote possibility.

Then, in the mid-80s an obscure program within the U.S. DOE, called the Subsurface Science Program (SSP) and led by Frank J. Wobber, started claiming that the subsurface was not sterile after all but instead was inhabited by an abundant and diverse community of bacteria and eukaryotes (e.g., protists and fungi). Unlike the earlier subsurface studies, the scientists working in the SSP had developed physical and chemical tracers to screen out the surface microbial contaminants. They were confident that they were finding indigenous bacteria everywhere, even in Triassic rocks from two miles beneath the surface of a farmer's field in Virginia. They claimed the abundance of this subsurface life rivaled that of surface life on our planet, and they soon began talking about a subsurface biosphere. The reports made big news with the general public, who found them as intriguing and fantastical as Jules Verne's journey to the center of the Earth. The broader scientific community, however, was extremely skeptical of the claims made by these DOE scientists and their program manager and also of the significance of the discovery. But the SSP microbiologists had isolated thousands of species of subterranean bacteria, which they planned to use to remediate the toxic underground legacy of the Cold War, a legacy that had started to migrate away from the DOE laboratories and toward the drinking-water supply of the surrounding communities. How ironic it is that a DOE program would not only discover a subsurface biosphere but also enlist its aid when its predecessor, the AEC, had unknowingly killed quintillions of subsurface bacteria in their homes with underground nuclear blasts. The SSP scientists also discovered that entire microbial communities in the subsurface were being powered from hydrogen (H_2) gas generated by the weathering of iron (Fe) minerals in water. Very soon thereafter, scientists started finding ecosystems in every subsurface corner of the planet. Some were three miles down and powered by radiation. Others were carving enormous underground cavities beneath mountains. Some were found trapped in ice thousands of feet beneath the surface of Antarctica. Even predatory, deep-dwelling worms were being discovered a mile beneath the surface. By then geomicrobiology had its own dedicated publication, and geomicrobiologists had founded a society for subsurface microbiology.

LIFE BENEATH THE SURFACE OF OTHER PLANETS

Now, as a geomicrobiologist, I look at all rocks as microcosms of tiny microorganisms, some of which may have been living in the rock since its formation hundreds of millions of years ago. When you look at the surface of Mars, you get the impression that it was once habitable for life. But currently, its surface is not habitable by life as we know it. Still you can't help but wonder if Mars, having had a surface biosphere at one time, still maintains a subsurface biosphere like that of the Earth's.

Could there be life beneath the surface of Mars? If you had openly asked a question like that in the sixteenth century you would have been burned at the stake, like the Italian Dominican friar and philosopher, Giordano Bruno. More than a hundred years ago, Percival

Lowell believed that he could see evidence of an extraterrestrial civilization living on the surface of Mars, but that optical illusion, along with his scientific credibility, was dispelled within fifteen years of his discovery. In 1901 H. G. Wells, a contemporary of Lowell, published *The First Men in the Moon*, in which he constructed a fantastically sophisticated society of insect-like creatures, Selenites, living beneath the lunar surface. Since then, the concept of subsurface life has figured frequently in science fiction novels and movies, even episodes of *Star Trek* and *Star Trek: The Next Generation*, but is there any scientific basis supporting it? It is certainly true that if you burrow miles into the Moon or Mars, you will reach a temperature at which fresh liquid water, the essential ingredient for life as we know it, can exist. But can life survive miles beneath a planet's surface even when that surface is inimical to it? With the discovery by SSP in 1996 that the H_2 generated from rocks and water could support a subsurface ecosystem, the answer seemed to be yes. Life beneath the surface of Mars was now no longer restricted to the realm of science fiction and could be treated as a real, tangible target for space probes. The National Aeronautics and Space Administration (NASA) quickly became engaged with designing drilling systems that could be sent to Mars.

All this narrative may sound to you like the script of a B-grade sci-fi film, and it could easily be one. But it is historic fact that in the space of twenty years scientific consensus concerning deep subsurface life on Earth and all its extraterrestrial implications switched from incredulity to active scientific endeavor. This book is in part about that remarkable scientific and philosophical transition as seen through the eyes of its protagonists, including myself.

But mostly in this book, you and I will actively explore the limits of life beneath the surface of our planet and search for anything that would prohibit life from existing beneath the surface of other planets, especially Mars. During our quest I will be taking you to places that few surface dwellers have seen. While we are hunting on our subterranean safaris, I want you to keep the following questions in mind, because my colleagues and I asked ourselves these same questions, not knowing what the answers would be.

Is it possible that subsurface life exists in such abundance that it exceeds the entire surface biosphere of our planet?
Just how deep down into the Earth do living organisms occur?
How long can a single subsurface microbe live? Can it live for a hundred years, a hundred thousand years, a million years?
Can microorganisms live on radiation?
How long can ecosystems survive beneath a planet's surface? Billions of years?
If it is for billions of years, can life exist beneath the surface of Mars?
Where did the microbes come from and how did they get down there? Can they travel for hundreds of miles beneath the surface?
Could life have originated beneath the surface of our planet, or any planet for that matter?
Is it possible that complex life forms and complex living ecosystems can exist beneath the surface of any planet?

To help us with the answers to these questions, I will be bringing plenty of scientific background with us.

In chapter 1 we begin with the discovery by SSP scientists of bacteria inhabiting rocks 9,000 feet beneath the surface of the Earth. We then flash back to the earlier years, when the SSP first started drilling and developing the tracer techniques that were pivotal in proving that subsurface bacteria were not contaminants.

In chapter 2 we follow the SSP to northern New Mexico, where we drill into an ancient volcano, and to western Colorado, where we drill more than a mile down into a tight gas formation near an underground nuclear test site. We will also begin our first Jules Vernian subsurface expedition down into the Waste Isolation Pilot Plant near Carlsbad, New Mexico. There we will explore the Permian salt formations in search of fluid inclusions containing 250-million-year-old seawater and, hopefully, living bacteria. We will also learn how scientists began to conceive of radiation-supported subsurface life.

In chapter 3 we will see how researchers used geological history to determine the origins of the microbes collected from the various SSP drilling campaigns. They deduced how long the microbes had resided down there, how far they had traveled to get there, and how quickly they moved. We will explore the boundary between life and death and what that means for a microbe through the use of the "Death-o-Meter." Finally we will learn of the discovery of the subsurface lithoautotrophic microbial ecosystems (SLiMEs), supported by H_2 gas derived from basaltic volcanic rocks.

The termination of the SSP in chapter 4 forces us to journey to the deep mines of South Africa in search of a way to obtain deep and potentially very ancient microbial ecosystems supported by radiation and to search for the origins of life itself. With the discovery of SLiMEs we will participate in the NASA meetings where the plans were made for drilling on Mars with the goal of detecting life beneath the Martian surface. We will see how, coincidently, that search effort was bolstered by the reports of fossil subsurface Martian life in a meteorite called ALH84001.

In chapters 5 through 8, we will explore the maze of tunnels in the ultradeep gold mines of South Africa and the challenges that greeted the scientists there. As we journey downward more than two miles in depth, we will learn what life must be like for a thermophilic subsurface bacterium. Finally, we reach our goal with the discovery of *Candidatus* "Desulforudis audaxviator", a novel new bacterium that travels beneath the surface of South Africa, living indefinitely on a chemical battery supported by the radioactive decay of uranium embedded in its rocky environments.

In chapter 9 we fly north of the Arctic Circle in Canada in order to answer the question of whether life could exist deep beneath the frozen surface of Mars. There we travel through the ice caves of the Lupin gold mine down to salty water a half mile below the frozen tundra to find chemolithoautotrophic bacteria, microorganisms that are able to obtain the carbon and nitrogen for biosynthesis by metabolizing inorganic elements, that cycle sulfur compounds. We also test the feasibility of drilling through permafrost to sample a deep biosphere on Mars.

Given the difficulties the researchers encountered drilling through permafrost, in chapter 10 we explore the possibility of using caves to gain access to subterranean life. We will go into the deep caverns of New Mexico, Romania, and Mexico in search of chemolithoautotrophic life and complex ecosystems. We see firsthand how microbial life has carved out its own habitat and what this could mean for Mars. Finally, we return to South Africa to discover multicellular life in the form of a predatory, hermaphroditic nematode named *Halicephalobus mephisto*, the worm from hell, living one mile beneath the surface of our planet.

In the epilogue, we will review the questions that scientists have answered and address the questions that remain to be answered and how they are going to do that. With each chapter, I also provide historical accounts of the earlier discoveries of subsurface life stretching back to 1793, which had been discounted but now, placed in the modern context of our current understanding, make sense. In the appendixes I also provide a chronology of subsurface life investigations and summaries of the seminal U.S. DOE meetings. The locations of subsurface microbiological field campaigns along with photographs from those expeditions can be found as a Google Earth document at http://press.princeton.edu/titles/10805.html.