

The Deep Time in the Depths of the Ocean

Discoveries of unusual marine microbes are radically changing our views about the evolution of life

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At the helm of the British ship *Endeavor*, James Cook departed England in 1768. He rounded Cape Horn in January 1769, entering the vast, unexplored Pacific and Southern Oceans and opening up an entirely new vista on the world.

Cook “added a hemisphere” to the body of European knowledge, said the naturalist Charles Darwin. He discovered new Pacific islands and Australia. He found never-seen-before animal species and more than 1,000 exotic species of plants.

In the 1830s, Darwin himself sailed aboard the *Beagle* to the Galápagos Islands. The observations he made there of animal life spurred his theory of natural selection, which revolutionized our understanding of the origin and evolution of species.

Centuries after these classical voyages, we are making discoveries that are similarly shaking and expanding prevailing ideas about life on our planet. Once again we have embarked on voyages to explore remote, unknown areas of our planet—this time in, rather than on, the oceans.

Wherever we have looked in the oceans, we have found previously unknown microorganisms. We have often found them living in conditions once thought to be incompatible with life, using unfamiliar physiologic and metabolic adaptations. These discoveries have radically changed our thinking about where and how life

may have originated and evolved on this planet, and where it might exist on others.

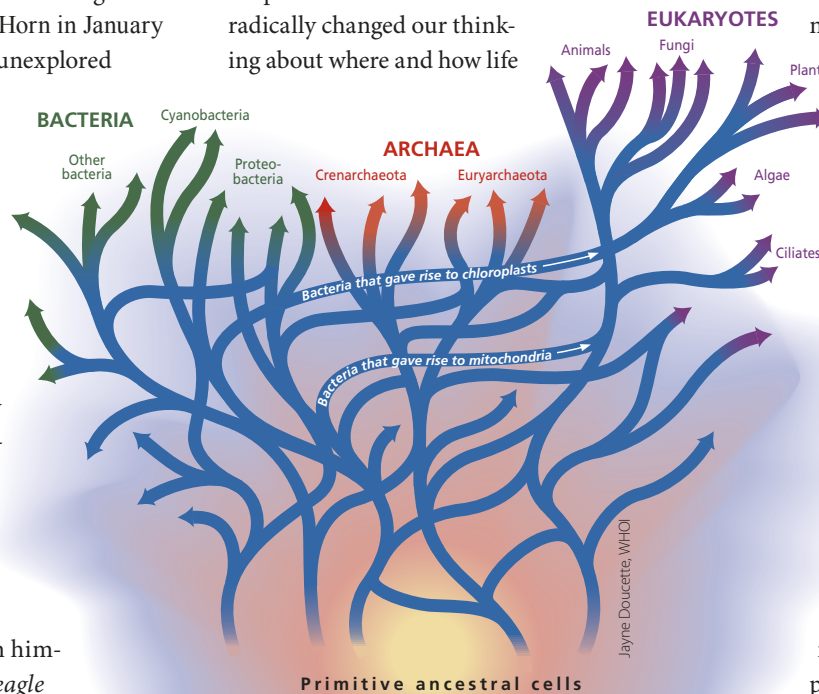
The seafloor and the rocky regions below it offer boundless new potential habitats to explore. With research submersibles, robotic vehicles, and new sampling

tools and techniques, marine microbiologists are making discoveries at an unprecedented rate. We are opening a wide window onto the immense, unexplored realm of the smallest, least-known, but most important life forms. We have entered the classical age of microbiology.

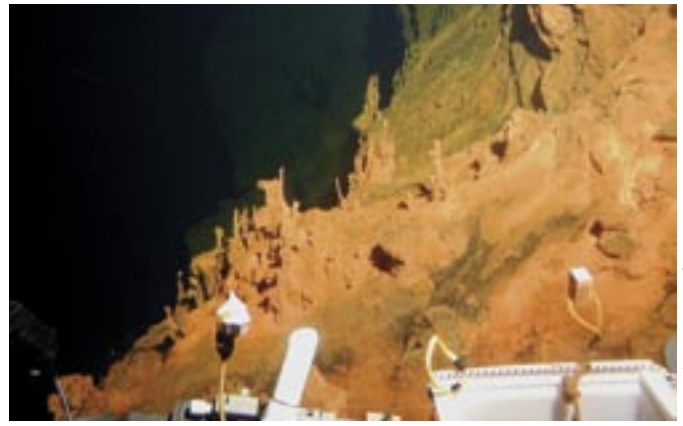
Recent discoveries

Without microorganisms, there would be no other life on Earth. Unseen, ubiquitous, and unicellular, microorganisms nevertheless keep the planet running. Photosynthesizing plankton form the base of the marine food chain and keep the biosphere well oxygenated. At the other end of the cycle, other microbes decompose organic molecules for reuse.

It was not until 1977 that we discovered cyanobacteria in the open ocean, which turn out to be among the most abundant and important bacteria on Earth. These bacteria were the photosynthetic pioneers responsible three billion years ago for infusing our planet’s atmosphere with oxygen. (See “Little Things Matter A Lot.”)



A COMPLEX TREE OF LIFE—Microbes are living archives of Earth’s evolutionary history. The discovery of a great variety of deep-sea microorganisms (using diverse metabolic strategies to live in diverse habitats) indicates that they evolved along different evolutionary pathways. Using genetic analyses, scientists can trace these pathways to reconstruct when various microbial biochemical and metabolic machinery developed, diverged, or intermingled in the three major domains of life: bacteria, archaea, and eukaryotes.



Terry Kirby, University of Hawaii

MICROBES IN MANY COLORS—Scientists have found a multitude of deep-sea microorganisms using a variety of chemical compounds to live. Yellow bacterial mats atop sediments in the Guaymas Basin in the Sea of California (left) are evidence of microbes that oxidize sulfide; the sediments underneath harbor methane-oxidizing archaea. The orange mats at right are made by microbes that live off iron in seafloor rocks off Hawaii.

As recently as the mid-1970s, scientists believed there were only two domains of life on Earth: prokaryotes (single-celled bacteria, without nuclei or complex cellular structures) and eukaryotes (organisms made of cells with nuclei, ranging from single-celled amoebae to all multicellular life, including fungi, plants, reptiles, and mammals). Then in 1977, Carl Woese of the University of Illinois identified a wholly new domain of single-celled life forms, called archaea, which are as genetically different from bacteria as bacteria are from trees and people.

Archaea, or “ancient ones,” have existed for billions of years on Earth. Many are extremophiles that thrive in hot, cold, salty, acidic, oxygen-deprived, or other extreme environments. Such conditions prevailed on an adolescent Earth, before cyanobacteria evolved and fundamentally changed Earth’s atmosphere.

Life in unexpected places

In the late 1970s, we also discovered microbial communities in the dark and high-pressure depths of the seafloor—living on superheated, acidic, sulfide-rich fluids emanating from hydrothermal vents. Since then, we have found microbes that thrive in polar ice; on ocean floor lava; buried beneath seafloor sediments; and in the rocky nooks beneath the seafloor. They exploit a wide range of chemical reactions, using hydrogen sulfide, iron

compounds, nitrites, methane, and other chemical compounds to obtain energy and resources to grow. (See “Revealing the Ocean’s Invisible Abundance” and “Is Life Thriving Deep Beneath the Seafloor?”)

This great variety of habitats and metabolic strategies indicates that microbes have taken a diversity of evolutionary pathways in the past. Ancient microbial lineages, which had their origin (and possible heyday) when different biogeochemical conditions prevailed on Earth, can survive today in diverse habitats that still exist in the mostly unknown deep subsurface of oceans. These microbes are living archives of Earth’s evolutionary history.

The novel microbial lineages we are finding on Earth are also expanding and guiding our search for life that may exist in the extreme environments on other planetary bodies. With our eyes opened wider to more possibilities, we can look for life in previously unsuspected places: in the iron-rich rocks of the red planet, Mars; beneath of ice-covered surface of Jupiter’s volcanic moon, Europa; or on Titan, Saturn’s moon, which now shows evidence of having liquid-methane lakes to go along with its methane-rich atmosphere.

Portals into microspace

Like Cook and Darwin, today’s scientists collect specimens in remote places, but studying microorganisms presents a new set of challenges. To study

microbes, scientists need to keep them alive, but it is often hard to reproduce undersea conditions in the laboratory, and only some microbial species have been successfully cultured.

Instead, microbiologists have exploited modern genetic techniques to search for, identify, and study newly found microbes. They examine samples from deep-sea environments containing unknown species of microbes, locate gene sequences within them, and compare these sequences with those of known, cultured microbial species.

An unknown organism in the wild can be identified—on the basis of how similar gene sequences are to those of known microbes—without scientists ever having to grow it in the laboratory. Fully half of the bacterial branches known today have never been cultured and have been identified only by gene sequences.

Genomic investigations

Gene sequences also allow scientists to trace microorganisms’ evolutionary history. All microorganisms share some common genetic equipment, including certain genes, known as “conserved genes,” which are the blueprints for basic biochemical functions. Mutations that change gene sequences accumulate in genes over evolutionary time, but this process occurs at a far slower rate in conserved genes than in other genes. Thus,

conserved genes are similar in closely related organisms and less similar in distantly related ones. The greater the differences in conserved genes shared by two organisms, the further back in time they diverged in evolutionary history.

By analyzing the DNA of conserved genes, scientists can place microorganisms in evolutionary trees that encompass deep evolutionary time and chronicle when various microbial biochemical and metabolic machinery developed and diverged. Surveying samples from marine environments, microbiologists are finding novel gene sequences from unknown organisms and accumulating libraries of gene sequences to reference newer discoveries.

Little microbes that could

At the same time, microbiologists are also extracting nucleic acids from microbes to determine what protein products the nucleic acids code for. By these means, we can find out something about what compounds and biochemical mechanisms the microorganisms use to obtain energy and carbon to live and grow.

In addition, microbiologists are analyzing isotopes of elements incorporated into microbes during their metabolic processes. These not only provide more clues to learn about the microbes' biochemical machinery, they also reveal how the microbes affect the rocks they live in, seawater chemistry, and even the atmosphere.

In 2000, for example, we found new species of microbes that live directly off minerals in seafloor rock. They oxidize iron in the rocks to obtain energy and convert carbon dioxide in seawater into organic matter to grow. (See "Living Large in Microscopic Nooks.")

If these previously unknown bacteria turn out to be as abundant as they seem to be, they may play a longstanding, important role in Earth's climate by extracting huge amounts of the greenhouse gas carbon dioxide from seawater and keeping it out of the atmosphere (while producing up to a million tons of biomass). They may have changed the geology of

the seafloor by changing the chemical composition of seafloor rocks. They may have been evolutionary pioneers on an iron-rich, oxygen-poor early Earth, or inhabitants of iron-rich, oxygen-poor planets today, such as Mars.

No oxygen, no problem

Over the past few years, for example, we have sampled and analyzed sediments in the Guaymas Basin in the Gulf of California, where hundreds of meters of sediments have piled on top of hydrothermal vents. We had expected to find the molecular signs of archaea adapted to high heat (hyperthermophiles), which are well known at hydrothermal vents.

But instead we found something completely different—a major new type of archaea, related to known methane-producing archaea, or methanogens. We believe that the high geothermal heat emanating from the hydrothermal vent site is breaking down organic matter in the sediments into short-chain fatty acids, ammonia, and more methane.

Some of these compounds percolate upward and are released from the sediments into the ocean—but not all

of them. In the sediments we also found isotopic and gene sequence signatures that reveal archaeal populations that use methane to grow in oxygen-free environments, such as those beneath the Guaymas sediments.

The discovery of these anaerobic methanotrophs fills a large gap in our knowledge of Earth's microbial and geochemical cycles. Microbes that generate methane, and others that consume it, play crucial roles in minimizing how much methane—a greenhouse gas more potent than carbon dioxide—is released from the ocean to the atmosphere.

These microorganisms complete a subsurface methane cycle that allows life to flourish at the seafloor, not only in the microbial oases of hydrothermal vent sites, but also in deep marine sediments and the subsurface biosphere. We are now exploring deep marine sediments in the Pacific to investigate whether this phenomenon is global.

Though the pace of microbial discoveries has increased, history warns us that we haven't seen everything yet. The book on microbial life, on Earth and elsewhere in the universe, is far from written.



Tom Kleindinst, WHOI

Andreas Teske earned a master's degree in biochemistry in his native Germany. After deciding to seek work that allowed exotic field trips, he spent a year in the cornfields at the University of Illinois, focusing on microbial evolution and diversity, before joining the Max Planck Institute for Marine Microbiology for his Ph.D. on microorganisms of the marine sulfur cycle. In 1996, his fascination with new and unusual hydrothermal vent microorganisms brought him to WHOI, as a postdoctoral scholar with the late Holger Jannasch and then as assistant scientist in the Biology Department. At WHOI, he became interested in the diversity and biogeochemical activity of microbes living in massive seafloor sediment layers. Teske now pursues the emerging field of deep-subsurface microbiology at the Department of Marine Sciences at the University of North Carolina, but he and his family return to Woods Hole every summer to keep up collaborations, and to sample Woods Hole's famous microbial life.



B. Tebo, Scripps

Katrina Edwards grew up in central Ohio, where she pursued an initial early career in the family business of running a small municipal airport just north of Columbus. She spent several years assisting her father and siblings in general airport operations (graduating to the role of chief flight instructor), which she continued as she pursued a bachelor's degree in geology at Ohio State University. Edwards then "retired" to attend the University of Wisconsin, Madison, where she earned a Ph.D. in geomicrobiology—the first degree in this field ever awarded by the university. Edwards and her family moved to Massachusetts in 1999 to join WHOI, where she established a geomicrobiology lab. It focuses on "the tooth decay of the solid Earth," she says, or more specifically, the transformation and degradation of Earth materials (rocks, minerals, organic matter) by microbes. Edwards now enjoys deep-sea exploration, as long as someone else "flies" the submarine and she can focus on geomicrobiological research.