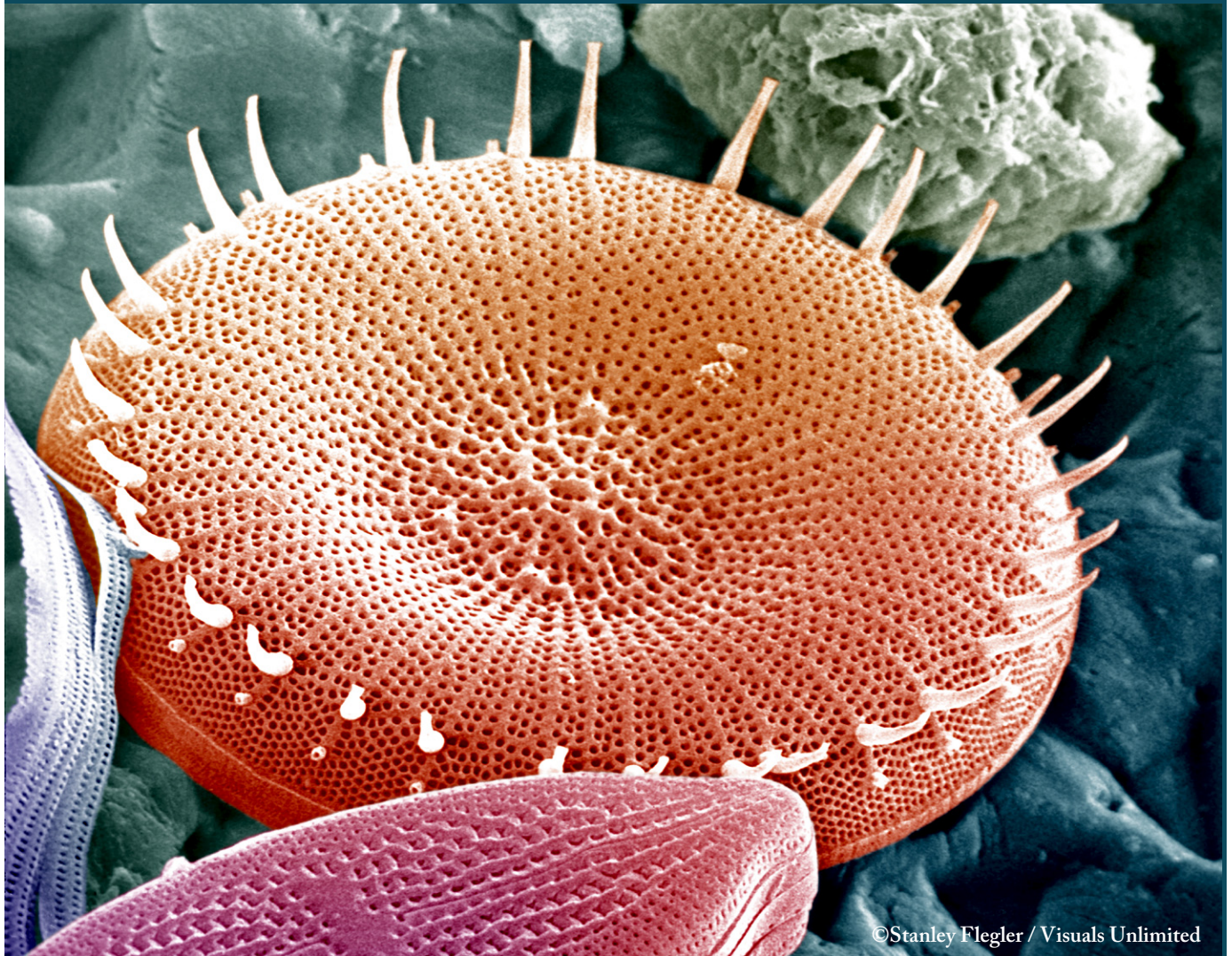


# The Microbial World: A Challenging Frontier



©Stanley Flegler / Visuals Unlimited

The physical diversity of microbes reflects a commensurate underlying genetic and functional diversity—yielding a broad range of biochemical capabilities that sustain the planet. Diatoms (pictured above) are photosynthetic microorganisms that play a role in global carbon cycling and sequestration. Famous for their wide variety of intricately shaped silica walls, these organisms are abundant in plankton and in marine and freshwater sediments, often being found in fossil deposits.

# THE MICROBIAL WORLD:

## A Vast and Genetically Rich Resource

**M**icrobes and their communities make up the foundation of the biosphere and sustain all life on earth. These single-celled organisms are masters at living in almost every environment and harvesting energy in almost any form, from solar radiation to photosynthesis-generated organic chemicals to minerals in the deep subsurface.

Microbes have evolved over 3.5 billion years, transforming the atmosphere with oxygen (a by-product of photosynthesis) more than a billion years ago to create the environment for life as we know it. Some microbes can thrive in either aerobic (with oxygen) or anaerobic (without oxygen) conditions. Microbes also capture nitrogen from the atmosphere, make it available to plants (and other life forms), and carry out processes responsible for soil fertility. Most do not cause disease. The unique microbial biochemistries amassed over eons in every niche on the planet now offer a deep and virtually limitless resource of capabilities that can be applied to national needs, including DOE energy and environmental missions.

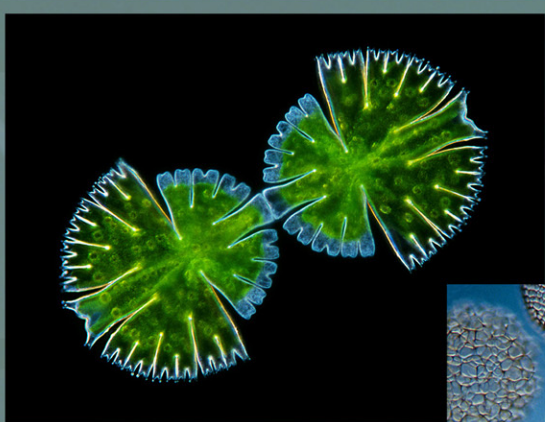
Although immense, the microbial world remains largely unexplored, a frontier of truly astronomical dimensions: The estimated nonillion or  $10^{30}$  individual bacteria on earth are  $10^9$  times more than the number of stars in the universe. The vast majority, however, cannot be studied using standard techniques. While 2000 to 3000 species are estimated to be present in a single gram of soil, we can cultivate for study only some 0.1 to 1% of the species in that or any other environment. About 5700 species have been described thus far.<sup>1-3</sup>

Investigators now are beginning to apply the tools of genomics to studying this enormous untapped natural treasure. Because microbes have modest-sized genomes (averaging 4 to 5 million bases compared with 3 billion bases in the human and other mammalian genomes), they represent a tractable life form we can use to explore and understand life processes at a whole-system level. Already, limited environmental sampling of microbes and their communities has led to the

discovery of millions of previously unknown genes and proteins, thousands of species, and innumerable variations in critical functionalities. As scientists begin to scratch the surface of the microbial world, they are finding analysis an enormous challenge.

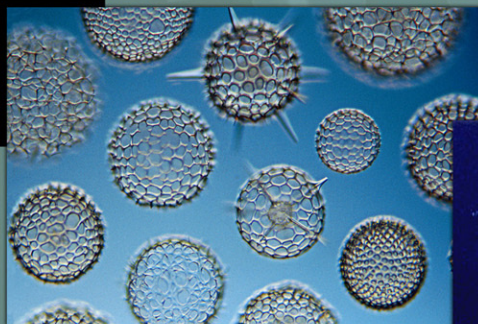
Recent discoveries from projects funded by DOE's Biological and Environmental Research program highlight the ubiquitous presence and critical importance of microbes in all ecosystems. For example:

- The cyanobacteria *Prochlorococcus* and *Synechococcus*, along with other ocean phytoplankton, account for about half of global photosynthesis.<sup>4</sup>



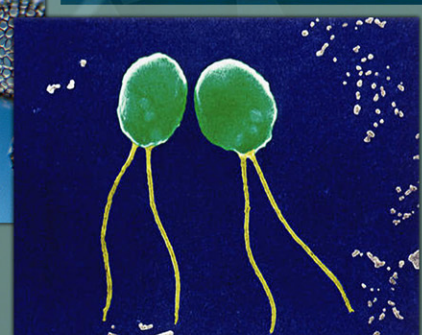
***Micrasterias rotata*, a Desmid Undergoing Cell Division or Cytokinesis.** Desmids are attractive unicellular freshwater green algae that have a distinct basic symmetry consisting of two semicells with the nucleus situated in the narrow center. When they divide, two new semicells are formed.

[© Wim van Egmond / Visuals Unlimited]



***Spumellarian radiolarian*, Skeletons from the Ocean Bottom.** Radiolarians are unicellular protists with strikingly beautiful siliceous skeletons showing radial symmetry.

[© Wim van Egmond / Visuals Unlimited]



***Chlamydomonas*, Green Algae with Two Flagella for Movement.** These microbes can generate hydrogen from light, water, and basic nutrients.

[Elias Greenbaum, Oak Ridge National Laboratory]

- Diatoms, ancient and intricately shaped ocean microbes, store an amount of carbon comparable to that in all the earth's rainforests combined. Over geological time, diatoms may have influenced the earth's climate.<sup>5</sup>

- More than a million previously undiscovered genes, possibly representing new biochemical functions, were the surprising find in sequencing DNA fragments from the Sargasso Sea—a region heretofore thought to sustain little life.<sup>6</sup> This discovery also was named one of *Science* magazine's "Breakthroughs of the Year."<sup>7</sup>

# A CHALLENGING FRONTIER

- Microbes thrive deep within the earth's subsurface and at extremes previously thought to extinguish life.<sup>8</sup>

Growing recognition of microbial capabilities and potential applications has made a compelling case for further investigations by DOE and other agencies and institutions.

Before we can harness their capabilities, microbes must be understood in far greater detail and in the realistic context of whole living systems—whether as individuals or communities of interacting microbes—rather than as isolated components such as single genes and proteins. Microbes already can be manipulated at the molecular, cellular, and system levels, but understanding and taking advantage of their complexities and surmounting the technical challenges of whole-systems biology is a daunting prospect.

## Understanding MICROBES and Their Communities

Most microbes live in highly organized and interactive communities that are versatile, complex, and difficult to analyze from many perspectives. Some of these challenges are outlined below.

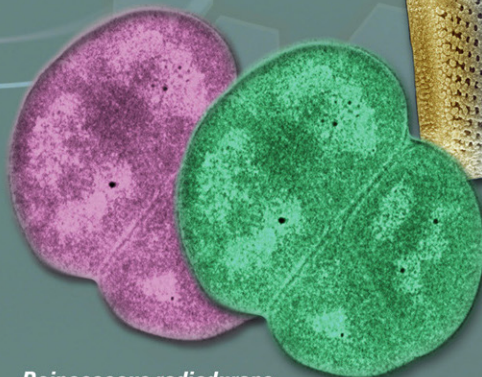
- Microbes are exceedingly small—only 1/8000th the volume of a human cell and spanning about 1/100th the diameter of a human hair. Investigating processes within this size range is challenging.
- The microbial world encompasses millions of genes from thousands of species, with hundreds of thousands of proteins and multimolecular machines operating in a web of hundreds of interacting processes in response to numerous physical and chemical environmental variables. Gene control is complex, with groups or “cassettes” of genes (operons) directing coordinated transcription and translation of genes into interacting proteins.
- Microbes adapt rapidly in response to environmental change, an ability that underlies their survival for billions of years. For example, various species of “extremophile” microbes have adapted to great extremes of pressure, temperature, pH, salinity, and radiation. Their high surface-to-volume ratio enhances interactions and supports adaptation. Unlike animal cells, they have no protective nucleus for their DNA, which leaves it more vulnerable to alteration. Genes move easily among species. Moreover, microbial communities are awash in genetic material from viruses that confer additional genetic properties and expand their range of adaptability.



**Rod-Shaped (Bacilli) and Spherical (Cocci) Bacteria Found in Compost.**

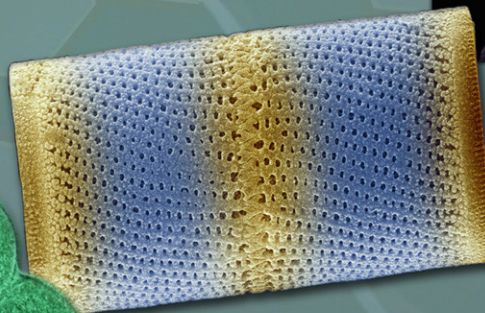
Decomposition of organic matter is an extremely important process in nature and a part of the global carbon and nutrient cycle.

[© Simko / Visuals Unlimited]



***Deinococcus radiodurans*, the Most Radiation-Resistant Microbe Known.**

[Michael Daly, Uniformed Services University of the Health Sciences]



**Diatom, a Unicellular Algae.**

The cell walls of diatoms are made of silica and come in a variety of shapes. These microscopic algae may be either fresh or saltwater, are photosynthetic, and play a role in carbon cycling.

[© Stanley Flegler / Visuals Unlimited]

- Microbial communities can extend in size from cubic millimeters (or smaller) to cubic kilometers. Even relatively simple communities can have millions of genes, giving them a genetic diversity substantially greater than that of higher life

forms, even humans. Recent investigations have focused on collecting DNA fragments from environmental samples in the sea and

other natural ecosystems. These “metagenomics” studies have given us a glimpse into the intricacies of these natural ecosystems and their diverse functions.

References noted on these pages are listed on the last page of this section.

## Microbes on the Move

### Chemotaxis: Sensing and Moving in a Chemical Gradient

Motile bacteria use sophisticated information-processing devices to detect and respond to changes in their chemical environments. *Escherichia coli* cells, for example, use a signaling cascade of protein phosphorylation and dephosphorylation reactions to control the stiff flagellar filaments responsible for cell motility. The filaments, made up of several individual molecular motors, rotate to propel the cell in favorable directions (called chemotaxis) or allow it to tumble randomly. Cells make motility decisions by comparing current conditions to those occurring previously. Chemical changes as minute as 1 part per 1000 can be detected.

The methylation state of transmembrane chemoreceptors (methyl-accepting chemotaxis proteins) encodes the memory of its chemical environment and controls the flux of phosphates through a signaling cascade. Two signals are produced: A feed-forward signal that alters the motor rotation and a feedback signal that updates the methylation record. Motor responses occur in a few hundred milliseconds, whereas sensory-adaptation machinery updating the methylation record takes several seconds. New in vivo experimental approaches are needed to better understand the functional anatomy of bacterial receptor clusters.

#### Reference

J. S. Parkinson, "Signal Amplification in Bacterial Chemotaxis Through Receptor Teamwork," *ASM News* (2004).

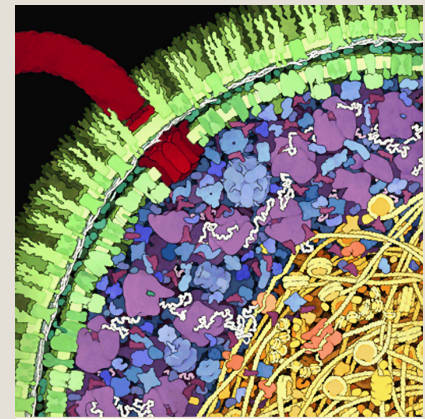


Illustration of *E. coli* Cell with Protruding Flagellum.

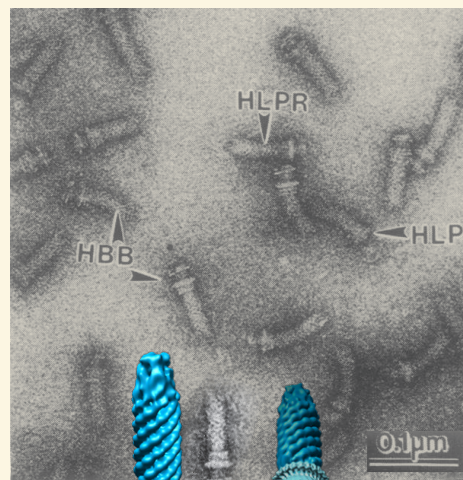
© 1999 D. Goodsell, Scripps Research Inst.

### Built for Motility: A Complex Molecular Motor

The bacterial flagellum consists of multiple copies of at least 13 different proteins. This multiprotein machine contains an axial structure running through the length and a set of ring structures within the basal body embedded in the cell envelope. At right is a conventional electron microscopic image of uranyl acetate-stained flagellar complexes and subcomplexes prepared from *Salmonella typhimurium*. The hook-basal body (HBB in photo) contains a hook and rings of proteins; HL-PR refers to the hook and L and P rings, while HLP refers to the complex containing hook, L and P rings, and distal portion of the rod.

#### Reference

G. E. Sosinsky et al., "Mass Determination and Estimation of Subunit Stoichiometry of the Bacterial Hook-Basal Body Flagellar Complex of *Salmonella typhimurium* by Scanning Transmission Electron Microscopy." *Proc. Natl. Acad. Sci. USA* 89(11), 4801-5 (1992).



Flagellar Complexes from *S. typhimurium*.

Inset: Surface Representation of a 3D Map of the Bacterial Hook-Basal Body Flagellar Complex of *S. typhimurium*.

Inset illustration: D. Thomas, N. Francis, and D. DeRosier, Brandeis Univ. Photomicrograph: C. Anderson, Brookhaven National Laboratory

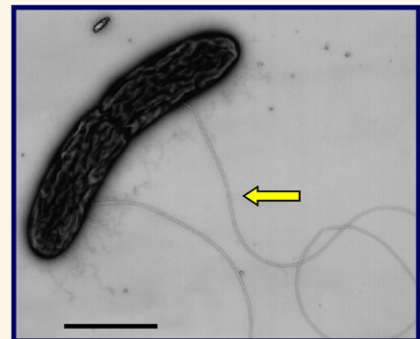
## Growing Flagella in a Pinch

Some microbes grow flagella only when they need to find their way to nutrients essential to metabolic processes. Such is the case with *Geobacter metallireducens*, which produces energy for biochemical reactions by transferring electrons to metals. Although originally thought to be nonmotile, *G. metallireducens* genome analysis turned up genes encoding flagella. Further investigations showed that this microbe produces flagella when faced with insoluble sources of iron or manganese [Fe(III) or Mn(IV)]. Genes for pili (fine hair-like structures on the microbe's surface) also are present and expressed during growth on insoluble oxides; studies indicate their role as facilitating movement toward and aiding attachment to iron oxides (see sidebar, Bacteria Use "Nanowires" to Facilitate Extracellular Electron Transfer, p. 73). Additional genes for chemotaxis also were apparent in the genome, leading to the discovery of a novel mechanism for chemotaxis to iron. Understanding *Geobacter's* physiology is important for optimizing strategies to use this organism to bioremediate metals such as uranium in contaminated subsurface environments. Global gene-expression studies are helping to identify regulatory circuits, specifically those involved in bioremediation pathways and electricity production. [Source: Derek Lovley, University of Massachusetts, Amherst]

### References

S. E. Childers, S. Ciuffo, and D. R. Lovley, "Geobacter metallireducens Accesses Insoluble Fe (III) Oxide by Chemotaxis," *Nature* 416, 767–69 (2002).

D. R. Lovley, "Cleaning Up with Genomics: Applying Molecular Biology to Bioremediation," *Natl. Rev. Microbiol.* 1, 35–44 (2003).



Flagella (arrow) Produced by *Geobacter* in the Presence of Insoluble Sources of Iron or Manganese.

D. Lovley, University of Massachusetts, Amherst

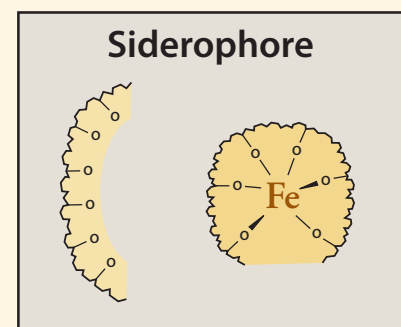
## Siderophores: Sending out Shuttles to Scout for Iron

Another efficient mechanism evolved by many microbes to obtain iron in limited environments such as marine surfaces is the production and secretion of siderophores. These low-molecular-weight chelating agents act as shuttles to bind insoluble iron [Fe(III)] and transport it back to the microbe, where it enters the cell by recognizing specific membrane receptor proteins and transport systems. More than 500 types of siderophores are known to exist. In addition to supplying essential nutrients, siderophores of one organism can lock up iron to achieve an advantage over their competitors. However, some microbes were discovered recently to have receptors for the siderophores of other organisms.

Until the genetic sequence of the ocean diatom *T. pseudonana* was determined in the DOE Microbial Genome Program and compared with sequences of other organisms, researchers were unaware that these organisms possessed siderophores. Diatoms, along with other ocean microbes, contribute to absorbing CO<sub>2</sub> in amounts comparable to that absorbed by all the world's tropical rain forests combined. Obtaining more detailed knowledge of their life processes will help us better understand their vital role in global carbon cycling.

### Reference

E. V. Armbrust et al., "The Genome of the Diatom *Thalassiosira pseudonana*: Ecology, Evolution, and Metabolism," *Science* 306, 79–86 (2004).



Schematic Representation of a Siderophore Before and After Iron Acquisition. [Adapted from H. Boukhalfa and A. L. Crumbliss, "Chemical Aspects of Siderophore-Mediated Iron Transport," *Biometals* 15, 25–39 (2002).]

# THE MICROBIAL WORLD

## Group Living and Communicating

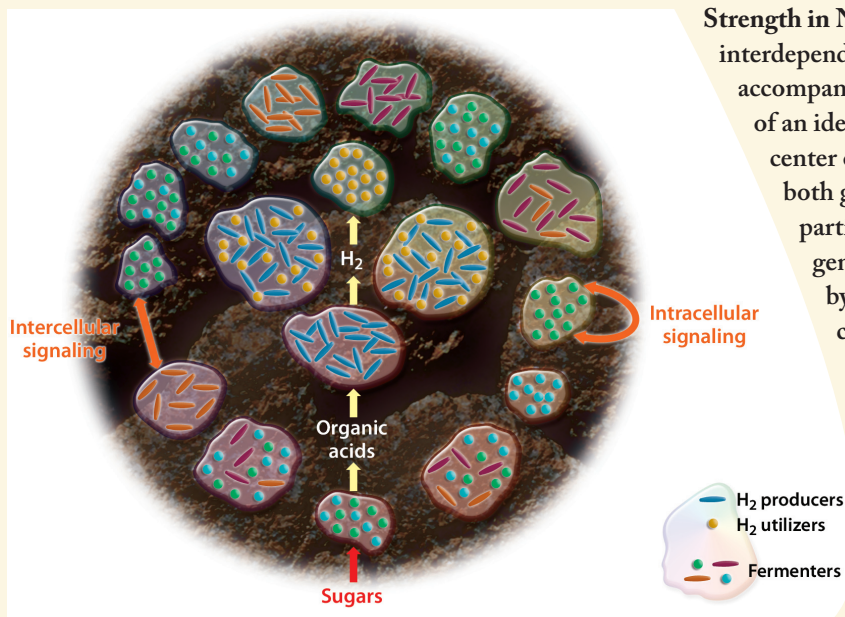
### Life in a Biofilm

Most microbes live attached to solid surfaces (biotic or abiotic) within highly organized and functionally interactive communities called biofilms. These biofilms can be composed of populations that developed from a single species or a community derived from multiple species. All exhibit collective and interdependent behavior, with different genes rapidly brought into play as conditions dictate (see figure below). Among the many advantages of biofilm living are nutrient availability with metabolic cooperation, acquisition of new genetic traits, and protection from the environment.

Researchers are only beginning to realize the prevalence and significance of biofilms. These communities probably play major roles in such complex natural processes as the cycling of nitrogen and sulfur and the degradation of environmental pollutants and organic matter, activities that require a range of metabolic capabilities. Recent metagenomic and metaproteomic studies focused on biofilm members in an acid mine drainage environment (see sidebar, Metagenomics, p. 62).

### Reference

M. E. Davey and G. A. O'Toole, "Microbial Biofilms: From Ecology to Molecular Genetics," *Microbiol. Mol. Biol.* 64(4), 847–67 (2000).



**Strength in Numbers.** Microbes in biofilms live an interdependent, community-based existence (see accompanying text above). In this overhead view of an idealized biofilm, four microcolonies in the center of the figure represent organisms that both generate and consume hydrogen. Two participate in syntrophism, in which hydrogen producers use organic acids generated by fermenting organisms that gain their carbon and energy by using various sugars. In addition to potential metabolic interactions, signaling molecules may aid in inter- and intraspecies communication. These genetic factors and environmental influences contribute to the biofilms' spatial organization. [Figure and caption adapted from Davey and O'Toole, 2000.]

## Quorum Sensing

Microbes communicate with each other by sending and detecting a wide variety of chemical signals (autoinducers). These molecules trigger group behaviors, including the formation and persistence of biofilms, symbiosis, and other processes. Many of these activities are density dependent, that is, when a threshold concentration of chemicals is detected (reflecting a certain number of cells), microbes respond with a change in gene expression. This process, called quorum sensing, facilitates coordination of gene expression by the entire community, in essence enabling it to behave like a multicellular organism. Quorum sensing allows microbial communities to adapt rapidly to environmental changes and reap benefits that would be unattainable as individuals.

Quorum sensing first was described in the bioluminescent marine bacterium *Vibrio fischeri*. This microbe lives in symbiotic association with several marine animal hosts, who use the light it produces to attract prey, avoid predators, or find a mate. In exchange, *V. fischeri* obtains a nutrient-rich home environment. *V. fischeri* emits light only inside a specialized light organ of the host, where the concentration of these organisms becomes dense; it does not give off light when free living in the ocean. Light production depends on producing, accumulating, and responding to a minimum-threshold concentration of an autoinducer (acylated homoserine lactone). Only under the nutrient-rich conditions of the light organ can *V. fischeri* grow to such high populations. Also, trapping the diffusible autoinducer molecule in the light organ with the bacterial cells allows it to accumulate to a concentration sufficient for *V. fischeri* to detect it.

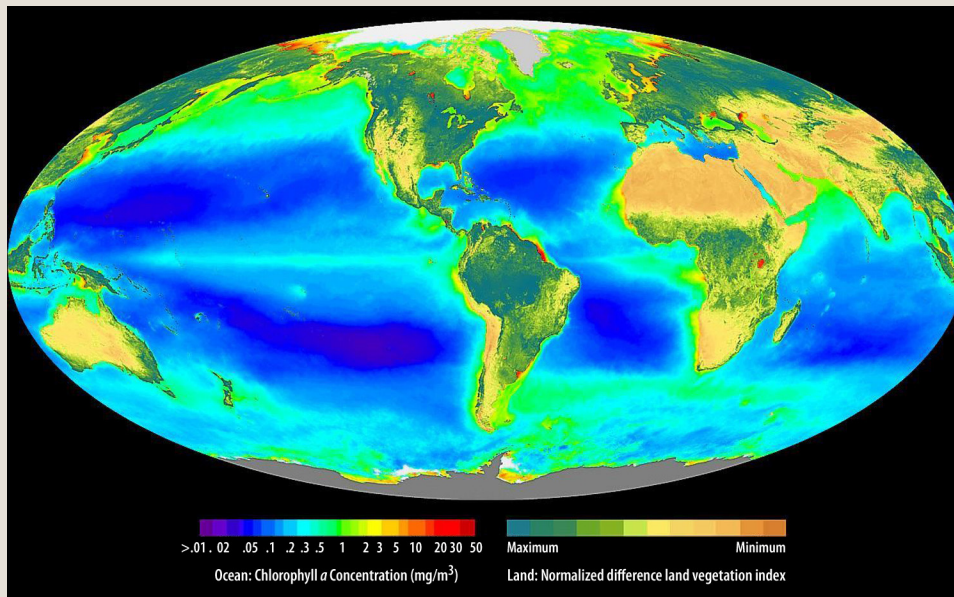
Recent studies have revealed diverse chemical languages that enable bacterial communication both within and between species (the latter called cross talk). The extracellular matrix surrounding mature biofilms (composed of glycans and other components) plays a crucial role in transmitting these chemical signals into and between cells. Biotechnological researchers are developing molecules structurally related to autoinducers to exploit quorum-sensing capabilities and possibly improve industrial production of natural products.

### References

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 S. Schauder and B. L. Bassler, "The Language of Bacteria," *Genes Devel.* 15, 1468–80 (2001).

## Photosynthetic Microbes—Major Contributors to Earth’s Life-Support System

When photosynthetic microbes, fungi, and plants convert light energy from the sun into glucose, they establish the foundation for the food chain on which all life, including human, depends. This NASA SeaWiFS image of the global biosphere shows the density of photosynthetic organisms on land and in the oceans. On land, the dark greens represent areas of abundant vegetation, with tans showing relatively sparse plant cover. In the oceans, red, yellow, and green pixels depict dense blooms of phytoplankton (photosynthetic microbes), while blues and purples show regions of lower productivity.



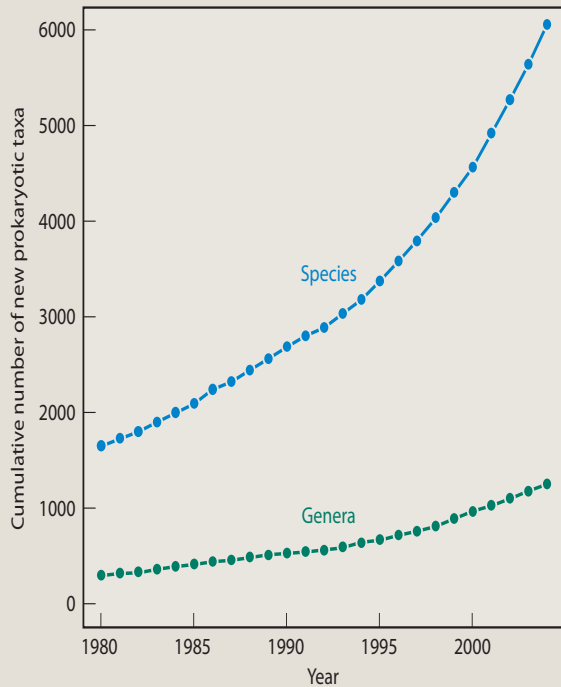
blooms of phytoplankton (photosynthetic microbes), while blues and purples show regions of lower productivity.

Ocean’s Long-Term Average Phytoplankton Chlorophyll Concentration. Image combines September 1997 through August 2000 concentration data with the SeaWiFS-derived Normalized Difference Vegetation Index over land; <http://oceancolor.gsfc.nasa.gov/SeaWiFS/>.

# THE MICROBIAL WORLD

## Cataloging Microbial Diversity

### What's in a Name? The Challenges of Tracking Microbial Species



Prokaryotic systematics is a dynamic field. The rate at which new species, genera, and higher taxa are described in the literature has increased dramatically since the 1990s (see figure at left), driven largely by advances in sequencing technology. Even with all this progress, scientists believe that 99% of the microbial world has yet to be discovered.

Just how many species (and genera) of *Bacteria* and *Archaea* are listed in *Bergey's Manual*, a widely used international reference for taxonomy? It seems a simple question, but, at present, the number of named species actually exceeds the true number of species having official standing in the nomenclatural record by about 22%. An explanation follows.

Many taxa bear two or more names, because when species or higher taxa are reassigned to existing or newly created taxa, both the new name and the old name are valid (in the original published context). Other types of nomenclatural synonyms exist as well. As a result, about 6900 species are listed in *Bergey's*, with information and data published in accordance with *The International Code of Bacterial Nomenclature*. The true number of named prokaryotes, however, is closer to 5700. [Source: George Garrity, *Bergey's Manual*]

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2. V. Torsvik and L. Øvreås, "Microbial Diversity and Function in Soil: From Genes to Ecosystems," *Curr. Opin. Microbiol.* 5(3), 240–45 (2002).
3. *Bergey's Manual*, 2<sup>nd</sup> edition (2001).
4. T. S. Bibby et al., "Low-Light-Adapted *Prochlorococcus* Species Possess Specific Antennae for each Photosystem," *Nature* 424, 1051–4 (2003).
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