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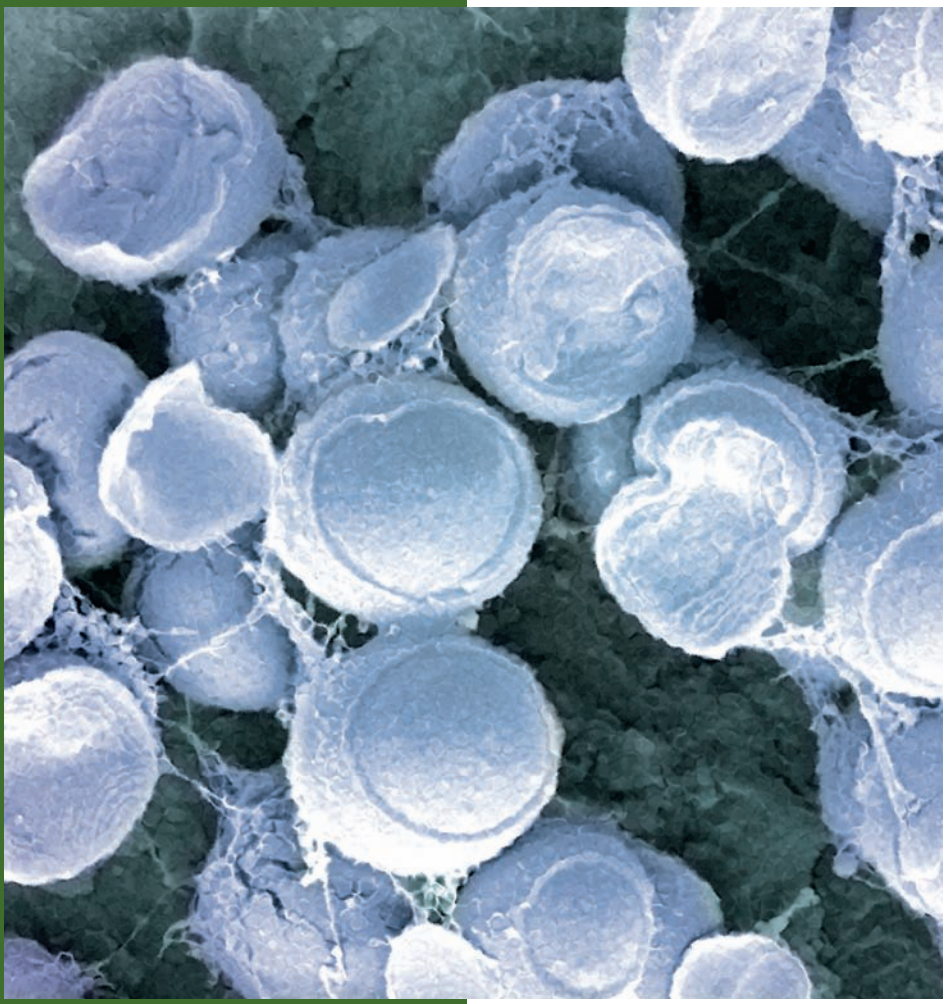
UNSEEN DIVERSITY:

THE WORLD OF BACTERIA

COURSE GUIDE



Professor Betsey Dexter Dyer
WHEATON COLLEGE

Unseen Diversity:
The World of Bacteria

Professor Betsey Dexter Dyer
Wheaton College

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Course Syllabus

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Photo courtesy of Betsey Dexter Dyer

About Your Professor

Betsey Dexter Dyer

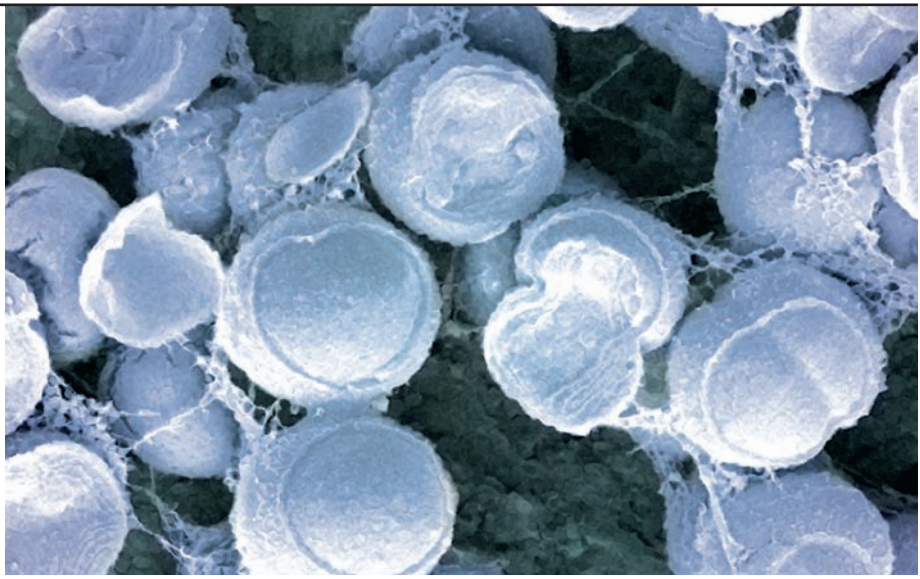
Betsey Dexter Dyer is a biology professor at Wheaton College in Norton, Massachusetts, where her courses include bacteriology, genetics, parasitology, and invertebrate evolution. She earned her Ph.D. in biology at Boston University in 1984. Dyer's research interests include DNA sequence analysis, cell evolution, symbiosis, and field microbiology. Dyer considers herself to be a curious naturalist and a generalist, with lots more to learn. She has written three books: *Perl for Exploring DNA* (with coauthor Mark LeBlanc, Oxford University Press, 2007), *A Field Guide to Bacteria* (Cornell University Press, 2003), and *Tracing the History of Eukaryotic Cells* (with coauthor Robert Obar, Columbia University Press, 1994).

In 1980, while still a graduate student, Dyer had the privilege of taking an intensive summer course in field microbiology taught by some of the world's experts. On one of the field trips, she was amazed at the confidence with which the various instructors were making field identifications and placing bacteria into environmental context. That was the origin of the idea of a field guide to bacteria; it is simply a compilation of the observation, procedures, and often unwritten advice from field microbiologists for seeking out bacteria in the field. Dyer's goal was to empower fellow naturalists to understand bacterial diversity in the context of nature studies.

Dyer grew up on a family farm in Rehoboth, Massachusetts, which was a great influence on her development as a biologist and naturalist. She lives in Walpole, Massachusetts, with husband Robert Obar, a protein chemist, two children, Alice and Sam, and a Brittany, Genevieve. She loves reading, writing, cooking, and dancing.

You will receive the greatest benefit from this course if you have the following text:

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Ithaca, NY: Cornell University Press, 2003.



An electron microscope image of cyanobacteria

Photosynthetic cyanobacteria are less than the size of a pollen grain. The microbes divide several times a day under optimal conditions.

Introduction

Bacteria are the most overlooked organisms on your nature walk. You see birds, trees, and wild flowers. You may even examine fungi, rock formations, mosses, lichens, nests, tracks, and insects. However, it is likely that you are not seeing bacteria even though you may know they are there in countless numbers, far outnumbering the other organisms, and that their influence on the environment is vast and profound.

The goal of this lecture series is to place the bacteria (and the closely associated archaea) firmly into their place as major players in Earth's biodiversity. The lectures include two on the history of microbiology, describing the most important early discoveries of bacteria and their activities. Also included is the four billion year history of bacteria and archaea as the dominant organisms on Earth. Finally, information on field identifications is provided in hopes that your nature walks will be enriched with new sightings of bacteria; in some cases their field marks were there, in plain sight, all along, but just in need of deciphering.

Pathogens, too, will be placed in the greater context of the bacterial world and there may be some surprises. While pathogenic bacteria are more likely than most bacteria and archaea to be featured in news stories, they are far from the majority. Indeed, their very rarity may hold some clues about their activities.

As for your own background, you need not be a scientist, just a curious naturalist like me. If you like to use field guides to enhance your nature walks, think of this as a sort of field guide experience and a friendly introduction to a world full of bacteria and archaea.

Lecture 1:
Introduction to the Bacterial World

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* introduction.

Bacteria are the unseen majority, comprising most of the biodiversity on Earth. However, observations about the diversity of organisms typically begin (and often end) with those of about our own size, morphology, and activity level. We humans, being primarily visual creatures, tend to appreciate organisms that are visually interesting with memorable, identifiable shapes and colors, and interpretable behaviors. There is already so much to look at on our own scale and within our own personal experiences with nature. Birds, flowering plants, mammals, trees, and insects dominate our landscapes and we even take the time to attract or cultivate them, as with birdfeeders and gardens. For those who collect and use field guides, it is most likely that these same accessible organisms dominate the bookshelf. The microbial world may seem to be an optional dimension with which one might (or might not) enrich an understanding of biodiversity. With so much of macroscopic biodiversity to see and appreciate and even protect, the picture of biodiversity may seem complete enough without seriously considering microbes.

A major goal of this course is that you might see microbial diversity as not simply one of many features of life on Earth, but as a predominant feature, well worth incorporating into a global view of diversity and indeed essential for a complete understanding. It is assumed that you already have an appreciation for the natural world and that throughout this course you might be inspired to add a bacterial dimension to your preexisting observations of nature.

Note that even a professional point of view on biodiversity may inadvertently leave out most microbes. If you have a college textbook for ecology available, try looking up certain keywords for microbes such as "bacteria" and "microorganisms." You will find only a few partial pages, mostly devoted to acknowledging a bacterial role in "nitrogen fixation" and "methane production." Under the topic of decomposition, bacteria may be discussed briefly in their role of recyclers of wastes, along with other "saprophytes" such as fungi. Other than

those mentions, an ecology textbook may give the appearance that the ecological world is dominated by the many interactions of animals and plants.

Meanwhile, there is one venue where bacteria are not at all neglected but are consistently represented as the primary villains in news stories or corporate press releases on the dangers of bacterial infections. It is a goal of this course to place such stories into the much greater context of the bacterial world. There may be millions of species of bacteria. (How to count the number of species is still a point of contention, to be discussed in Lecture 5.) There are only about fifty pathogens of humans and of those fifty, only a few make their primary living with pathogenic activities. Lecture 10 is about bacterial pathogens and how they might have evolved into the rare niches of pathogenicity. Research on understanding and controlling pathogens increasingly is grounded in an appreciation for the majority of bacteria that are benign (from a human point of view) or even in some cases essential to our well-being.

The goals and aspirations for this course are

- to add a bacterial dimension to nature studies and to the appreciation of biodiversity.
- to show-off some of the astounding versatility and diversity of the bacterial world, with a focus on accessible examples that naturalists may be able to observe themselves.
- to place bacteria into the context of other microorganisms, especially soil fungi, which dwell side by side with soil bacteria and which occupy similar niches.
- to place the few pathogenic bacteria into the greater context of bacterial diversity.

Three-dimensional rendered image of bacteria.

Broad Generalizations About Bacteria

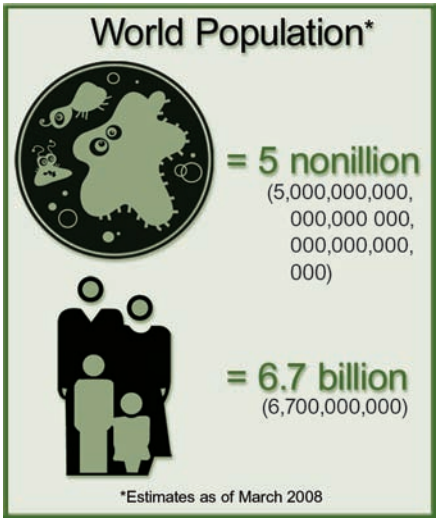
Many sweeping generalizations will be made in this lecture about bacteria, including some that may seem so obvious that they need not be said. However, sometimes it is the most obvious that gets overlooked. The bacterial world is a cryptic one (at least from our point of view as large organisms) and in need of deciphering if we are to appreciate it. Bacteria are not just tinier, simpler, single-celled versions of bigger organisms. Being tiny has its own deep meanings and implications. A goal of this lecture is to recognize just how different bacteria are from ourselves and how we might use that to get a better understanding of them. Consider, even, the mental exercise of putting yourself (hypothetically, of course) in their place, empathizing perhaps or even thinking like a bacterium.

Five Generalizations About Bacteria

First Generalization

Bacteria are the most numerous of all organisms on Earth. How numerous? Microbiologists at the University of Georgia calculated 5×10^{30}, a number so large that it is difficult to imagine. We tend to use numbers like "million" and "billion" to indicate lots of something. A billion has nine zeros in it: 1,000,000,000. In contrast, the calculated number of bacteria has thirty zeros (5,000,000,000,000,000,000,000,000,000,000). How did microbiologists come up with this fantastic number? They did hundreds of calculations, a few of which went something like this:

1. Count all the bacteria in a cubic centimeter of ocean water at the surface. Now take that number and multiply it by the total surface area of all oceans.
2. Count all of the bacteria in a cubic centimeter of soil. Now multiply times the volume of all of the soil in the world. And so on and so on until they accounted for every imaginable surface (including the surfaces of all animals, plants, fungi, and protists[1]) and every cubic centimeter of available space.



Second Generalization

Bacteria are the most diverse of all organisms on Earth. We know this because when the family (phylogenetic) tree of all organisms is constructed

1. *Protists* are any of a group of eukaryotic organisms belonging to the kingdom Protista according to some widely used modern taxonomic systems. The protists include a variety of unicellular, coenocytic, colonial, and multicellular organisms, such as the protozoans, slime molds, brown algae, and red algae.

based on the differences in DNA sequences, bacteria and archaea (which together comprise all of the simple, single-celled organisms) form the majority of main branches of the tree. Complex, mostly multicellular organisms such as ourselves and other animals plus plants and fungi, occupy a small section of the tree. Bacteria and archaea have been evolving and diverging for about four billion years and apparently have done so with great abandon, resulting in a wide diversity of activities and by implication a wide diversity of DNA sequences. Meanwhile, multicellular organisms have been evolving for only about 750 to 500 million years and apparently with less variability in their genomes. Thus their (our) occupation of just one branch from the much larger tree.

Third Generalization

Bacteria and archaea are the most ancient of all organisms, already mentioned in the second generalization, but reiterated here in a different context. For most of the history of life on Earth, it has been a microbial world. Four billion years ago life originated. It was microbial. Two-and-a-half billion years ago, complex cells evolved, but were still single cells, that is, microbial. Around one billion years ago, small fungi, plants, and animals (or perhaps their precursors) most likely began to evolve, although definitive fossil evidence does not occur until 750 to 500 million years ago. Even then those first animals, plants, and fungi were small. Our own enormous size (along with that of other large animals and of plants) is anomalous. It always has been a microbial world and by evidence of numbers and diversity, it still is.

A Digression

Why the quibbling about bacteria and archaea? What are archaea? For the purpose of this lecture series, which is meant to be an introduction to bacteria, both bacteria and their relatives the archaea typically will be considered under the single heading of “bacteria.” However, archaea will be given their own lecture (Lecture 7: Extremophiles) as well as parts of others and I will take care to point them out by the name archaea. This is because they are a large and separate group unto themselves, occupying a considerable portion of the tree of life, by virtue of their different DNA sequences and sometimes very different activities. However archaea have many other similar functions and nearly identical morphologies to bacteria and have traditionally been encompassed into courses on bacteria, as they will be in this one.

Fourth Generalization

Bacteria are featureless, disappointing even. If you have ever seen them under the microscope, you know that they are just tiny dots and dashes. I could depict them thusly [· ‧ ~ -], using some of the punctuation keys of my keyboard. Clearly, all that diversity and variability of DNA is not going into morphology. That’s because bacteria are all about what they do and not so much about what they look like. For organisms as morphologically definitive as ourselves and as appreciative of morphologies we can visualize, bacteria are really in a different world. That world is not smoothly continuous with our own, but rather is at the other end of a broken continuum. Organisms do not get smaller and smaller, yet still retain a certain repertoire of miniature morphologies from their larger relatives. This would be something like doll house

furniture that can be tinier and tinier but still retain the recognizable feature of dining room chairs. Rather, there is a dichotomy of multicellular versus single celled and many distinctive differences between the lifestyles of the two. We large multicellular, terrestrial creatures must work hard to imagine the bacterial world.

Fifth Generalization

Bacteria are tiny! Why bother saying it? Well, because it has so many implications for the bacterial lifestyle. It is something that we (enormous beings) need to understand if we are to understand bacterial ways. Being tiny means being intimate with every nuance of the environment and highly responsive to even slight changes in pH, temperature, ions, and water. In contrast we have layers and layers of cells between most of ourselves and the outside world. What we do perceive is mostly through the specific portals of sensory neurons. The bacterial environment consists of many physical parameters, some of which are caused by constant inputs and outputs of the bacterial cell itself. It also consists of other bacteria and their inputs and outputs. Bacteria are great communicators, even across species boundaries. They are constantly sampling and adjusting to their environments. Alternatively, they may be in dormancy (for example, if desiccated), sometimes almost indefinitely, reestablishing activity only in response to some significant change such as an influx of water. Being tiny also often means being able to get through reproduction quickly. A bacterial generation may be only an hour if conditions are favorable.

Anthropocentrism is a challenge in any area of nature studies. It is almost unavoidable, because we have nothing but our own peculiar human senses with which to experience the world. One way for naturalists to get around this a little is to try not to allow the human state and point of view to take too central a role. For example, rather than thinking of organisms as being larger or smaller relative to our own size, it might be more helpful and more accurate to think of humans as anomalously large. That is, we are not at some median between dinosaurs and sequoias at one end and microbes at the other. Being a tiny bacterium is the normal state. We are outliers! The lack of distinctive morphologies in bacteria (in contrast to our own baroque morphologies) belies an underlying complexity and diversity of activities. Mere “looks” are a truly superficial trait in bacteria. A goal for the microbially oriented naturalist might be “bacteriocentricity” as a means of approaching the microbial world and catching a glimpse of the way things are.

FOR GREATER UNDERSTANDING

Questions

1. Why will it be worthwhile to incorporate microbial diversity into a global view of diversity?
2. Why might microbes be so frequently left out of discussions on ecology?
3. How has our world always been a microbial one?
4. In terms of morphology, how are bacteria in a different world than humans?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Introduction. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Dixon, Bernard. *Magnificent Microbes*. New York: Harcourt, Brace & Co., 1976.

———. Power *Unseen: How Microbes Rule the World*. New York: W.H. Freeman, 1994.

Postgate, John. *Microbes and Man.* 3rd ed. Cambridge: Cambridge University Press, 2003.

———. *The Outer Reaches of Life.* Cambridge: Cambridge University Press, 1994.

Article of Interest

Whitman, William B., David C. Coleman, and William J. Wiebe. “Prokaryotes: The Unseen Majority.” *The Proceedings of the National Academy of Sciences*, vol. 95, pp. 6578–6583, 1998.

Lecture 2:
Hidden in Plain Sight

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* introduction.

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The blue bands visible on the surface of a Finnish lake are from the blue pigment phycocyanin, which is leaching from decomposing cyanobacteria cells.

The field marks of bacteria often are overlooked by amateur naturalists and professional biologists alike. Like an encoded message, the evidence for bacteria is "hidden in plain sight," requiring decoding or interpretation. However, for nearly every bacteria or archaea there is a community of specialists who if asked could lead you directly to the very best site and most compelling field marks of their particular favorite organism. For example, an expert on sulfate-reducing bacteria would say, let's go to a salt marsh and sniff the air and look at the color of the sediments. If we smell reduced sulfate, in the form of gaseous (and odiferous) hydrogen sulfide, and if we see reduced sulfate in the form of black iron sulfide in the sediments, then we are in the habitat of and in the presence of sulfate-reducing bacteria. Take all of that expertise in particular microbes and the favorite rules of thumb for detecting diverse bacteria and archaea and you have the beginnings of a field guide by which anyone (even an amateur) might begin to notice the field marks everywhere.

Some might argue that although you might smell hydrogen sulfide and see iron sulfide and thereby extrapolate the presence of the bacteria, you do not actually have the bacteria in hand (or more likely in culture) and therefore have not really made any proper identification. So let me call in the methods of ornithologists who (thanks to Roger Tory Peterson) essentially pioneered the idea of a field guide and field marks and field identification of birds, making birds accessible to amateurs at all levels. Note that ornithologists and their amateur counterparts rarely have the bird in hand either (unless deliberately capturing in mist nets for banding). Rather they have the concept of "JIZZ," borrowed from a military acronym from World War II: "General

Impression of Shape and Size," which at first was "GISS" and then morphed into a more pronounceable (and inside joke) acronym "JIZZ." The term was used by plane-spotters to describe the overall impression of an incoming plane seen and heard only faintly in the distance and yet identifiable by a particular combination of colors, sound, and shape. Ornithologists use JIZZ all the time. They name birds by sounds, by markings glimpsed sometimes only briefly, by silhouette, by patterns of flight, by behaviors and almost always at a distance, almost never by closely examining a bird in hand. Just as important, although sometimes not acknowledged per se, is location, which is an integral part of field identification. An ornithologist spotting a flash of pink in a New England hedgerow does not need to sort mentally through an entire list of all pink birds in the world. The fact that we are in New England at the edge of a hedgerow is a major identifier and helps to narrow the choice down to "male House Finch."

Returning to bacteria and our ability to spot them in the field, there are some locations more abundant in bacterial field marks and you should not hesitate to use location as a primary aspect of field identification. Extreme environments (from the point of view of large animals like ourselves) are good choices. Therefore, marine mud flats and estuaries, hot springs, deserts, deep anoxic sediments, and salterns are all good choices. There will be fewer animals and plants obscuring the view, as most do not tolerate extremes of heat, dryness, salt, and lack of oxygen. The slimes, scums, flocs, bubbles, and crusts that are often visible in such environments often are interpretable field marks of bacteria. These will be described in greater detail in later lectures. Even in your own backyard or neighborhood, microenvironments may be interpreted as extreme (for example, the warm, fermenting microcosm of organisms that comprise your compost heap or the black, murky depths of a goldfish pond). Furthermore, every animal and plant may be interpreted to owe its existence directly or indirectly to the activities of microbes, especially those associated with digestive systems and roots. For example, the rotund, fermentation vat-like shapes of bovine animals advertise (field-mark like) the teeming myriads of microbes that dwell within. Other examples will be discussed in future lectures.

A final suggestion for embarking on a project to identify bacteria in the field is to use all of your senses. We humans are primarily visual animals, but bacteria are not always identifiable by merely looking. Olfaction is the universal sense and for most microbes it is the primary sense, essential for the intimate interactions with the chemicals of the environment. So sniff the air and taste sometimes (for example, at a deli that makes fermented foods the old-fashioned way with bacteria and fungi). Go ahead and touch the scums and crusts, perhaps after listening to Lecture 10 on pathogens in which my goal is to assure you that pathogens are an exception in the microbial world and that you are rarely in danger if you soil your hands. You may even listen for the subtle effervescence of bubbles or startling eructions of gasses released by metabolizing bacteria.

FOR GREATER UNDERSTANDING

Questions

1. From where did the term "JIZZ" originate and why is it relevant to identifying bacteria in the field?
2. Why are extreme environments conducive to finding field marks of bacteria?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Introduction. Ithaca, NY: Cornell University Press, 2003.

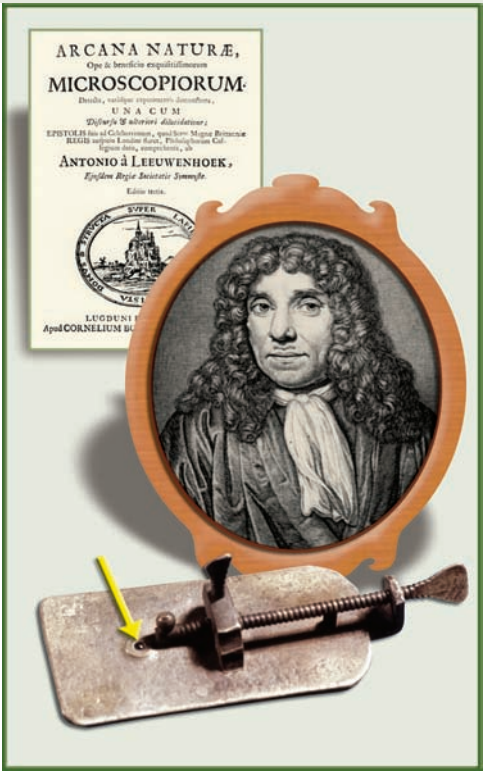
Lecture 3:
Seventeenth-Century Microscopy and the Discovery of Bacteria

The Suggested Readings for this lecture are Clifford Dobell's *Anthony van Leeuwenhoek and His "Little Animals"* and Brian Ford's *The Leeuwenhoek Legacy*.

It is not known which seventeenth-century European invented the microscope. Lenses were being made and used throughout the seventeenth century as aids for poor eyesight, as the means for seeing great distances (including the moons of Jupiter) and for magnifying tiny objects.

Anton van Leeuwenhoek

Anton van Leeuwenhoek of Delft, Holland, used lenses in his business as a draper (a dealer in linens) to examine the quality of weave in his fabrics. At some point unrecorded, van Leeuwenhoek began making hundreds of little microscopes and applied them to hundreds of different specimens. The method at the time for this sort of microscope was to build a dedicated one for each permanent specimen (for example, a bit of fish muscle or a slice of a tooth). While van Leeuwenhoek did not invent the microscope, he was the most extensive user and innovator of the microscope in his century and well beyond into the nineteenth century. His extraordinary achievements included the first observations of bacteria as well as of protists (larger single-celled organisms) and details of other cells. Everything that you might expect to see under the microscope in an introductory biology class was seen and accurately described and depicted by van Leeuwenhoek. In addition, quite a few things that you are not likely to see in introductory biology were also observed by the intrepid van Leeuwenhoek, including tiny explosions of chemicals that burned his eyebrows. What about Robert Hooke, the great English naturalist and polymath, author of *Micrographia*, early and influential member of the first scientific society, and coiner of the word "cell"? What Hooke did not



Antonie Philips van Leeuwenhoek (1632–1723)

A portrait drawing of van Leeuwenhoek with the frontispiece of his book on microscopy and one of his microscopes. The tiny glass bead lens is indicated by a yellow arrow.

do as well as van Leeuwenhoek was to observe the truly microscopic world, well below the range of a strong magnifying glass. Van Leeuwenhoek saw bacteria, an astonishing accomplishment. The microscopic images most associated with Hooke are of an enormous exquisitely detailed flea and of a pair of fly eyes filling the page, images that may be produced using a very good magnifying glass. The difference is in the type of microscope. Hooke's was a sort of telescope-like design used in reverse. Look through a telescope (or binoculars) backwards to get a glimpse of what seems likely to have been the seminal idea for Hooke's style of microscope, which looks very much like a modern microscope. However, in the seventeenth century, lens-grinding techniques could not produce convex lenses for Hooke's microscope sufficient to see bacteria. Van Leeuwenhoek used a different lensmaking technique based on glass blowing to produce tiny spherical glass beads. Looking through such a bead gives the effect of looking through two tiny lenses, one concave, the other convex, and the effect (if the bead is nearly perfectly spherical) is excellent magnification. By the way, Hooke was quite aware of van Leeuwenhoek's work because it was published in the form of letters to the first and only scientific journal of the time, *The Philosophical Transactions of the Royal Society of London*, of which Hooke was a prominent member.

So what did Anton van Leeuwenhoek see of the bacterial world with his tiny (but nearly perfectly spherical) glass bead lenses? He made thousands of detailed observations on microbes, many of which were bacteria. In many cases, for example, throughout letter #18 to the Royal Society, he remarked upon how small some of the "little animals" were: one million were equal in bulk to a grain of sand. In letter #19, he estimates that the "little animacules" are "twenty-five times smaller than one of those blood globules that make the blood red." Blood cells are about seven micrometers in diameter, suggesting that van Leeuwenhoek was definitely observing in the range of bacterial sizes, about one micrometer. Van Leeuwenhoek even tried to estimate the sizes of his tiniest animacules in feet, rods, and miles.

Robert Hooke tried to repeat some of van Leeuwenhoek's observations and himself saw little animals, but it is not clear from Hooke's description that he was seeing something as small as bacteria; he was most likely observing protists. Van Leeuwenhoek used not only exceptional glass bead lenses but also a lighting system that he considered proprietary (to the dismay of the Royal Society) and therefore it may have been difficult for anyone to exactly match van Leeuwenhoek's observations.

In letter #39, van Leeuwenhoek described bacteria obtained by removing debris from between his teeth with a toothpick. The engravings along with that letter and the descriptions are unmistakably of bacteria. "I then most always saw, with great wonder, that in the said matter were many very little animacules very prettily a-moving." He went on to explore the teeth of other people, filling dozens of pages with descriptions. He took a sample from one man who, when asked how often he cleaned his teeth, replied that he'd "never washed his mouth in all his life." "I found an unbelievably great company of living animacules. . . . The biggest sort (whereof there were a great plenty) bent their body into curves in going forward." Van Leeuwenhoek figured that although he kept his own teeth quite clean, "all the people living in our United Netherlands are not so many as the living animals that I carry in my own

mouth." By the way, van Leeuwenhoek also wrote many hundreds of pages vividly describing protists of all sorts, in such detail that modern names can be assigned to them.

In the greater context of seventeenth-century Europe, the accomplishments of van Leeuwenhoek are parallel to the great astronomers like Galileo—and perhaps as challenging to doctrines of the Catholic church. Galileo showed that Earth was not the center of the celestial bodies. Van Leeuwenhoek showed that there were other worlds of organisms in which humans could not possibly be the central figures. Galileo was persecuted for his heretical scientific work, but van Leeuwenhoek was working in the relatively liberal and tolerant Netherlands, perhaps somewhat under the radar and out of reach of the Catholic church. Indeed, seventeenth-century Holland was an exceptional place in the history of science, a place where philosophers with radical ideas such as René Descartes and Baruch Spinoza were able to live and produce important bodies of work.

Huygens and Vermeer

Constantijn Huygens, a Dutch essayist, described microscopic discoveries as "a new theatre of nature, another world" and a "second treasure house of nature" and "a newly discovered continent of our globe," albeit one designed by "the great architect." Huygens pondered the implications of such discoveries:

> *"Let us in short be aware that it is impossible to call anything 'little' or 'large' except by comparison. And, then, as a result, let us firmly establish the proposition that the multiplying of bodies . . . is infinite."*
>
> ~Svetlana Alpers
> *The Art of Describing*

The use of lenses had an influence on the arts of the seventeenth century. One striking example is Jan Vermeer, an exact contemporary of van Leeuwenhoek, born in the same small city and in the same year, 1632. Vermeer's wonderfully detailed paintings of ordinary scenes in households (some as clear and spontaneous as photographs) may have been set up with a lens system called a camera obscura. Did Vermeer and van Leeuwenhoek know each other? Both were lifelong and famous inhabitants of Delft. Historians have tried to find concrete evidence for any relationship, such as the unsubstantiated hypothesis that van Leeuwenhoek is the subject of Vermeer's paintings "The Astronomer" and "The Geographer." One tantalizing connection is known: van Leeuwenhoek acted as an executor for Vermeer's estate, strongly suggesting other connections may have existed as well.

The many microscopic observations published in the *Transactions of the Royal Society* by van Leeuwenhoek and others influenced not only philosophers, but poets such as Alexander Pope. Pope's long multipart poem "An Essay on Man" is well worth reading for its many allusions to all of the emerging branches of science of his time. For example:

> *See, thro' this air, this ocean, and this Earth,*
> *All matter quick and bursting into birth.*
> *Above, how high progressive life may go!*
> *Around, how wide! How deep extend below!*

FOR GREATER UNDERSTANDING

Questions

1. Why might van Leeuwenhoek not have been persecuted in the manner that Galileo was?
2. In what ways did the science of microscopy influence the art world?

Suggested Reading

Dobell, Clifford. *Anthony van Leeuwenhoek and His "Little Animals."* Mineola, NY: Dover Publications, 1960 (1932).

Ford, Brian. *The Leeuwenhoek Legacy*. Bristol, UK: Biopress, Ltd., 1991.

Other Books of Interest

Alpers, Svetlana. *The Art of Describing*. Chicago: University of Chicago Press, 1983.

Gaskell, Ivan, and Michiel Jonker, eds. *Vermeer Studies*. Washington, DC: National Gallery, 1998.

Pope, Alexander. *Essay on Man and Other Poems*. New ed. Mineola, NY: Dover Publications, 1994.

Lecture 4:
A Brief History of Bacteriology

The Suggested Reading for this lecture is Paul De Kruif's *Microbe Hunters*.

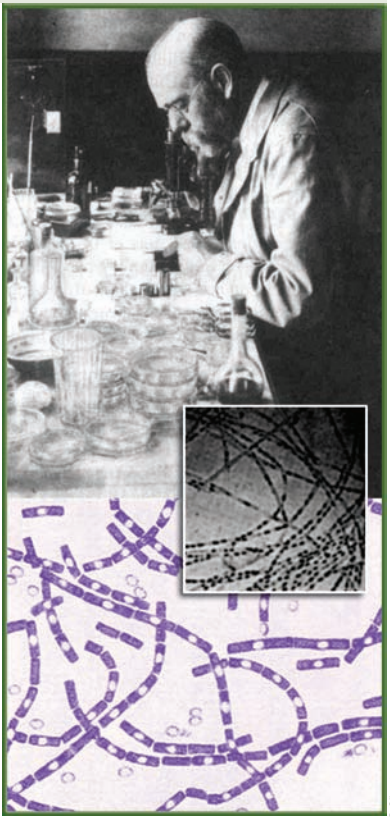
In this lecture we leap ahead from the seventeenth-century world of Anton van Leeuwenhoek to the nineteenth century, the beginning of modern bacteriology. Major players described in this brief history are Robert Koch, Louis Pasteur, Christian Gram, and Vladimir Vernadsky. Their accomplishments were to place bacteria more securely into the greater context of all of life by identifying some bacterial activities and interactions. This was accomplished in part by new insights into culturing and staining bacteria.

Robert Koch

The German physician Robert Koch was able to connect the presence of bacteria with particular diseases by using an especially thorough protocol to demonstrate the association unequivocally. Koch was awarded a Nobel prize in 1905 for the protocol that bears his name.

Koch's Postulates

1. Bacteria were found in all cases of the disease (such as *Mycobacterium tuberculosis* found in all tubercular patients).
2. Bacteria could be isolated into pure culture (Koch was able to do this for the bacteria he worked with, although if he had chosen extremely fastidious bacteria with unknown requirements this might have been impossible).
3. Cultured bacteria could be reintroduced into an animal and would cause the disease.
4. Bacteria could again be isolated from the animal and placed again into culture.



Robert Koch (1843–1910) at work in his laboratory ca. 1900.

In 1877, Robert Koch grew the anthrax bacillus organism (shown stained purple at the bottom of the image) in pure culture, demonstrated its ability to form endospores, and produced experimental anthrax by injecting it into animals. *Bacillus anthracis* was the first bacterium shown to be the cause of a disease.

Inset: Original photomicrographs of Bacillus anthracis taken by Koch.

Koch's culture method of growing bacterial colonies on the firm surface of nutrient agar was derived from observations that anyone can make in a kitchen. Indeed, Koch's colleague, Walter Hesse, passed the idea along from his wife Angelina Hesse, who had made the connection. Microorganisms grow nicely on the surfaces of jellies and aspics as well as on the moist surfaces of all sorts of foods, such as potatoes. These often may be easily observed as tiny, discrete, often colorful colonies. Later, the entire food surface may become completely covered with slime or fuzz; however, there is an opportunity early on to see individual colonies of microbes, presumably having been founded by one or a very few individuals. The Hesses and Koch experimented with various culinary gelling agents, including pectins (from plants), and gelatins (from animal tissues), but settled on what is now a microbiology standard: agar (from seaweed). All sorts of specific nutrients, such as sugars and proteins, could be dissolved along with agar and then allowed to solidify into a nutritious surface upon which discrete microbial colonies could be made to grow. Beef broth was a favorite source of nutrients; combined with agar, it made a sort of beef aspic of the kind that was a popular food in the nineteenth century. Lots of bacteria grow extremely well on it, especially the ones Koch was studying. Beef broth nutrients are a close match to those nutrients that pathogens might find in the human body.

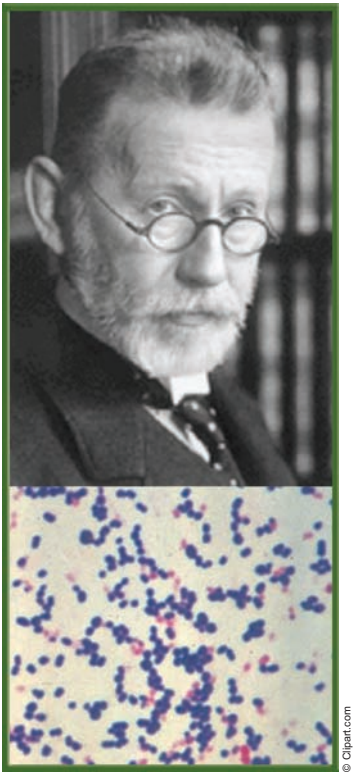
However, beef broth makes an "undefined" medium with various amounts of sugars and proteins along with a host of unknown nutrients forming a complex mixture. Later, microbiologists worked hard to establish a much more difficult but informative "defined medium." Constructing a defined medium is difficult because it necessitates understanding the bacterium well enough to know precisely what it needs and in what proportions and quantities. Often defined media are made from long recipes containing not only the obvious nutrients but also many trace quantities of salts and other ingredients in minute quantities. Microbiology labs today use both defined and undefined media; both have their good uses. For example, if you wanted to find bacteria that break down bird feathers, you might concoct an undefined medium of ground feathers as the only source of nutrients and see what grows on it. However, if you wanted to get a more precise idea of what the feather-eating bacteria were consuming, you might attempt a defined medium using various chemicals such as keratin (the major protein in feathers) and other nutrients known to be in feathers.

Another good idea that came from Koch's lab was the "Petri dish," named for Koch's assistant Julius Richard Petri. It turns out that only a thin layer of solidified nutrients (in a low dish) is needed to support colonies of many bacteria, and in many cases the lid need not be tight. Extensions of Koch's culture methods were subsequently developed by others to accommodate bacteria that did not thrive in an oxygen-rich environment on the surface of agar. These methods included culturing beneath the agar and culturing in the presence of oxygen-scavenging chemicals to render the environment oxygen-free or the replacement of oxygen with an inert gas.

Hans Christian Joachim Gram

A contemporary of Koch, Hans Christian Joachim Gram, a Danish physician, was among those experimenting with various dyes as means to make bacte-

ria more visible under the microscope. The European dye industry was thriving and there were many new synthetic dyes from which to choose. Gram tried a dye called “Crystal violet” to distinguish various types of pneumonia-causing bacteria in lung tissue. His method is called “Gram staining.” Of all the staining methods that were being experimented with at the time, many of which are still in use, Gram staining turned out to be unusual in that it divided most bacteria into one or another of two major groups that seemed to follow along taxonomic groupings. Gram staining became one of the first steps in identifying almost any bacteria by means of dichotomous (or binary) decision trees. Bacteria could be placed in one or the other of two major categories: “Gram negative” and “Gram positive.” A result of DNA sequencing work more than one hundred years later confirmed the importance of the two categories. It turns out that Gram-positive bacteria are all members of one large cohesive taxonomic group. Nearly all the rest are Gram negative. The exceptions, however, are important. None of the archaea (an enormous phylogenetic group) respond in any taxonomically meaningful way to Gram staining. However, ordinary clinical laboratories and most microbiology classes typically do not encounter archaea and therefore Gram staining continues to be a favorite way to begin a characterization of a new bacterium.



Hans Christian Joachim Gram (1853–1938)

Professor Hans Gram in his office at the University of Copenhagen (ca. 1902), where he lectured in medicine. Below his picture is an image of cocci, microorganisms (usually bacteria) whose overall shape is spherical or nearly spherical. The dark bluish-purple are Gram-positive while the reddish organisms are Gram-negative.

Another contemporary of both Koch and Gram was the French scientist Louis Pasteur, who worked on fungi and viruses as well as bacteria. Among his many accomplishments was the demonstration that fermentation (for example, of wine) was a microbial process. He also did considerable work on vaccines, the method by which small quantities of infectious (but often weakened) microbes or viruses would be injected into a patient, conferring an immunity against future encounters with the pathogen.

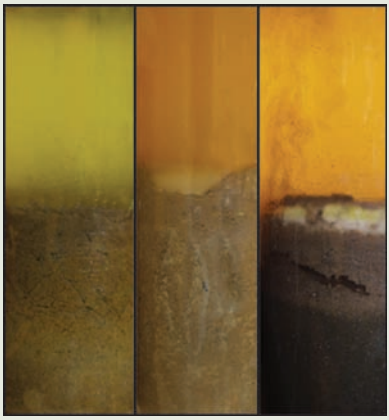
Sergei Nikolaievich Winogradsky

Meanwhile, in a sort of parallel universe of microbiology, was the Russian microbiologist and soil scientist Sergei Nikolaievich Winogradsky. He was using an entirely different culture method from Koch and his colleagues and was not in any particular communication with Koch. Rather than deciphering the activities of individual species isolated from other species in solitary colonies on Petri dishes (“pure cultures”), Winogradsky cultivated mixed

communities or “mixed cultures.” While Koch had “domesticated bacteria,” Winogradsky’s approach was to turn them loose in a simulation of “wild” conditions to see what happens. His method is now referred to as a “Winogradsky column.” It is a tube full of sediment and water and the microbes that happened to be present. The tube of sediment is allowed to develop in the presence of various conditions that might include light, particular minerals, and particular sources of nutrients. Winogradsky found that bacteria interact with each other (almost impossible to see on most Petri plates) and that important functions occur through those interactions. For example, the cycling of nitrogen through the biosphere is a consequence of mostly microbial activities in communities.

Winogradsky was the first to describe a strange sort of metabolism in some of his bacteria, chemoautotrophy, by which sugars are synthesized from carbon dioxide using energy not from the sun (as photosynthesizers do), but rather from energy in chemical bonds of particular compounds such as minerals. After the Russian revolution in 1917, Winogradsky lived in exile in France and worked at the Pasteur Institute. Thus began his influence on European and American microbiology and a gradual synthesis that eventually included both culture methods. However, to this day, there are many microbiology labs that would never consider mixing cultures, and when it happens by accident, it is not considered informative, but rather a contamination.

Three Winogradsky Columns



Initial

This image shows the initial appearance of three different Winogradsky columns containing soil and water samples from a river. The column on the left was unaltered, the middle and right columns were modified with phosphate, nitrate, and sulfur additives. These additions promoted the growth of various bacteria specific to the aerobic and anaerobic regions of the columns.

Both images © T.J.M. Hay

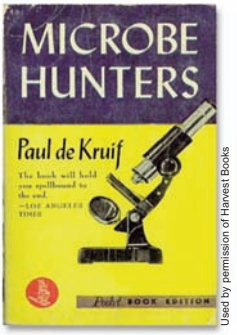
After Seven Weeks

The same three columns are shown in this image after a seven-week period. Each column grew algae, cyanobacteria, and other bacterial colonies. Of specific interest are the reddish regions of the middle column, which indicates the presence of purple nonsulfur bacteria (in this case, *Rhodospirillaceae*). In the column on the right, the red growth along the side is a purple sulfur bacterium, *Chromatium*.

Literature Popularizes Bacteria

Many popular authors have captured the excitement of early developments in microbiology both in nonfiction and fiction accounts. Paul de Kruif (1890–1971) inspired generations of microbiologists with his 1926 book *Microbe Hunters*, still well worth reading today. Among those featured as adventuresome scientists are van Leeuwenhoek, Pasteur, and Koch, described in breathless accounts full of superlatives and with frequent use of the word "gorgeous."

In the fiction category is *Arrowsmith* by Sinclair Lewis (1925), who was greatly influenced by de Kruif. The novel is full of the politics of science and the personal and professional pressures around scientific training, all in the context of a microbiology lab. It too is well worth reading today for its contemporary themes.



Used by permission of Harvest Books

A 1940 edition of *Microbe Hunters* by Paul de Kruif

Microbiology versus Bacteriology

Throughout this account of the history of the field, the broader, more inclusive word "microbiology" rather than "bacteriology" has been used. Microbiology includes all of the organisms that are best seen under a microscope, including protists and fungi as well as bacteria and archaea. It has also encompassed (especially in some microbiology courses) the study of viruses and the immune system. The first scientists exploring the microbial world delved into all they could see or detect and thus the field as it developed was much broader that just "bacteriology." Pasteur, for example, did most of his work on viruses and fungi. However, the focus for this series of lectures is bacteriology with the semantically awkward inclusion of the archaea. Terminology has simply not yet caught up to or conformed to the realities of the microbial world. We do not yet have an "-ology" that properly acknowledges a course that includes both the bacteria and archaea together. Combined they are sometimes referred to as "the prokaryotes," but that name is falling out of favor and there is no commonly used "prokaryotology." Meanwhile, other microbes such as fungi will be included in this course only in their roles as community members along with bacteria and archaea, the soil community being a good example. What about the viruses? They are extraordinary genetic entities unto themselves and will have their own lecture in this course to place them into context.

FOR GREATER UNDERSTANDING

Questions

1. What is Gram staining?
2. What happens during chemoautotrophy?

Suggested Reading

De Kruif, Paul. *Microbe Hunters.* 70th anniversary ed. Orlando, FL: Harvest Books, 2002.

Other Books of Interest

Brock, Thomas D. *Robert Koch: A Life in Medicine and Bacteriology*. Reprint. Washington, D.C.: American Society for Microbiology, 1999 (1988).

Debré, Patrice. *Louis Pasteur*. New ed. Trans. Elborg Forster. The Johns Hopkins University Press, 2000.

Lewis, Sinclair. *Arrowsmith*. Reprint ed. New York: Signet Classics, 2008.

Websites to Visit

Sumanas, Inc. provides "The Winogradsky Column: An Animated Tutorial" — http://www.sumanasinc.com/webcontent/anisamples/microbiology/winogradsky.html

Lecture 5:
The Family Tree of Bacteria

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* introduction.

Early Taxonomy

In addition to being tiny, bacteria (as individual cells) are essentially featureless, almost entirely lacking in morphology and therefore missing the visual identifiers that are so often used to classify organisms. The cells come in one or another of a short list of shapes (sphere, rod, curved rod, spiraled rod, and amorphous or shapeless), which are by no means sufficient to categorize millions of species. Instead, microbiologists have characterized and identified bacteria based mostly on what they do. Especially valuable skills in microbiology include being able to get bacteria to live as normally as can be arranged in a laboratory setting (in a flask or on a Petri dish) and to get them to perform a suite of typical functions and activities. These include consuming and producing acids or gasses from particular sugars and converting certain compounds from one to another such as nitrates to nitrites. In addition, bacteria may be identified by their reactions to a series of colorful dyes either becoming stained or not. Certain aspects of morphology in addition to shape may be considered such as presence or absence of flagella or of protective capsules around the cell as well as the ability to stain (or not) with Gram stain. For many decades, bacterial classification was based almost entirely on characteristics such as these. An efficient microbiology lab traditionally was set up to take any unknown bacterium (able to be grown in a lab) through a battery of tests and queries to arrive at an identification. Indeed a famous, often culminating, lab project in the education of any microbiologist is the identification of an "unknown" culture, assigned by the professor from a collection of relatively easy to grow bacteria. Out of these techniques for getting bacteria to grow and function in the laboratory came most of what was known for decades about bacteria taxonomy. Entire groups of bacteria were clustered and arranged on family trees according to what they consumed, what their waste products were, and whether they became stained with certain chemicals. Also arranged on the family tree were some bacteria known mostly in the field, not as easily grown in the lab, and often bearing certain colorful pigments and performing certain distinctive activities.

DNA Sequencing

That was the state of bacterial taxonomy until DNA sequencing became fast and inexpensive. Then came some surprises and a complete rearrangement of many well-established taxonomic groups. It might be expected that sequences of entire genomes (complete sets of DNA) would reflect the arrangement of the phylogenetic tree of bacteria. They do reflect it. Indeed, the reasoning is even a bit circular in that we assume that sequence differences ought to be arrangeable in a tree-like format that in turn ought to

reflect phylogeny. The surprise was how little it mattered what specialized metabolisms might be occurring. For example, the seemingly exotic ability to perform chemoautotrophy (the ability to make food out of carbon dioxide using the energy from chemical bonds in minerals) is not a good identifying characteristic for any particular grouping of bacteria. That exotic metabolism occurs throughout the phylogenetic tree.

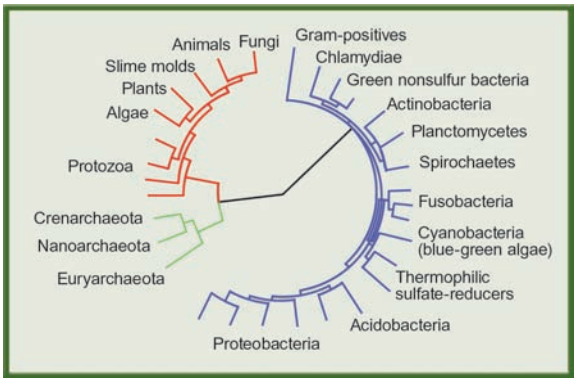
All up-to-date phylogenetic trees reflect DNA sequence data and since those data are still coming in, the tree structure is not yet settled. However, the general shape of the tree is known with some confidence and it looks like three enormous many-branched, many-twigged trunks that may be labeled “The Bacteria,” “The Archaea,” and “The Eukaryotes.”

The Bacteria

One trunk comprises all of the bacteria and seems to have sprouted at the base of the tree, as close as we can imagine to the origin of life. There are about eleven major branches of bacteria. The two branches that emerge closest to the base of the trunk include bacteria that thrive in extreme heat, boiling or near boiling temperatures. This is suggestive of a hot origin of life, perhaps in a thermal spring like the sort that occur in deep sea vents or in Yellowstone National Park, where such ancient lineages of bacteria still thrive. The other nine bacterial branches emerge in no discernable order, at least by the current methods for distinguishing DNA sequences. These include the proteobacteria, the Gram-positive bacteria, and the cyanobacteria, each of which will be the topic of its own lecture. Finally, six branches of bacteria will be touched upon only briefly to show off some of their intriguing features to conclude this lecture series.

The Archaea

The archaea once were included with the bacteria and are still included with bacteria in a colloquial or popular sense. DNA sequences reveal them to be a large and distinctive trunk of the three-part tree. However, they may be easily mistaken for bacteria being of the same size, the same extraordinary range of diversity, and present in the same unfathomable numbers. They are subjects side-by-side with bacteria in any microbiology or bacteriology course. And (whether or not experts approve of this) in common parlance they are often called “bacteria.” Biological terminology has a history of failing to keep up efficiently or even at all with changes in understanding and necessary adjustments in definitions. This is a good example.



Simplified Bacteria Family Tree

Phylogenetic tree showing the relationship between the archaea and other forms of life. Eukaryotes are colored red, archaea green, and bacteria blue.

Many traits make the archaea distinctively different from bacteria. For example, important cell structures such as cell walls, cell membranes, and flagella are made of a different suite of chemicals from bacteria, suggesting a divergence from bacteria deep at the base of the tree. While some bacterial and archaeal metabolisms have characteristics in common, many other archaeal metabolisms are strikingly different. For example, many archaea dwell deep in anoxic sediments and are part of a world based on the production of methane gas, which they generate in several ways using variations on autotrophies and heterotrophies. Other archaea have specialized in some of the most extreme environments on Earth, including salt crystals, boiling springs, and extremely deep sediments. These extremophilic archaea will be the focus of Lecture 6. It is further evidence of a hot origin of life that the extreme heat-lovers among the archaea tend to branch from the base of the tree, suggesting an ancient lineage.

Digression: Natural Genetic Engineering or Horizontal Transfer

Before describing the third trunk, "The Eukaryotes," there is a necessary digression to reveal that the image of a tree actually is too simple. The image will now will be complexified to reflect a strange reality: bacteria and archaea are extraordinarily promiscuous with their DNA. They readily pick up stray bits of DNA from their environments, regardless of whose DNA it is. This trait (called horizontal transfer) has made "genetic engineering" in the laboratory very easy because it is such a normal and seemingly ubiquitous function; it was not something invented by human researchers. This is also in marked contrast to the form of DNA exchange that we humans know best, that is, sexual reproduction, and which is actually quite an anomalous activity compared to what most organisms are doing with their DNA. Sex tends to be strictly limited to receiving DNA only from one's own species. Bacteria and archaea have no such restrictions. Therefore a new development in the phylogenetic tree is the realization that it should be drawn with anastomosing (fusing) branches throughout, to reflect horizontal transfers and a much more complex picture of relatedness.

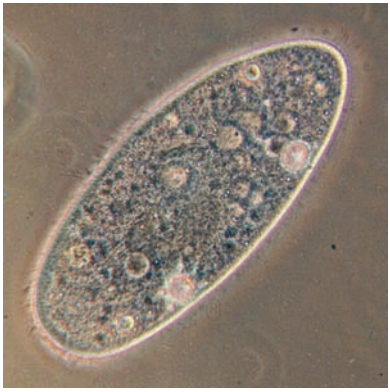
One interesting consequence of horizontal transfer is that the phylogenetic tree may be a sort of snapshot in time of a flow of DNA that mostly diverges branch-like, but that blurs the boundaries of "species." Indeed, "species" as a concept developed primarily for sexual animals, especially mammals, insects (such as fruit flies), and birds. Within these groups the idea of discrete species with clear boundaries and very limited exchange of DNA seems to work. Otherwise (for the rest of life, which is mostly bacteria and archaea) species is a fuzzy concept. That may be why it will continue to be difficult to pin down how many "species" there are. Depending on your time frame it could be relatively few, because the longer they have to interact the more flow of DNA there is.

On the other hand, microbiology has become a field for explorers and adventurers, descending to ocean depths in submersibles, drilling, diving beneath polar ice, and investigating deep caves. Maybe there are many more "types" of bacteria left to be found. Perhaps we have been severely restricted by our own non-aquatic, temperate, oxygen-breathing physiologies on a planet that is almost entirely aquatic and with deep, fractal, inaccessible subsurfaces.

The Eukaryotes

The third major trunk of the phylogenetic tree of all organisms is "The Eukaryotes." It comprises familiar groups that owe their very existence to the extraordinary promiscuity of bacteria and archaea (sometimes referred to as "The Prokaryotes"). Indeed, some extremely bacteriocentric and archaeocentric scientists would suggest that the eukaryotes are just another example of the vast range of consequences of horizontal transfer between bacteria and archaea and therefore not unusual enough to warrant their own large-scale taxonomic nomenclature. Here, we will be more inclusive. Approximately two and a half billion years ago some archaea and some bacteria formed intimate relationships that had the particular quality of making the new resulting cells more complex, more compartmentalized, and ultimately larger. These cells may also have been predisposed to multicellularity. About one billion years ago at least two branches of eukaryotes did indeed assemble into complex multicellular, often macroscopic organisms. The eukaryotes comprise four major sub branches: protists, fungi, animals, and plants, the later two of which are always multicellular.

Protists are predominantly single celled, fascinatingly diverse in form and function and are favorite topics for microscopy. They include amoebae, paramecium, euglena, green algae, and many others. This is the original, deep lineage of eukaryotes that seems to have come about at first from a symbiosis between an archaeal lineage and a bacteria lineage to make a new heterotroph. Then some of these symbioses seem to have taken on a third symbiont, a photosynthetic bacterium (perhaps in several lineages in several separate events) to make photosynthesizers. A third symbiosis that might have occurred is one still embroiled in scientific controversy, namely that spirochete bacteria may have joined the symbiosis as well, conferring their own particular undulating form of motility.



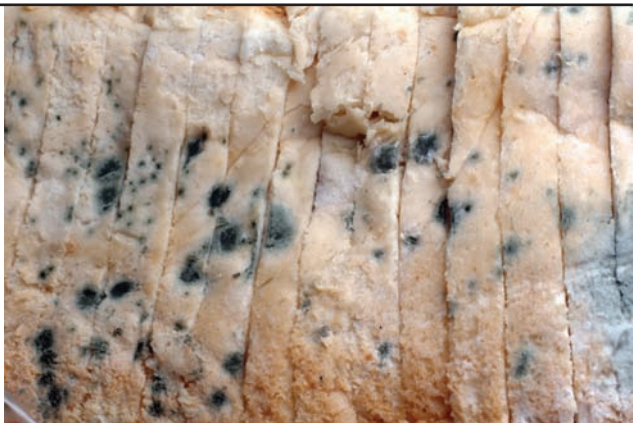
© Josh Grosse

An optical microscope image of a paramecium. This eukaryotic cell, which is full of inclusions and compartments, is about 100 micrometers in length—about one hundred times the size of a bacterium.

Fungi and animals are sister groups, both heterotrophic, both multicellular (except for those fungi that are not) and both originating from a particular lineage of protists, the choanoflagellates. Clearly being a multicellular, pre-animal one billion years ago was a powerful position from which to diverge, as evidenced by the explosion of diverse morphologies throughout the many invertebrate and vertebrate phyla. The fungi include molds, yeasts, and mushrooms.

Plants come from a lineage of green algal protists and have also distinguished themselves in a fantastic array of adaptations and morphologies.

The classification of organisms traditionally has been either a five kingdom system (prokaryotes, protists, fungi, plants, and animals) or six kingdom system (with prokaryotes split into bacteria and archaea). I continue to argue



Penicillium fungi (mold) growing on bread.

strenuously for a five or six kingdom system in textbooks, not because of their respective "weights" on the phylogenetic tree, which clearly shows only three big groups, but because naming is so important in how we value things. "Obscure" groups like protists, fungi, most invertebrates (that is, most animals), and most inconspicuous plants are easily ignored by textbook writers who favor large charismatic organisms of limited diversity. For a few decades the five or six kingdom system came to prominence in even the most ordinary high school textbooks and I think biology education was better for it. As fascinating as archaea and bacteria are, their mere weights on the phylogenetic tree should not be primary measures for how they are weighted in textbooks. Terminology (like the loaded word "Kingdom") counts in this and I fear we are in danger of losing whatever we had gained with protists and fungi as groups in their own right.

The completion of the human genome project brought with it a host of speculations and musings about the potential meanings and consequences of it all. These include the possibilities of "designer babies," cloned humans, medical ID cards with complete genomic information, and other such topics. However, the greatest consequence that I have seen—actually manifested manifold—is that sequencing has become much easier and much cheaper and with all those sequencing labs temporarily idle after the human genome was finished, microbial genomes are being completed by the hundreds. It is a sort of "side effect" of the human genome project, but an extremely powerful and meaningful one. Not only do we have a much richer version of the phylogenetic tree, complete with anastomoses, but we also have a new stream-lined method for identifying bacteria that previously could not be easily cultured in the lab. We can now pick up a handful of soil (an entire microbial community with all its proportions and interactions of members) and analyze the sequences within and come up with a list of who is there. And that is exactly what microbiologists are doing all over the world in habitats ranging from the open ocean to our own teeming digestive systems. All of the sequences (at least those not considered proprietary by a company) go into the publicly accessible national database NCBI (National Center for Biotechnology Information) for anyone to use. These are exciting times in microbiology newly enriched by DNA sequencing.

FOR GREATER UNDERSTANDING

Questions

1. What is meant by horizontal transfer?
2. What is perhaps the greatest consequence of the human genome project in the opinion of this bacteriocentric professor?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Introduction. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Fenchel, Thomas. *Origin and Early Evolution of Life*. Oxford: Oxford University Press, 2002.

Knoll, Andrew. *Life on a Young Planet.* Princeton: Princeton University Press, 2003.

Margulis, Lynn, and Michael F. Dolan. *Early Life: Evolution on the Precambrian Earth.* Sudbury, MA: Jones and Bartlett, 2002.

Margulis, Lynn, and Karlene Schwartz. *Five Kingdoms*. New York: Freeman and Co., 1987.

Websites to Visit

National Center for Biotechnology Information website — http://www.ncbi.nlm.nih.gov/

Lecture 6:
The Extremophiles

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* chapters 2 through 4.

Extremophiles include those archaea and bacteria that live in conditions that we would consider extreme, such as boiling water, salt crystals, and deep anoxic habitats. Many of them are archaea, so this will be an opportunity to introduce some of the more visible members of that enormous and diverse lineage.

Thermophiles and Hyperthermophiles

Four and a half billion years ago, Earth was a newly accreted planet still molten on its surface, and therefore much too hot for liquid water. By four billion years ago, enough cooling had occurred that Earth had a thin crust and shallow seas of liquid water. It was still hot; molten rock was just below the crust. Geysers, hot springs, and volcanos steamed, bubbled, and probably erupted to the surface in many places. Furthermore, the atmosphere was devoid of oxygen. By about three and a half billion years ago, there were organisms substantial enough to have become fossilized. These appear to be bacteria or at least are of bacterial size and are of limited shapes, as is typical of bacteria. Note that this approximate date of three and a half billion years ago should not be considered a date for the "origin of life." It is merely the first date at which we have reliable fossils preserved enough to interpret and that we were fortunate to find in the only two locations where sedimentary rocks of

Grand Prismatic Spring in Yellowstone National Park is a favorite of visitors for its brilliantly colored hyperthermophiles.

that age may be found: South Africa and Western Australia. We can dare to extrapolate the origin of life as being sometime shortly after the cooling of the Earth four billion years ago, but before the dates of the first fossils at three and a half billion. I often round off the putative date of the origin of life to "four billion years ago." Any details as to how the origin of life may have come about and what the very first life may have looked like are part of a complex topic that would need much more than a short digression.

So instead we will skip ahead a few million years to speculations about what early bacteria and archaea might have been like, once they had evolved into forms that we would recognize. They were hyperthermophiles (is the good guess of many microbiologists), that is, they loved high heat and many descendents still love it and are thriving in boiling hot springs and geysers and in deep sea vents. These are the branches of bacteria and archaea described in the previous lecture as originating near the base of the phylogenetic tree. Where can you view them? Deep sea vents are off limits to most people except for those researchers who descend in deep sea submersibles. For the rest of us there is a rich collection of photographs and videos by which we can imagine what it is like to be next to a boiling vent, three miles down in cold, black ocean water.

However, you can go to Yellowstone National Park in Wyoming, the most pristine, most extensive, and most safely accessible hot spring and geyser area in the world. The system of boardwalks will take you to within feet of erupting geysers, steaming fumaroles, boiling mud, and bubbling hot springs. The National Park Service is increasingly aware of the importance of the thermophilic archaea and bacteria as being among the main organisms of the park (along with some buffalo, elk, wolves, bears, and lodge pole pines). Not only does the park service support microbiology researchers, but they are also working hard to educate the public about what bacteria may be viewed at a safe distance with a system of signage, guided tours, and literature. What can you expect to see? Gorgeous colors characterizing nearly every thermal feature are an important indicator along with the temperature of that feature for determining which archaea or bacteria you are observing.

At temperatures greater than 80 degrees C (176 degrees F) (the range of hyperthermophiles) in waters of neutral to alkaline pH, the reds, pinks, yellows, and oranges you see are likely to be bacteria of the ancient hyperthermophilic lineages mentioned in the previous lecture. If water of that temperature is acidic, it is also likely to look muddy and if so, is the habitat of acid-loving, heat-loving archaea such as *Sulfolobus*, also from the deepest known lineages. The famous *Thermus aquaticus* was isolated from near-boiling waters at Yellowstone and went on to become a Nobel Prize-winning bacterium for Kary Mullis, who used its heat-tolerant enzymes to develop PCR (the polymerase chain reaction.)

Between 60 and 80 degrees (the range of thermophiles) may be found more bacterial and archael types, including green and beige-yellow-orange colors of some photosynthesizers. All photosynthesizers have some form of green chlorophyll, but many have an abundance of carotenoid pigments in shades of yellows and oranges that mask underlying green pigments. One important photosynthesizer, *Chloroflexus*, has its own main branch, "The Green Non-Sulfur Bacteria," and is especially distinguishable from other organisms if

observed above 60 degrees. They are often yellow-flesh colored and are positioned next to the greener colors of heat-loving cyanobacteria, some of which can live in temperatures up to 75 degrees.

Below 60 degrees you are getting away from the exclusive realm of thermophiles and hyperthermophiles and become increasingly likely to encounter eukaryotes. Bacteria and archaea are still there, of course, but sometimes your view may be obstructed.

Halophiles

What grows on crystals of salt? Halophilic archaea love salt and display themselves in lovely shades of pink and red in the hot, dry, evaporitic environments where salt crystals naturally form or are made to form in commercial salterns. The colors of halophiles are why salterns can look like beautiful patchworks of color from above. The pigment is used in an intriguing type of metabolism that is not easily categorized using terms like heterotrophy and phototrophy. It is a hybrid of the two that allows the halophile to get its nutrition by consuming food compounds (as we fellow-heterotrophs do); however, it has a back-up method for getting energy that takes advantage of the baking sun. It uses its red pigment rhodopsin (the same family of pigments that we have in our retinas) to make a light-driven pump that accumulates protons such that they can be stored in energy-rich bonds. Note that this is not what photosynthesizers do. They synthesize sugars (after having baked in the sun collecting photons and storing them in energy-rich bonds). Halophiles don't make their own food; they're heterotrophs; they just have a few extra energy-rich molecules to use.

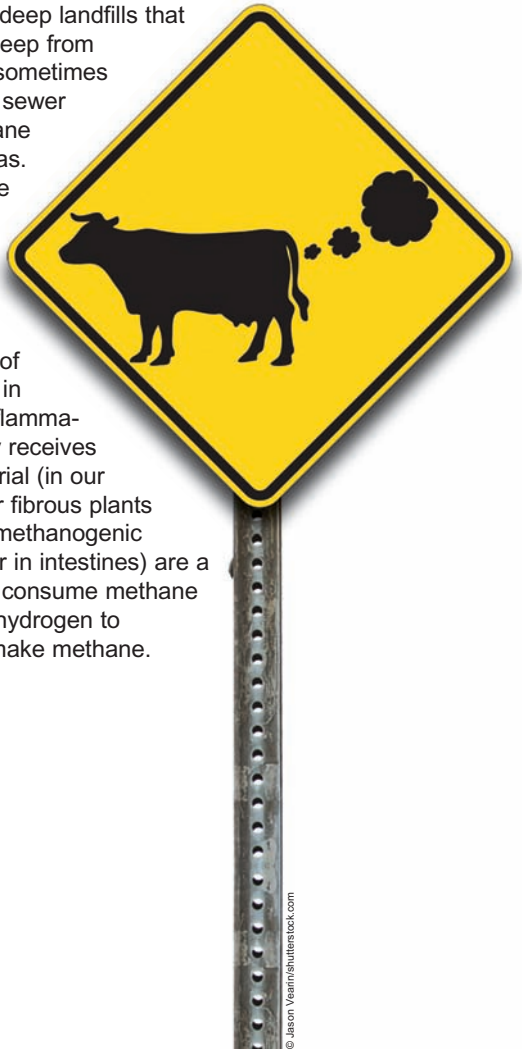
Go to a commercial or natural saltern to see the lovely pinks and reds of halophiles. If you are allowed to investigate the salt flats closely, notice that a community of bacteria and archaea accompany the pink halophilic area right below the salt layer in black, sulfurous salty muds. Alternatively, you might check out the heaps of road salt that some towns use to de-ice roads. If these are left exposed to moisture, they may show pink colors of halophiles, the same that are in salterns and perhaps even transported to the department of public works from some distant saltern.

A salt works evaporation pond on the margin of Lake Tyrrell, Australia. The lake has a pink cast because of the presence of halophilic microorganisms. The salt works salt piles can be seen in the distance.

Methanogens

Methanogens are extremophiles in the sense that they dwell in deep sediments devoid of oxygen where we humans cannot easily explore. However, Earth abounds in deep waters and deep sediments and therefore abounds in methanogens and many other anaerobes. Methanogens are archaea that produce methane gas as the product of one or another of several metabolisms that resemble heterotrophies and autotrophies. Detecting them is as easy as detecting methane, which is odorless but quite flammable. In the still waters of a swamp or other stagnant fresh water (such as a goldfish pond), stir deeply with a stick. Up may come bubbles, very likely of methane released from the methane community thriving below. Various clever methods of collecting swamp methane include allowing it to displace water in an inverted container.

Other sources of methane are deep landfills that require methane vent pipes to keep from exploding and sewer lines that sometimes accidently explode. That is why sewer line workers usually wear methane detectors to warn them of the gas. If you have access to a methane detector, hold it close to emerging bubbles of swamp gas to get a reading. Unusual as methanogens might seem to be, we and other animals usually carry lots of them in our intestines such that in some cases flatulence may be flammable. In a swamp, the community receives and processes dead plant material (in our intestines they receive whatever fibrous plants we are eating). Accompanying methanogenic archaea (whether in a swamp or in intestines) are a host of bacteria, some of which consume methane and some of which pass along hydrogen to methanogens that they use to make methane.



FOR GREATER UNDERSTANDING

Questions

1. What is the defining characteristic of a hyperthermophile?
2. What can the pinks and reds of a commercial or natural saltern be attributed to?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Chapters 2–4. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Brock, Thomas D. *Life at High Temperatures*. Yellowstone National Park, WY: Yellowstone Association for Natural Science, History & Education, Inc., 1994.

Friend, Tim. *The Third Domain: The Untold Story of Archaea and the Future of Biotechnology*. Washington, D.C.: Joseph Henry Press, 2007.

Schreier, Carl. *A Field Guide to Yellowstone's Geysers, Hot Springs, and Fumaroles.* Moose, WY: Homestead Publishing, 1992.

Sheehan, Kathy, David Patterson, Bret Leigh Dicks, and Joan Henson. *Seen and Unseen: Discovering the Microbes of Yellowstone*. Guilford, CT: Falcon, 2005.

Websites to Visit

1. An online version of *Life at High Temperatures*, a booklet by Thomas D. Brock (E.B. Fred Professor of Natural Sciences-Emeritus at the University of Wisconsin-Madison), is available at the University of Wisconsin website — http://www.bact.wisc.edu/themicrobialworld/LAHT/B1
2. The official website of the Yellowstone National Park by the National Park Service — http://www.nps.gov/yell

Lecture 7:
An Enormous and Diverse Group: The Proteobacteria

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* chapters 6 through 9.

The proteobacteria are among the best known of bacteria. Many are easily grown in the lab and therefore have been thoroughly categorized based on their activities (mostly eating and excreting) on Petri plates and in flasks. When the sequencing of entire genomes of DNA became relatively easy and inexpensive, many proteobacteria were among the first to be sequenced. The public database of DNA sequences at the National Center for Biotechnology Information (NCBI) hosts a growing number of bacterial genome sequences. At this writing, about six hundred genomes are included and about half are proteobacteria. Does this mean that proteobacteria are the predominant bacterial group? Probably not. Rather, this reflects the ease by which many can be cultured. In future years, we might expect that database to grow with many other groups of bacteria that are being collected from exotic locations, some of which are adding more branches to the phylogenetic tree.

Many of our best known and most serious pathogens are proteobacteria, and that is another good reason that they are so numerously represented in the database. However, proteobacteria are diverse and the vast majority are not pathogenic, but rather are out in the environment doing many interesting and sometimes highly visible activities. This lecture will focus on those non-pathogenic proteobacteria. Indeed, in keeping with the spirit of this lecture series, the focus will be on those proteobacteria that are most likely to be memorable and observable by a diligent layperson. Meanwhile, pathogens will be the topic of Lecture 10.

The proteobacterial group is a wonderful example of the upheaval and confusion that occurred when DNA sequencing became the primary method for building family trees. Suddenly it became apparent that groups of proteobacteria who had some activities in common (mostly around

eating and excreting) were in fact not closely related, as had been supposed. Meanwhile, other proteobacteria with rather different activities were now realized to be close relatives. One aspect of the problem is that when we say "activity" we really do mean mostly those few things that can be readily observed by a human in a lab. That leaves out a great many other more subtle activities that may help to classify a group. DNA sequences give us a more complete picture of those relationships.

So shaken was the community of bacteria researchers by the new DNA-based proteobacterial classification (requiring the old classification to be mostly thrown away) that (uncharacteristically) they did not coin a set of new multi-syllabic jargon based on Latin and Greek for the new relationships. Instead, a very cautious set of names based on the first five letters of the Greek alphabet was offered and accepted: alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria, delta-proteobacteria, and epsilon-proteobacteria. These are sometimes nicknamed by their letters, as in "the alphas" or the "beta-proteos." None of these five proteobacterial groups have any particular outstanding characteristic activity that would allow a more specific name.

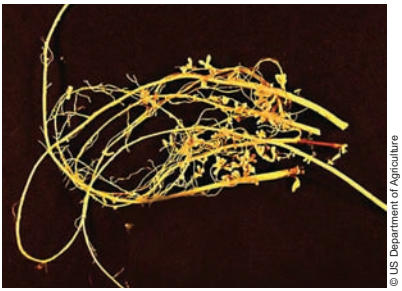
Four "Charismatic" Proteobacteria

Here are descriptions of four charismatic proteobacteria that have in common some interesting stories and accessible field marks. A goal for this lecture is that you might feel empowered to talk about them (to retell the stories) and maybe even to confidently point some out on your next nature walk).

Nitrogen Fixers

Earth's atmosphere contains 79 percent nitrogen gas that is of no direct use to animals, plants, fungi, and protists. We breathe in nitrogen gas and we breathe it out again, unchanged. Meanwhile, we organisms all need lots of nitrogen in the right form to build our proteins and DNA and RNA. How do we get it? Bacteria of several groups are able to capture nitrogen gas and convert it via a series of expensive (but worthwhile) chemical reactions, "nitrogen fixation," to form ammonia, a sort of nitrogen fertilizer that then may be incorporated into protein, DNA, and RNA, and passed around as organisms consume each other.

Some alpha-proteobacteria such as the nitrogen-fixer *Rhizobium* have formed symbiotic partnerships with plants by which nitrogen fixation is facilitated and the excess fixed nitrogen (ammonia) is passed off to the plant. From the point of view of the plant it is like having a fertilizer factory. To see these partnerships, dig up some clover, peas, beans, or other leguminous plants. Try to keep the roots intact by leaving plenty of soil around them. Gently rinse the roots in a bucket of water until you can see the tiny root hairs. Then look closely with either your naked eye or a hand lens to see nodules on the roots;



Soybean root nodules, containing nitrogen-fixing *Rhizobium* bacteria.

those are formed by the combined efforts of bacteria and plant and serve as tiny habitats for the bacteria. Inside they are fixing nitrogen, enough for themselves and for the plant that absorbs the fixed nitrogen through its roots. Use your thumbnail to break open a nodule and look closely for a faint pink color. That is a type of hemoglobin, similar to the hemoglobin carrying oxygen in our bloodstreams. In this function, hemoglobin is keeping oxygen tightly bound lest it interfere with the oxygen-sensitive nitrogen fixation reaction.

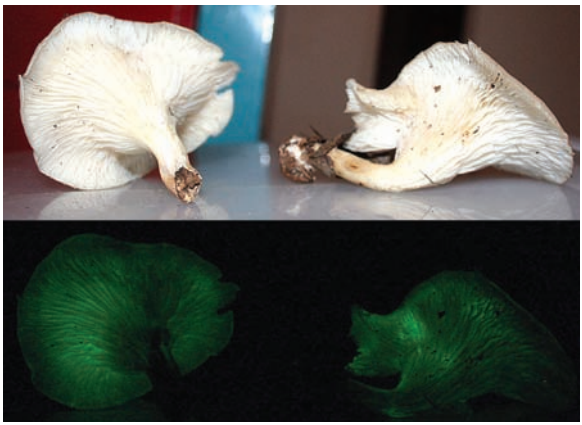
Magnetotactic Bacteria

Magnetotactic bacteria are alpha-proteobacteria that contain sets of tiny iron magnets in a row by which they can orient themselves by Earth's magnetic field, especially if they are in one or another of the Earth's hemispheres where the magnetic force not only points toward the north or south pole, but also curves down. These bacteria seem to be using magnetotaxis to orient in respect to up and down in swamps and bogs and other sediments. They favor a position between oxygen-poor sediments below and oxygen-rich sediments above. Magnetotactic bacteria in the southern hemisphere tend to be south-seeking and in the northern hemisphere, north-seeking. Both north and south-seeking magnetotactic have been found at the equator and their relative abundances seem to follow local magnetic differences in sediments.

So that's the story, but can you actually see them? Try setting up the following experiment and you might get lucky with the results. Fill a jar about two-thirds full of sediment from a body of somewhat stagnant fresh water. Top it off with some stagnant water. Tape several magnets to the outside of the jar at several different levels. If you are in the northern hemisphere, the north end of each magnet should touch the glass. If in the southern hemisphere, use the south end of the magnet. Cover the jar with foil. Every few days (up to weeks) take a peek behind each magnet. If magnetotactic bacteria are present, you may see a tiny spot of grey (a group of magnetotactic bacteria) behind one or more of your magnets.

Bioluminescent Bacteria

Some gamma-proteobacteria are bioluminescent, using similar reactions to light up as fireflies (or lightning bugs). When these bacteria get together in sufficiently high numbers (often congregating and multiplying at a food source), they glow. Your best view is when you are in darkness with your eyes adjusted and ready to spot even the faintest pale glow.



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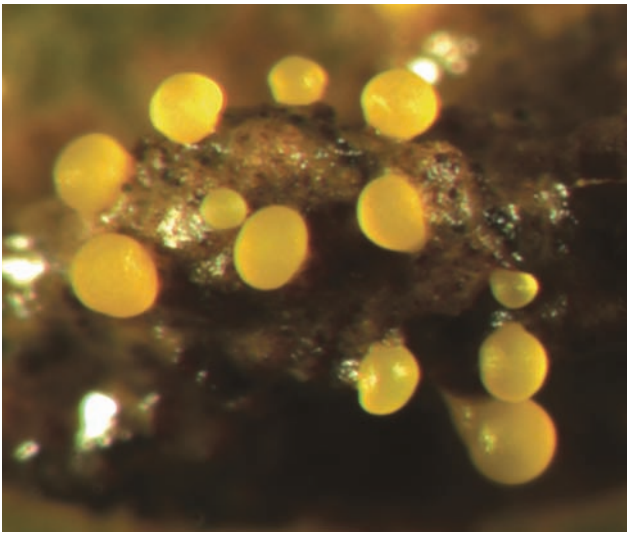
Omphalotus nidiformis, or "ghost fungus," glows in the dark because of its bioluminescence.

Bioluminescence is prevalent in many groups of organisms. You may have seen such glows in the wake of a boat, especially in the tropics due to luminescent dinoflagellates, which are eukaryotes, not bacteria. You may also have seen the faint glow of luminescent fungi in some woods. To see bioluminescent bacteria, it helps to have a decaying marine fish on a beach or crustaceans such as a bucket of old shrimp destined to be used as bait. If you are lucky and if it is dark enough, the fish or shrimp may glow faintly.

The other concentrated area where bioluminescent bacteria may be seen is inside of certain marine fish and invertebrates. Sometimes the bacteria merely reside in the digestive system and are not especially visible. However, in many marine animals, the relationship has evolved into a real symbiosis with the glowing bacteria being kept in culture-chamber-like pockets of the digestive system. They are part of the communication and behavior of the host animal by which mates are attracted, prey is lured, and predators are startled. The connection between such symbioses and that glowing fish on the beach may be this: a dead glowing animal is that much more attractive to the animal that might eat it, as is well known to fisherman who use light sticks as bait. Some luminescent bacteria seem to be involved in a cycle that takes them from animal to animal. When the first host dies, the bacterial glow is readily spotted and fish and bacteria together are consumed by the next host.

Lilliputian Bacterial Gardens

Delta-proteobacteria include myxobacteria, which form enormous structures during part of their life cycle. By enormous, I mean as tall as a millimeter (although more likely half a millimeter) and often colorful (yellows, oranges) and with little branches and knobs. The life cycle goes like this. Throughout the leaf litter and soil on the forest floor are many bacteria, including highly motile rod-shaped myxobacteria that travel around in pack-like formations, consuming whatever nutrients they encounter. Sometimes nutrients are scarce, in which case thousands of myxobacteria converge and erect an enormous structure (sometimes called a fruiting structure) into the air on which tiny spores are formed. Water or wind carries these spores to some distant place (perhaps meters away) and tiny rods emerge and continue their lives in the soil. The structures of myxobacteria are so unbacteria-like that



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Myxococcus xanthus cells amassed into a fruiting body with spores.

they have been mistaken for tiny fungi or slime molds (myxomycetes, which are fascinating in their own right). Some myxobacteria may even be found preserved in herbarium collections. There is certainly a learning curve with identifying any tiny fungal-looking structure in the woods, but some naturalists love doing exactly that. I recommend getting some books on lichens, fungi, and slime molds (often incorporated into fungal guides) to get a clear idea of what you are not looking for if you are seeking myxobacteria. Many myxomycetes (slime molds) are bigger (by a few millimeters) than the one millimeter or less size of myxobacteria. So (with guidebooks in hand) the intrepid naturalist is advised to look very closely, perhaps with hand lens at that rotting log or bit of leaf litter. The good news is that the community of fungi, lichens, myxomycetes, and myxobacteria, along with the tiny associated invertebrates, is so fascinating and worth examining that even not spotting an actual myxobacterial structure may still be rewarding.

A thirty-minute lecture is not sufficient to describe enough proteobacteria. Watch out for them in subsequent lectures. Keep in mind that the generic Greek letter nomenclature (alpha, beta, and so on) reflects an extraordinary diversity of functions, so expect them to be doing something interesting when they are discussed again.

FOR GREATER UNDERSTANDING

Questions

1. What is the role of alpha-proteobacteria in nitrogen fixation?
2. How does bioluminescence facilitate the movement of bacteria from animal to animal in many marine animals?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Chapters 6–9. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Sagan, Dorion, and Lyn Margulis. *Garden of Microbial Delights: A Practical Guide to the Subvisible World*. Dubuque, IA: Kendall/Hunt Publishing Company, 1993.

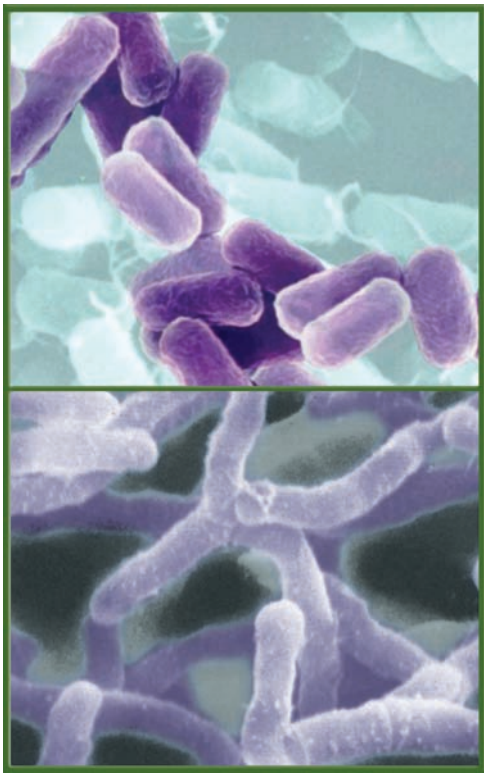
Lecture 8:
An Enormous and Diverse Group: The Gram Positives

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* chapters 10 and 12.

When Dr. Hans Christian Joachim Gram discovered that the textile dye crystal violet (later named Gram stain) colored some bacteria but not others, he did not realize the broad taxonomic significance or that this seemingly trivial staining technique was different from most others that were being developed in the late nineteenth century. Gram-positive bacteria, those that become permanently stained with crystal violet, have different cell walls from Gram-negative bacteria, and those walls cause Gram stain to be retained, coloring the bacteria purple. Furthermore, there are enough other significant differences, separating Gram positives from all the rest of bacteria and archaea, that Gram positives occupy their own major, well-defined branch on the phylogenetic tree.

Like the proteobacteria, the Gram positives are an enormous and diverse group. The focus of this lecture will be just a few charismatic examples that you might have a chance of observing yourself once you know the field marks. Like the proteobacteria, the Gram positives have had a long history of intensive study in the lab. About a quarter of the bacterial genomes sequenced and stored at NCBI are of Gram positives. As with the proteobacteria, the prominence of Gram positives in the database may reflect their accessibility to human researchers and the medical and economic importance of some of them and may not necessarily represent any majority status among all bacteria and archaea.

Gram positives are divided into two sub-groups, Firmicutes (meaning firm skin) and Actinobacteria (meaning thread-like bacteria). Both groups have members that are easily observed, as they



False-colored scanning electron micrographs showing examples of Firmicutes (top) and Actinobacteria (bottom).

comprise a large part of our own personal bacteria (our normal microbiota), and as a natural extension, they comprise the majority of bacteria that enhance our foods and drinks through fermentations. The Gram positives also are important inhabitants of the soil community of microbes, the topic of the next lecture.

Our own natural microbiota is just that: "natural," completely normal, and even highly desirable compared to the opposite situation of our bodies being devoid (essentially sterile) of any bacteria. We have bacteria all over our skin, especially in moist areas such as any part covered by clothing and abundantly under the arms and in the genital area. For example, a square centimeter of an armpit may harbor almost 2.5 million bacteria. Our moist mouths and noses are teeming with bacteria and our digestive system is essentially a culture chamber for a vast metropolis of them. Our bacteria comprise about 10 percent of our body weight and far outnumber our own cells. We have about 10^{13} of our own cells and are carrying around an additional 10^{14} bacterial cells. That means 90 percent of "our" cells are bacterial. Despite their constant intimacy with us, we know relatively little about our bacteria and their activities, most of which seem benign and many of which appear to be beneficial. A DNA sequencing initiative is underway to explore and identify (and perhaps better appreciate) the thousands of species that are estimated to be on and within us.

The world might seem full of pathogenic bacteria, but that is an illusion created by industries that depend upon a heightened (and constantly renewed) anxiety resulting in (they hope) the purchase of their anti-bacterial products. Even news reporting on bacteria is not necessarily independent of business interests. In some cases a news story may come almost verbatim from an industry press release promoting some new product against bacteria. I am not cavalier about real bacterial infections and I certainly use antibiotics when they are warranted. I have no wish to return to the days before antibiotics, when people died from what we now think of as routine bacterial infections. However, I do not think that we all need to be scrubbing like surgeons several times a day (sometimes with harsh chemicals not even rinsed off) or keeping the play environments of babies and children as sterile as possible. On the contrary, a growing body of convincing research suggests that the normal bacteria in our lives keep our immune systems healthy, on the alert, well prepared and ready to go when we need to fight off serious infections. Allergies and asthma (and other indications of immune systems gone awry) may develop more readily in children who are not allowed to play in the dirt or have pets. Furthermore, by having nearly every habitable space on and in our bodies taken up by our benign or beneficial bacteria, there is little room left for a pathogen to get access. That inaccessibility plus our vigilant immune systems go a long way to keeping us healthy.

If our normal body bacteria can be described as benign and even beneficial, what exactly are they all doing, besides occupying space such that pathogens cannot get access and keeping our immune systems on alert? In short, we represent an enormous supply of nutrients in the form of our many excretions (sweat, oil, saliva) and the constant renewal of cells, especially of the skin and digestive system. Furthermore, we have a long tube down the center of our bodies, which we tend to keep packed with food in various stages of digestion.

The Gram positives that have been identified as beneficial include some that help to convert and make more available to us certain nutrients. Many of our Gram positives and proteobacteria produce Vitamin B_{12}. In addition, the proteobacterium *Escherichia coli* synthesizes vitamin K, which we then absorb. There are likely to be many more beneficial functions, to be discovered when we have a better idea of the full diversity of our microbiota.

At their most benign, our myriad bacteria are enjoying (in their various specialized ways) our secretions, our discarded cells, and especially all that abundance of food we take in. Sometimes their activities contribute to our normal body odors (which may be considered field marks) including those odorous gasses emitted from the digestive system at either end. (We have been taught that such odors are undesirable and entire industries thrive on keeping those odors in check. However, various body odors typically are not signs of any pathogenicity whatsoever, but are indicators of our microbiota at work.)

Here are some experiments on your body bacteria that you can do or that you in fact have already done.

1. You can experiment with different foods to find out their effects on the emissions of your digestive community. Actually, you probably do this experiment all the time. Some fibrous foods and legumes cause your bacteria to produce more gas than usual. Some especially flavorful foods, such as those made with garlic and onions (which have trace amounts of savory sulfur compounds), can cause your sulfur metabolizing bacteria to release trace (but detectable) amounts of odorous sulfur-rich gasses.
2. Your “experiments” with antibiotics have most likely been done out of necessity under orders from a doctor. Some courses of antibiotics kill many more than the harmful bacteria from which they were prescribed. A side effect may be acute intestinal distress as your metropolis of benign and beneficial bacteria are displaced and disrupted.
3. Experiments with natural prebiotics and probiotics are encouraged. Prebiotics are those foods that seem to promote the well-being of your intestinal bacteria and therefore of yourself. Your bacteria enjoy certain fiber-rich foods such as whole grains. Have a bowl of oatmeal for them! Probiotics are doses of the bacteria themselves. You can consume yogurt and other bacterially fermented products and also freeze-dried bacterial cultures in capsules or tablets, now commonly sold in drug stores or health food stores. Probiotics are recommended to counteract the distressing side effects of some courses of antibiotics and to more quickly restore your microbiota to its normal function.

It is not just coincidence that the very same bacteria of fermented foods and drinks are the ones that can restore the bacteria of your body. From a bacterial point of view our bodies and our food are on a continuum. Our body bacteria are the same ones to be found around a kitchen, tumbling into a pail of milk, destined to be cheese. Our ancestors did not so much invent all the great fermented cuisines of the world but rather tolerated and then appreciated the inevitable, unstoppable presence of bacteria in food.

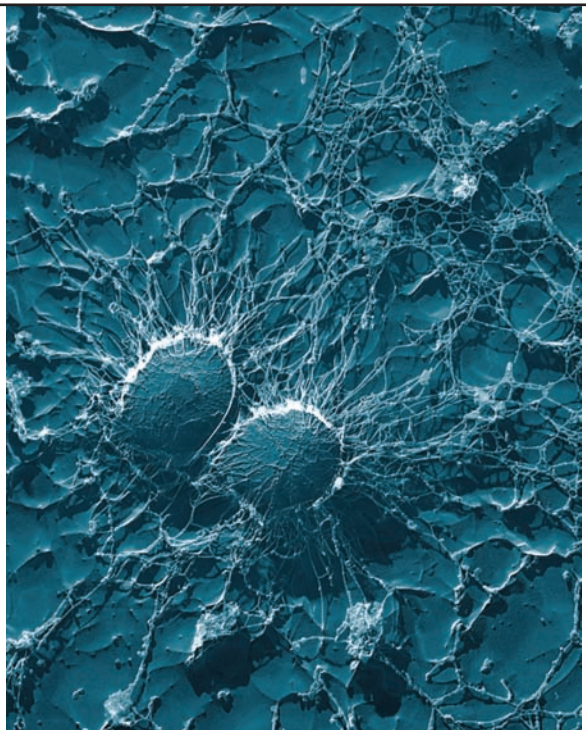
Refrigeration as a preservation method is very recent. Most food storage throughout the history of humans and throughout many parts of the world today is at room temperature. Furthermore, food gathering and saving was a major occupation of our hungry ancestors. If they were hungry, they were not likely to discard items that had a few discolorations, bubbles, and scums due to microbial activities. Thus cheeses were developed over and over again throughout all milk-drinking cultures. Asian cultures in which soy beans are important have fermented soy products of all kinds. Likewise, there are fermented fish products, fermented grains (ranging from sour-dough bread to beer), fermented fruits (including wine), fermented vegetables (such as kimchi, sauerkraut, and pickles), and on and on—representing all of the most flavorful and delicious foods in all cultures.

Fermented foods constitute an effective preservation method in that the first colonizers tend to be benign members of our own microbiota that then occupy every available space in the food, thereby preventing subsequent invasions by less desirable, competing bacteria. It is a similar role to that of taking up all available space in our bodies. By the way, we also harbor many species of fungi and these too live in a continuum with ourselves and our food. Although a long digression on fungi will not fit here, I will briefly acknowledge them for all of their fungal fermentations without which some of our most important foods and drinks would not be possible: bread, wine, beer, and all of the cheeses with blue and white fuzzy molds enhancing them.

Fermented foods are also powerful shibboleths, defining cultures by their preferences for particular fermented flavors introduced in childhood. No matter how assimilated an adult might want to be into a new culture, a challenging step is to truly enjoy and even crave some particularly odiferous fermented dish (like limburger cheese or aged, fermented shark) and not merely politely tolerate it.

Some of the bacteria of our bodies and of our cuisines are worth mentioning by name because several have a sort of dual role as opportunistic pathogens under some very specific circumstances. This will be further elaborated upon in Lecture 10 on pathogenicity.

Some red wines are mellowed by the activities of malo-lactic fermenting Gram-positive bacteria. Most flavorful cheeses are bacterial products. In addition, fungi (eukaryotes) make major contributions to our fermented foods and drinks. Blue *Penicillium* fungi are in the blue cheese and the primary fermenters of the red wine are yeast, which are also fungi.



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False-colored scanning electron micrograph of *Staphylococcus aureus* (literally "Golden Cluster Seed" and also known as "golden staph") is the most common cause of staph infections.

Among the Firmicutes (one of the two main groups of Gram positives) of our bodies are *Staphylococcus* and *Streptococcus*, nicknamed "staph" and "strep" and often implicated with skin and throat infections of those names. How could they be both normal inhabitants and well-known pathogens? They are among the "opportunistic" pathogens that most typically lead non-pathogenic existences. Our healthy immune systems, unbroken skin, plus our hoards of benign bacterial inhabitants keep most of the opportunistically, pathogenic Gram positives in check. Opportunities arise, unfortunately, in patients whose immune systems are already taxed or compromised by fighting other infections, enduring debilitating therapies, and healing deep wounds. However, our daily, normal encounters do not typically put us into danger.

Lactobacillus and *Lactococcus* are among the beneficial Firmicutes of both fermented milk products and our digestive systems. Look at the ingredients of a container of yogurt and you will most likely see some variations on those names and others with a "lacto" prefix. As yogurt makers become more and more conscious of their product being not only a food, but also a probiotic, these labels have been getting longer and more specific with names of bacteria. All are in the Firmicutes and include such genera as *Streptococcus* (of a species that never is pathogenic) and *Bifidobacterium*.

Of the actinobacteria (the other main group of Gram positives) are *Propionibacterium* and *Brevibacterium*, both excellent examples of bacteria enjoying a continuum from their human hosts to the fermented foods of their hosts. *Propionibacterium* on the skin dwells in active sebaceous glands consuming the secretions. At their worst, they are implicated in acute acne infections. However, that same genus fermenting milk produces the nutty flavors and big holes of swiss cheese. *Brevibacterium* dwells between the toes breaking down the protein of the skin cells that are regularly discarded. Protein typically contains some sulfur and these bacteria release odiferous sulfur compounds as a waste product. That smell of foot odor is replicated in

a group of delicious cheeses called “surface washed.” They often have a light pink scum of brevibacteria on their surfaces and include notorious (shibboleth-like) examples such as munster and limburger.

Not a Gram positive, but an extremely important inhabitant of our digestive system is the genus *Bacteroides*. It has its own distinctive main branch on the phylogenetic tree. About a quarter of the weight of your feces (which reflect the contents of your intestines) is the bacterial group *Bacteroides* and its relatives. What is notable is that this important group has virtually no pathogenic members, but rather seems to have had a long benign history of co-evolution with us. The sole exception is their accidental introduction into surgical wounds of the abdomen, in which case they may cause a treatable infection.

Escherishia coli of the proteobacteria deserves a brief mention too. In its normal place and in normal numbers it is a beneficial bacterium in our digestive system, supplying us with vitamin K. So why do we close down beaches and declare emergencies for our water supply when “coliform” counts are up? Too much *E coli* (and its close “coliform” relatives), for example, ingested via a big gulp of water, can be extremely disruptive of the normal balance of microbiota, with consequent intestinal distress. A high coliform count is also often indicative of sewage contamination to a beach or water supply and that in itself is worth correcting not just because of *E coli*, but because of all the other contents of sewage, including viruses, household chemicals, and odiferous and foul-tasting molecules. Note that travellers’ diarrhea, often caused by *E coli* and its relatives in the water supply, is (as the name implies) a problem for newcomers to an area and not necessarily to the regular inhabitants. Remain in an area long enough and your system (and microbiota) may adjust such that the normal bacteria in the water no longer gives you trouble.

FOR GREATER UNDERSTANDING

Questions

1. How do bacteria help to protect our bodies?
2. How have bacteria left a distinctive mark on the relationship between food and various cultures?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Chapters 10 and 12. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Katz, Sandor Ellix. *Wild Fermentation: The Flavor, Nutrition, and Craft of Live-Culture Foods*. White River Jct., VT: Chelsea Green Publishing Company, 2003.

Marples, Mary J. *The Ecology of the Human Skin*. Springfield. IL: Charles C. Thomas, 1965.

McFall-Ngai, Margaret, Brian Henderson, and Edward G. Ruby, eds. *The Influence of Cooperative Bacteria on Animal Host Biology*. Cambridge: Cambridge University Press, 2005.

Tannock, G.W. *Normal Microflora: An Introduction to Microbes Inhabiting the Human Body*. New York: Chapman and Hall, 1995.

Van Slyke, Lucius, and Charles Publow. *Science and Practice of Cheese-Making*. Reprint. Carlisle, MA: Applewood Books, 2001 (1913).

Lecture 9:
Gram Positives in the Soil Community

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* chapter 11.

The Harvard naturalist E.O. Wilson has said "Scoop up a handful of soil . . . and you could be holding ten million bacteria, representing five to six thousand different species. Scoop up a ton of soil and the number of varieties of bacteria could jump to four billion, considerably more than the number of animals and plants now known" (from the *Harvard University Gazette*, June 15, 2006).

Soil is a vast microcosm of tiny organisms, operating in plain sight, but with many of the intricate, complex interactions still poorly understood and with most of the microorganisms still unnamed. It is a biological frontier, as mysterious (and as challenging to access) as the deep sea and the rain forest canopy. Indeed, "access" to the soil microscopic community may always be indirect, an extrapolation via informed imagination. So, imagine creeping or gliding through a subterranean fractal-like network of moist passages in and amongst boulder-like sand grains. Now and then you come upon island-like rafts of nutritious materials of diverse kinds, from trees, from feathers, from feces, from pretty much anything that has fallen to the forest floor, perhaps after all the most tender and digestible parts have been consumed by large animals. Some of your fellow microbes are engaged in a deadly competition for those nutrients. Others are huddled in a temporary arrangement of close interactions that might look like "collaboration," by which multiple strategies release nutrients from especially tough and intractable materials. These are the "decomposers," and their activity is decay. They are not only makers of soil, but they are themselves an integral, defining part of the soil. Take up a handful of the richest compost or garden dirt or forest floor rotting litter that you can find. Look closely and smell it. You will most likely recognize the familiar earthy odor that actually has a name, "geosmin" (odor of the Earth), and which is a product of, and therefore a field mark of, the Gram-positive actinobacteria that are abundant in good soils. If decomposition is especially active (as in a compost heap), you may also see fine white threads weaving through. The threads are fungi, the major eukaryotic decomposers that operate side by side (often in competition) with the soil bacteria. Actinobacteria also have thread-like morphologies, although much too small to be seen with the naked eye. Both actinobacteria and fungi, each on their own scale, use their threads to encompass and enclose as much nutrient material as possible in a sort of possessive network that establishes boundaries and limits access of other decomposers.

Furthermore, both actinobacteria and fungi synthesize and release toxic, often lethal compounds, antibiotics, into their environment to stop other microbes from encroaching on a nutrient particle. The antibiotics of soil microbes are the same ones (either natural or synthetic based on natural

structures) that have been an essential part of modern medicine ever since their discovery. Robert Koch and Louis Pasteur were among the early microbiologists who noticed that colonies of bacteria and fungi grown close together on Petri plates often inhibit each other, forming clear zones where neither can advance toward the other. In 1928, Alexander Fleming was among those who had the insight that such activities could be used as treatments of microbial infections. He developed penicillin from *Penicillium* fungi, commonly observed on old bread as blue fuzzy colonies. *Penicillium*, with its long threads, coursing throughout the bread, plus the release of its antibiotic, can outcompete many other kitchen microbes for that nutrient bonanza. Following the discovery of penicillin, many other soil microbes were mined for their antibiotics, and as a result, we have hundreds to choose from. Thus soil microbiology became an important subject, not just for its intrinsic interest, but as a source of medicines, as in the prolific work of Selman Abraham Waksman and colleagues who discovered many new antibiotics throughout the twentieth century.

One catch, though, is that the microbes have evolved many mechanisms, coded for by genes, by which antibiotics may be resisted. Horizontal transfer of DNA is one of the ways that communities of soil bacteria (or alas communities of hospital bacteria) can pass around among themselves snippets of DNA, some of which just happen to carry genes for resisting antibiotics. Those microbes that by chance receive those resistant genes thrive if the environment happens to be full of antibiotics. The following point will be reiterated in Lecture 10 on bacterial pathogens. Having genes for resisting antibiotics confers no special advantage if there are no antibiotics in the environment. Indeed, such extra genes may be considered excess baggage and a disadvantage. Antibiotic-resistant genes are an important advantage in environments full of

antibiotics. Which environments are full of antibiotics? The soil environment is one and plenty of horizontal transfer is going on between those soil organisms by which resistant genes get passed around. The hospital environment is one in which, of necessity, large amounts of antibiotics are in use to treat seriously ill patients and there is plenty of horizontal transfer going on between hospital bacteria. Furthermore, those bacteria that unfortunately for them do not receive resistant genes in a hospital are soon dead. It is a very effective way to establish a large population of resistant bacteria. Two other environments that are sometimes full of antibiotics (with predictable consequences) will be addressed in Lecture 10; they are industrial-scale animal stockyards and our own bodies when we misuse antibiotics for non-bacterial ailments.

In addition to all that competition by soil microbes armed with invasive threads and antibiotics against each other, there are plenty of examples of interactions ranging from brief exchanges to full-fledged symbioses. The word "collaboration" has human-centered connotations and is maybe not exactly right to describe relationships between microorganisms. However, there are numerous examples of associations that allow mixed groups of microbes to live more efficiently and ultimately to leave more offspring, both of which count for a lot in evolution.

One example (of many) is the community that undertakes the decomposition of protein-rich (therefore nitrogen-rich) nutritious material. The trick seems to be specialization. No single microbe in the group can completely break down and completely utilize every part of the food. Together, through a network of reactions, they can each participate in some small part of the process. In this simplified sequence, first "ammonifiers" attack the newly deposited protein (a bit of animal waste) using protease enzymes to break down the nutrient into ammonia. (The smell of ammonia, if it is not overpowered by other products of decay, is a field mark. Sometimes you can smell ammonia in a chicken coop or cat litter box.)

Then two types of "nitrifiers," one after another, convert the ammonia into nitrite and then the nitrite into nitrate. Both of these are chemoautotrophs, which use the energy in the bonds of the ammonia or nitrite to make sugars. You can observe field marks of nitrifiers when you establish them in a natural aquarium filter to decompose waste products from fish. The aquarium tests that you use to detect levels of ammonia, nitrate, and nitrite help you to visualize the field marks of bacterial activity.

Next are the "denitrifiers," who take up that nitrate and use it as we use oxygen in our own metabolism. (You could say they are "breathing" nitrate as we breathe oxygen.) They release nitrogen gas into the atmosphere. At this point, the nitrogen from that starting material (the animal waste) has been broken down into its smallest unit: N_2. Recall from Lecture 7 that plenty of bacteria can use nitrogen gas in their own metabolism. This will also be reviewed in Lecture 12 on cyanobacteria. Thus the cycle goes on.)

By the way, fungi have not been done justice in this lecture. They were mentioned only briefly as forming large (visible) white threads and antibiotics with which they compete with various bacteria. Fungi are prodigious decomposers with many specialized digestive enzymes by which they can reduce entire logs to soil in a few years. The focus was on bacteria, but fungi should not be overlooked for their often dominating role in soil formation.

FOR GREATER UNDERSTANDING

Questions

1. How do actinobacteria and fungi stop other microbes from encroaching on nutrient particles?
2. What did Robert Koch and Louis Pasteur notice about colonies of bacteria and fungi grown close to each other?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Chapter 11. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Scott, Nicky. *Composting: An Easy Household Guide*. White River Jct., VT: Chelsea Green Publishing Company, 2007.

Lecture 10:
Bacteria as Pathogens

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* introduction.

When I think about bacterial pathogens, I like to turn some of the usual questions around. I do not ask why there are so many (because there aren't), but rather I ask why there are so few. Who are those rare microbes that are able to get through the dry, inhospitable boundary of our skin and past the vigilant and multifaceted components of our immune systems to establish themselves as pathogens within our bodies? How are pathogens different from the vast majority of bacteria, which either cannot or will not have any sort of relationship with us (or any other host), whether pathogenic or not. How did human pathogens become associated with us to begin with? We've been evolving as a species less than one million years. What were those bacteria of ancient lineages doing previously? And if we manage to answer these questions, will we be a little further along to understanding pathogenic bacteria and perhaps controlling infections better?

How unusual are pathogens? Recall from Lecture 5 that estimates vary widely as to how many total species or types of bacteria there are. This is because "species" as a concept is difficult to define in the fluid genetic landscape of the bacterial world, where genes are handed around with such promiscuity. It is also because we are still looking and have not yet exhaustively searched all possible habitats, including many that are deep in oceans and sediments. However, let's say it is about one million species of bacteria, which is a memorable round number, but probably quite conservative. There must be at least one million different habitats for bacteria to occupy if you count every animal, plant, fungus, and protist as potential surfaces covered with bacteria, not to mention the vast fractal dimensions of the physical world. In contrast (to a conservative one million) there are about fifty pathogenic bacteria of humans. If each pathogen were given its own collectible trading card with its photo on the

Escherichia coli (*E. coli*)

Escherichia coli or *E. coli* is a typical and normal inhabitant of our gastrointestinal tract, producing, among other things, Vitamin K. It is rarely a pathogen and usually only an opportunistic pathogen, gaining access in situations when the immune system is compromised, for example. However, some particularly invasive and antibiotic resistant strains have also developed, perhaps in connection with misuses of antibiotics.

front and vital statistics on the back, the cards would form a stack about the size of a deck of playing cards, which is a good mnemonic aid for remembering the number of pathogens. Indeed, the American Society for Microbiology sells a set of cards for each bacterial pathogen (as well as for other types of pathogens such as some viruses, fungi, and protists). Master those fifty bacterial cards and you are on your way to having a pretty good idea of which bacterium is causing an infection. When a hospital lab runs tests to identify the source of an infection, they are not starting with a list of all possible bacteria, but rather a very short list of likely candidates.

What do the pathogen trading cards tell us?

There are twenty-three Gram positives (firmicutes and actinobacteria). They include various species of *Streptococcus* (strep), of *Staphylococcus* (staph), of *Bacillus*, and of *Clostridium*, common inhabitants of soil and of our skin and digestive tracts as well as those of all animals. The majority are opportunists that reveal pathogenic characteristics only in those circumstances when immunity is down or there is a serious breach in the barrier of our skin.

There are twenty-six pathogenic proteobacteria in the deck of cards. Many are inhabitants of our digestive system and nasal passages and are opportunistically pathogenic, awaiting the unfortunate circumstances of a compromised immune system, deep wounds, and invasive, disruptive therapies. The vast majority of "our" bacteria seem to have coevolved with ourselves (and our immune system). Our coexistences are mostly peaceful or tolerant. The exceptions are well worth looking at for their exceptionally unstable relationships with us.

I sorted the cards to remove all the bacteria that typically do not become highly invasive. Their symptoms tend to be transient and controllable, albeit unpleasant, causing, for example, diarrheas, skin infections, ear aches, and respiratory infections. Several are capable of causing pneumonia, which can be fatal if left untreated or if immunity is down, but even these pneumonia-causing bacteria tend not to be obligately pathogenic and consistently invasive. I focused on the truly invasive proteobacteria and Gram positives, which manage to slip by our defenses and get deep into our organs.

The most pathogenic of proteobacteria are few and most seem to require injection, not mere inhalation or ingestion. An additional factor with these serious pathogens seems to be newness of the relationship with the host, us. *Yersinia pestis* causes bubonic plague and is injected by blood-sucking fleas. Its enormous success in killing a quarter of the population of Europe during the Middle Ages suggests that it had newly arrived from a different host and was experiencing an extremely dysfunctional relationship with its new host, humans. Indeed, that was the case. Overcrowded cities bred rats (the usual host in which *Yersinia pestis* is endemic and not especially lethal or even debilitating) and rat fleas. Encounters of rats and fleas with humans were greatly facilitated.

Another proteobacterial pathogen is *Francisella tularensis*, which causes plague-like tularemia and is transmitted by ticks or deerflies from rabbits in which it is not particularly pathogenic. The proteobacteria *Rickettsia rickettsii* and *Rickettsia prowazekii* are injected by ticks and lice, respectively. These two are additionally interesting for the greatly reduced sizes of their genomes

(total DNA) (compared to less pathogenic relatives). This is often a hallmark of a close obligate relationship between host and bacteria such that most of what the bacteria require is provided by the host, precluding the need for certain sets of genes. Both of these *Rickettsia* dwell inside of cells, suggesting a long evolution of intimacy with a host, although not necessarily a human host, wherein lies the problem. The relatively new and intimate relationship with us results in Rocky Mountain spotted fever and typhus, respectively.

The most pathogenic of the Gram positives in the collection are the mycobacteria, some of which have evolved an obligate relationship inside of our cells. That means they do not have any significant free-living existence. The two most important are *Mycobacterium leprae*, which causes leprosy and *Mycobacterium tuberculosis*, which causes tuberculosis. How did we get into such a negative relationship with these two? In many cases people may be exposed to mycobacteria, but will successfully fight off infection with a healthy immune system. However, the obligate association with a host combined with pathogenicity again suggests a fairly recent and unsettled relationship between us and them.

The remaining cards are of two other groups of bacteria, the chlamydia, which are obligate occupiers of their host's cells and have no free living existence outside of a host, and the spirochetes. There are three major pathogenic spirochetes of humans. Their long, thin morphology with a corkscrew-like motility enables spirochetes to bore into the densest of tissues. Their invasiveness combined with a tendency for two of them to be injected as well as their lack of a free-living existence makes them among the most serious pathogens in the set of cards. They are *Borrelia burgdorferi*, injected by ticks and causers of Lyme disease; *Borrelia recurrentis*, injected by lice and causers of relapsing fever; and *Treponema pallidum*, bearer of syphilis and related diseases.

So in general, injectable and/or invasive pathogens with no other particular life-style other than pathogenicity and especially those that seem to be in a relatively new relationship with us are the truly serious ones to watch out for. Ironically, these are not usually the ones that manufacturers of hand scrubbing and home sterilization products are focusing on. It is a good thing that most people have functional immune systems that provide important protection. Studies suggest

Treponema pallidum spirochetes

Electron micrograph of *Treponema pallidum* spirochetes using a modified Steiner silver stain.

Treponema pallidum is a Gram-negative spirochete bacterium. There are at least four known subspecies: *T. pallidum pallidum*, which causes syphilis; *T. pallidum pertenue*, which causes yaws (a tropical infection of the skin, bones, and joints); *T. pallidum carateum*, which causes pinta (a human skin disease endemic to Mexico, Central America, and South America); and *T. pallidum endemicum*, which causes bejel (or endemic syphilis).

that in order to keep our immune systems vigilant, healthy, and responsive, we may actually need frequent encounters with most of the ordinary (non-pathogenic or rarely pathogenic) organisms that abound in soil and are a natural part of our skin and digestive microbiota.

However, there is an additional point concerning antibiotic resistance, which will be reiterated from Lecture 9 on soil bacteria.

Bacteria pass around bits of DNA in a process called horizontal transfer. Some of that DNA contains genes for resistance to antibiotics. Having genes for resisting antibiotics confers no special advantage if there are no antibiotics in the environment. Indeed, such extra genes may be considered excess baggage and a disadvantage. Antibiotic-resistant genes are an important advantage in environments full of antibiotics. Which environments are full of antibiotics? I have already mentioned soil and (unfortunately) hospitals, where antibiotics must be in constant use, a situation that is unavoidable.

Additional environments abundant in antibiotics include our own bodies when we are taking a course of antibiotics for a bacterial infection. Finishing the entire course of antibiotics no matter how good you begin to feel is recommended to be sure there is no potential for resistant forms (via horizontal transfer) remaining. Flooding your body with antibiotics when you do not have a bacterial infection (when, for example, you have a viral infection like a cold or flu) is a terrible idea. It sets up conditions nicely by which some of your normally benign bacteria may pick up and keep (under the circumstances) some new antibiotic-resistant genes. A few misuses of antibiotics are not egregious, but to make it a regular habit is to play with a powerful system, horizontal gene transfer, by which resistant strains are easily created.

The worst and most dangerous abuse of antibiotics that I know of occurs regularly (and with very little control) in agricultural industries that have scaled up and mechanized and streamlined some of their routine practices. Cattle feed lots are an excellent example. There, large numbers of crowded animals are fed an unnatural diet (vast amounts of grain) to fatten them quickly. Many animals get seriously ill and most just have constant intestinal problems from the diet. To keep the animals eating constantly and alive long enough for slaughter, huge amounts of antibiotics are added to the diet. That, in brief, is why some of the most horrific mutant forms of extraordinarily pathogenic and antibiotic-resistant bacteria are found from time to time in our food supply. Sometimes the pathogens arrive via contaminated meat of animals fed antibiotics. Sometimes they arrive via vegetables, fertilized with manure from animals fed antibiotics. And here the identities of the bacteria are the most disconcerting of all: they are one and the same with species that I have just finished describing as mere opportunists, usually easily controlled by our immune systems and typically benign inhabitants of ourselves, our fermented foods, and good soils. They include highly invasive, sometimes deadly, and distressingly antibiotic-resistant forms of *Escherischia coli* and *Staphylococcus aureus*. It is a preventable situation whether on the individual level by insisting on eating locally or on a larger scale through legislation. Meanwhile, the situation is that my pack of pathogen identification cards may have to grow by a few cards to keep up with the new pathogenic forms that we are creating ourselves.

FOR GREATER UNDERSTANDING

Questions

1. What are some of the common characteristics of the most seriously pathogenic bacteria?
2. How does the agricultural industry produce horrific mutant forms of pathogenic and antibiotic-resistant bacteria?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Introduction. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Peppler, Mark S. *MicrobeCards: Medical Microbiology and Infectious Diseases Study Cards*. Washington, D.C.: American Society for Microbiology Press, 2003.

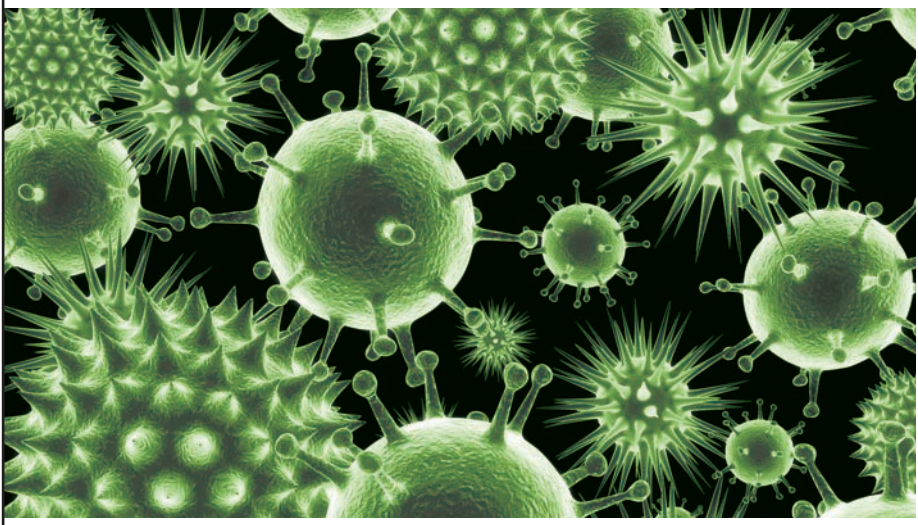
Lecture 11:
What About the Viruses?

Professor Dyer suggests that students interested in pursuing further study on bacteria, archaea, and viruses should investigate any up-to-date college textbook on microbiology.

What Is a Virus?

Viruses are not organisms. They are tiny snippets of DNA or RNA. Essentially, they are genetic information. Viruses may be found inside all organisms. In fact, they have no active existence outside of an organism. Archaea, bacteria, protists, fungi, animals, and plants all have their particular repertoires of viral DNA and RNA. The more we understand about viruses, especially now that entire genomes of organisms can easily be sequenced, the more we realize that most viruses are a normal component of the genetic information of an organism. This is quite a different idea from the more traditional interpretation of viruses as pathogens.

Viruses were discovered indirectly in the late nineteenth and early twentieth centuries by observing the damage they caused to other organisms. For example, some viruses of bacteria cause the bacteria to break open. Some viruses of plants cause major changes in plant cell function sometimes resulting in discolorations and distortions of plant tissue. The viruses themselves were not seen. For many decades the only way to detect a virus was to look for some sort of damage to a host organism. There simply was no convenient way to look for benign or beneficial viruses. Therefore, in the logic of somewhat circular reasoning, all viruses were pathogens.



An artist's rendering of several types of viruses based on electron microscope scans.

When electron microscopy was developed for laboratory use in the mid-twentieth century, scientists got a look at some viruses for the first time. These particular viruses all had one important property: the ability to produce a protective protein coat around the DNA or RNA. The electron microscope photos revealed wonderful geometric structures in a range of fantastic shapes from “geodesic” spheres to spirals. As a result, viruses became physical entities, as well as pathogens. Indeed, there was no convenient way to seek out viruses that never make beautiful protein coats.

Billions and Billions

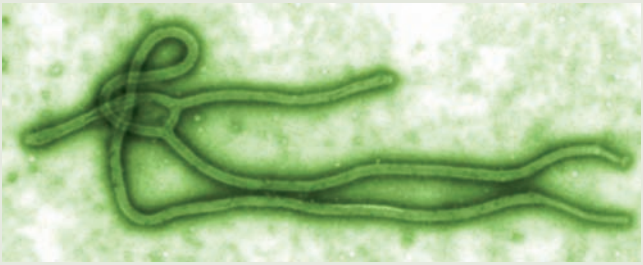
A surprising revelation about viruses came about through the routine sequencing of entire genomes. We organisms (especially eukaryotic organisms) are loaded with viruses and they are mostly not pathogenic and mostly not capable of producing protein coats. Humans have about three billion DNA bases (As, Cs, Gs, and Ts) comprising their genomes. Less than 10 percent of all that DNA codes for genes, in spite of their having long been considered to be the most important functional parts of our DNA. The big surprise is that about one-third of our genome is viral DNA and it is currently quite a mystery as to the significance of all that viral information. Much of it may be benign, but some of it seems to be beneficial. About half of our DNA is another enigmatic type of genetic entity called “transposons” or “jumping genes,” which may turn out to be some evolutionary derivative or precursor of viral DNA. Therefore (adding together one-half and one-third) we find that five-sixths of our genomes are viral or something close to viral. This is really a topic for a genetics class and indeed that is where I typically teach about viruses. I decided to bring viruses briefly into this course about bacteria because there are so many misconceptions about them, often centered around confusing bacteria and viruses because of the prevalent idea that all viruses and bacteria are just one version or another of pathogens.

Are Viruses Alive?

A question that I sometimes get about viruses is, “Are they alive or not?” The way to answer that is that the question presents a false dichotomy and is essentially not answerable as asked. Viruses are genetic information. Being alive or not is not the question, just as you would not ask, “Is DNA alive or not?” Lately, I’ve been answering that question by presenting viruses as a real mystery, a frontier in biology, and I love to tell the extraordinary fact of our own genomes being so full of viruses, most with completely unknown functions. It is all so much more interesting than, “Are they alive or not?” Neither! They are genetic information. That said, some of the best-studied viruses are serious pathogens and they deserve their own subset of explanations.

Pathogenic Viruses

The pathogenic viruses (a minority of all viruses) are fascinating, and it is well worth comparing and contrasting their versions of pathogenicity with those of the pathogenic bacteria. In doing so we perhaps gain a better understanding of pathogenicity in general. For that purpose, I took out my pack of microbial pathogen cards again. The set of cards includes not only bacteria, but also viruses (twenty-five of them), pathogenic fungi, and some parasites.



© CDC/Cynthia Goldsmith

A false-colored transmission electron micrograph of the RNA virus *Ebola*. It is the virus responsible for hemorrhagic fever in humans.

The first thing to notice in examining the twenty-five major pathogenic viruses is that they have been traditionally classified according to their protein coats and according to whether their information is in DNA or RNA form. That means that in light of the newest genomic information on viruses (most of which do not make protein coats) the entire classification of viruses will have to be completely revised. It is an exciting time to be a virologist or a geneticist, tackling such a problem.

Characteristics of Viral Pathogens

One of the characteristics that is common for viral pathogens is that they all have protein coats. This is why they were first noticed and how they were characterized. The significance of this is that such coats are protective of the viral DNA or RNA, allowing the viral information to get around from one host to another. Furthermore, many protein coats acquire a layer of host molecules, producing a sort of "Trojan horse" effect. A susceptible host cell sometimes "recognizes" a viral particle as being a friend rather than a foe and takes the virus in. The HIV virus is a good example of this "Trojan horse" stealth.

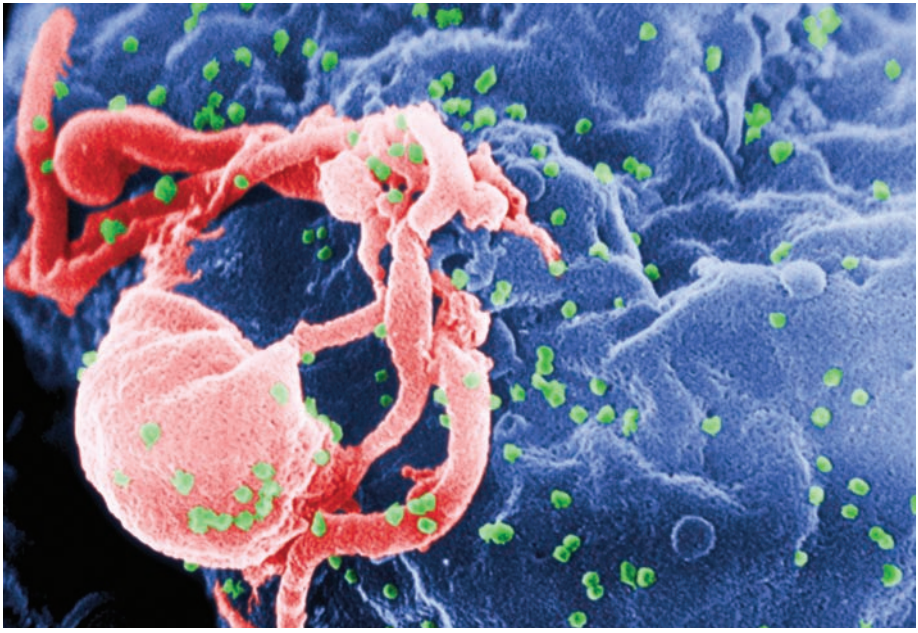
Many viruses are extraordinarily "careless" with their DNA, shuffling it around and making all sorts of new combinations. Imagine cutting up the pages of a short book and re-pasting them into new configurations. Some pathogenic viruses do that regularly and the result is that it can be difficult to pin down their identities and precise characteristics. This is one of the reasons that it is such a challenge to come up with the right vaccine for each year's new version of the flu virus. It is also one of the reasons that there are hundreds of versions of common cold viruses and therefore no single vaccine effective against the common cold. Our immune systems also have difficulty in identifying a virus, such as HIV, which can shuffle its DNA, and so we are not able to mount a sufficient defense against the virus.

The very worst of the viral pathogens seem to have relatively new and therefore somewhat dysfunctional relationships with their hosts. Sometimes a newly pathogenic virus is a novel combination of viruses from two or more other animals, as seems to be the case for flu viruses. Sometimes viruses are introduced directly from another animal where the relationship was benign, but which suddenly becomes pathogenic in the new host. Smallpox is an intriguing example of a virus that is suspected to have arrived suddenly and pathogenically into humans thousands of years ago from another host animal, but that host animal is now extinct. In the case of HIV, however, the

original host animal can be identified and appears to have been an African green monkey.

Misunderstandings about bacterial and viral pathogens sometimes lead to misuse of antibiotics. Antibiotics are ineffective against viruses and should never be used against them. Doing so only sets up conditions by which new strains of antibiotic-resistant bacteria might be created. The exception is any condition in which a viral infection has led to a secondary bacterial infection. For example, a common cold viral infection might lead eventually to a bacterial sinus infection. Under that circumstance, antibiotics might be useful to control the sinus bacteria.

In conclusion, I will reiterate that this entire fascinating topic, which only gets more and more interesting as we analyze new genomes, belongs either in its own dedicated course on virology, or in a genetics course. This lecture has offered only a tantalizing sample of a few of the myriad details about viruses and briefly sorts out the differences between viruses and bacteria. This topic is well worth pursuing in another venue.



Scanning electron micrograph of HIV-1 virus budding from a cultured lymphocyte. This image has been colored to highlight important features. The multiple green-colored round bumps on the cell surface represent sites of assembly and budding of virions. Virions are virus particles. They are the inert carriers of the genome and are assembled inside cells from virus-specified components. They do not grow and do not form by division.

FOR GREATER UNDERSTANDING

Questions

1. What is a virus?
2. Why is “Are viruses alive?” not the right question?

Suggested Reading

Professor Dyer suggests that students interested in pursuing further study on bacteria, archaea, and viruses should investigate any up-to-date college textbook on microbiology.

Other Books of Interest

Peppler, Mark S. *MicrobeCards: Medical Microbiology and Infectious Diseases Study Cards*. Washington, D.C.: American Society for Microbiology Press, 2003.

Villarreal, Luis P. *Viruses and the Evolution of Life*. Washington, D.C.: American Society for Microbiology Press, 2005.

Lecture 12:
Cyanobacteria:
The Original Photosynthesizers

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria,* chapters 13 and 14.

The cyanobacteria have always been the dominant photosynthesizers on Earth and still are, with the exception perhaps of a brief window of time right after the origin of life, when their particular form of photosynthesis had not yet evolved. What cyanobacteria do is absolutely marvelous and explains their enormous success and profound impact on our planet. Cyanobacteria take two of the most unnutritious, unuseful molecules available (water and carbon dioxide) and using the enormous power of light energy from our sun, they cobble together these two molecules and make sugars that are completely nutritious, eminently useful, and actually essential for building themselves and almost all of the rest of the living world. And cyanobacteria do it in such abundance that the rest of the world itself is abundant. The waste product for this marvelous synthesis is oxygen and is the only major drawback, albeit one that has been "solved" in many different ways by diverse organisms such that we all manage to one degree or another on our oxygen-rich planet.

Anabaena sphaerica (Nostocales)

Anabaena is a genus of filamentous cyanobacteria, or blue-greens, found as plankton. It is known for its nitrogen-fixing abilities, which occur in the rounded bead-like cell, the heterocyst, in the center. They also form symbiotic relationships with certain plants, such as the mosquito fern.

One of the generic terms for what cyanobacteria are doing is "autotrophy," a term implying that they need only themselves and a few simple ingredients (carbon dioxide and water) to synthesize all of the sugars they need. The complementary term "heterotrophy" refers to many of the rest of us, who must consume other organisms to get our nutrition. The prefix "photo" or "chemo" may be added to autotrophy to indicate the source of energy for the synthesis of sugar. It is light energy in the case of photosynthesis and chemical bond

energy in the case of chemosynthesis. Bacteria and archaea have evolved several forms of autotrophy, some of which may be evolutionary forerunners of cyanobacterial autotrophy. These other autotrophs may be found abundantly in many particular environments, especially those (such as dark ones) that are not dominated by cyanobacteria. The next lecture (13) will be an overview of major types of bacterial metabolism and will compare and contrast the various autotrophies as well as some diverse heterotrophies.

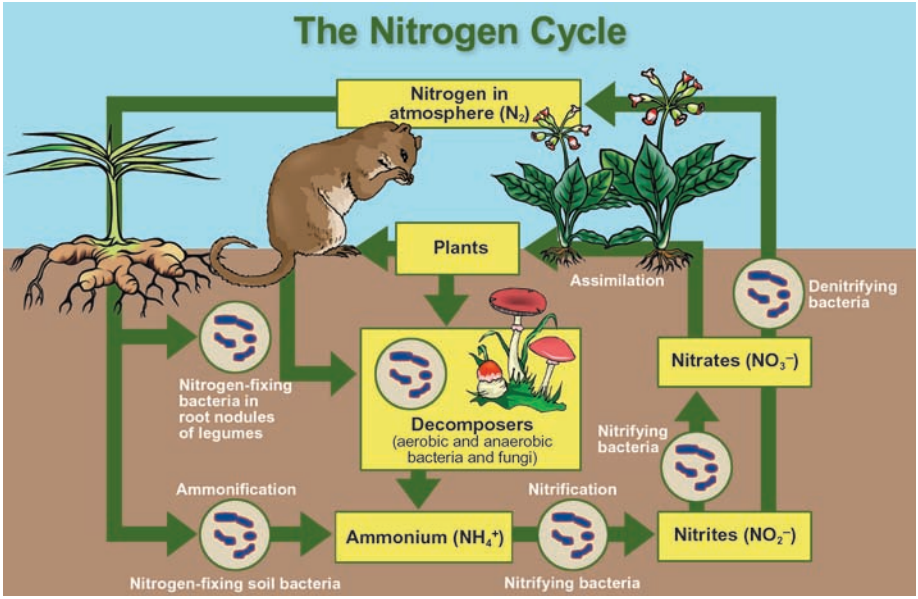
The first microfossils (the ones mentioned in Lecture 6), found only in particular rocks of Western Australia and South Africa, may be of cyanobacteria. In addition to a similar morphology (which is a tricky criterion in organisms with not much morphology), the microfossils have thick protective sheaths. Indeed, the fossils are almost nothing but sheath-like material and this tough outer covering may be the reason they were able to withstand fossilization in the first place. Autotrophs and in particular photoautotrophs are often sheath-makers. They produce such an abundance of material that they can "afford" extra structures like sheaths and bulkier sizes. So if those first microfossils are cyanobacteria, then oxygen-producing photosynthesis must have evolved soon after the putative origin of life at four billion years ago. There is additional evidence: oxygen generated by cyanobacterial photosynthesis began to slowly accumulate in all of the waters, sediments, and finally atmosphere of Earth. At the origin of life, there was none. Now we have about 20 percent oxygen. The consequences of all that oxygen for bacterial metabolism (and actually all metabolism) will be addressed further in the next lecture.

Eukaryotic photosynthesizers (algae and plants) do an oxygen-generating type of photosynthesis too. Therefore, you might ask why we have been so bacteriocentric in giving so much credit to cyanobacteria. The reason is that all algae and plants are descended from one (or perhaps more than one) symbiotic event between an early single-celled, heterotrophic eukaryote and cyanobacteria. This may have happened 2 to 2.5 billion years ago. It was a very powerful symbiotic event (or events) that allowed the host eukaryote to have its own little sugar-producing cells, precluding the need for heterotrophy. Just basking in the sun was sufficient. Indeed, it is still a very successful type of symbiosis: a heterotrophic host with a photosynthesizer. There are many examples, including lichens (fungi plus algae), and some marine flat worms and slugs, which are green with photosynthetic symbionts. The symbiosis between the forebears of algae and plants became extremely well-established obligate. The cyanobacterial symbionts became well integrated enough to be cell organelles, called chloroplasts. However, they still carry their own cyanobacterial DNA, a remnant of their once independent pasts. So yes, from a bacteriocentric point of view, algae and plants are all versions of cyanobacterial photosynthesis. Therefore, all oxygen in our atmosphere and the enormous biomass of plant material is cyanobacterial either directly or indirectly through symbiosis.

To get a look at cyanobacteria, seek out blue-green or even brown-black pigmentation in very dim light, such as the north side of a building where water is dripping, or just inside a moist cave, or a little deeper in a pond compared to where the brighter green eukaryotic algae are growing. Or look for pale blue-green on a well-trodden but damp path where it is difficult for larger photosynthesizers to get established. At the seashore, examine salt flats

closely for blue-green on the surface, sometimes thick enough to be felt-like. However, the fact that you have to look carefully to see them or decipher their presence does not mean that they are rare. They are among the most common and ubiquitous photosynthesizers on Earth. Being tiny, they are easily overlooked. One example is climate modeling, in which scientists attempt to account for every bit of carbon dioxide that is being removed from the atmosphere and every bit that is being released back into the atmosphere. Some models have not taken global cyanobacterial activity sufficiently into account and have ended up with "missing carbon," and therefore an incomplete understanding of potential climate changes.

In Lecture 7 on proteobacteria, nitrogen fixation was discussed as an essential bacterial activity without which there would be no cycling on nitrogen from the atmosphere (of which it is 79 percent) to organisms that must have nitrogen for building proteins, DNA, and RNA. Many proteobacteria and Gram positives are nitrogen fixers. So are many cyanobacteria. These too must be accounted for if we are to get accurate models of the nitrogen cycle. Temperate forests seem to depend mostly on nitrogen fixation from proteobacteria and Gram positives, while tropical forests seem to be more dependent on cyanobacteria, although these can be difficult to see past all the extravagant biodiversity of a tropical forest. Another adaptation in the tropics is to keep the nitrogen cycle very tight and close such that as soon as a nitrogen-rich organism dies, it is decomposed extremely quickly (soil organisms such as were described in Lecture 9) and sent right back into the biomass. There is not much leaf litter or even soil at the base of a tropical forest.



The importance of bacteria in the nitrogen cycle is immediately recognized as being a key element providing different forms of nitrogen compounds assimilable by higher organisms.

FOR GREATER UNDERSTANDING

Questions

1. What is the origin of the chloroplasts (the photosynthetic compartments) of algae and plants?
2. Where can one get a good look at cyanobacteria?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Chapters 13–14. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Rai, Amar Nath. *CRC Handbook of Symbiotic Cyanobacteria*. Boca Raton, FL: CRC Press, Inc., 1990.

Whitton, Brian A., and Malcolm Potts, eds. *The Ecology of Cyanobacteria: Their Diversity in Time and Space*. Norwell, MA: Kluwer Academic Publishers, 2000.

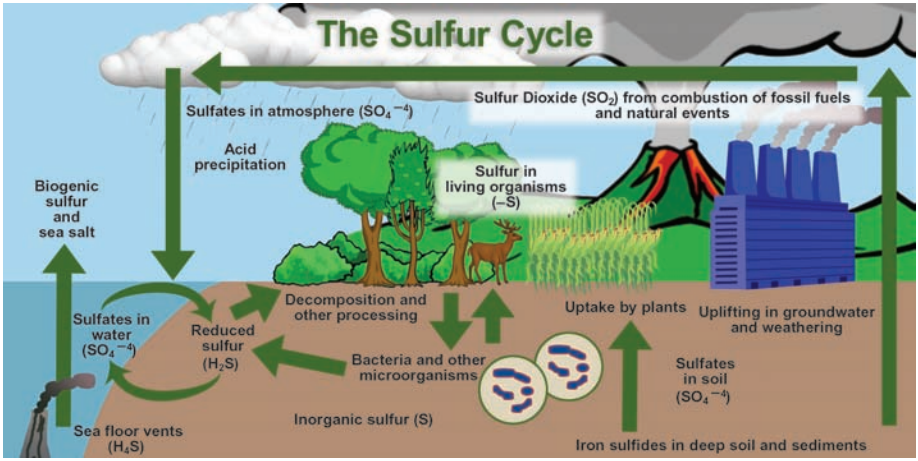
Lecture 13:
Diverse Metabolisms

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria*.

Bacteria and archaea show off most of their diversity by what they do (for example, their metabolisms) and not by what they look like (morphologies).

One of the best places to observe a suite of interrelated metabolisms, autotrophies, and heterotrophies is any sulfur-rich environment (a sulfuretum). These include sulfur springs, estuaries, some deep freshwater pools in an area of carbonate caves, and low-tide marine mudflats. A smell of hydrogen sulfide in the air is a good indicator of a sulfur-rich environment or sulfuretum and often is a field mark of sulfate-reducing bacteria dwelling within. Let's take as an example a low-tide marine mudflat. A close examination of the surface of the mud may show various colors: greens, blue-greens, whites, pinks, and purples. Are those minerals or bacteria? Find out by examining the colored areas for textures indicative of microbes: some of the colors may be in the form of felt-like mats, some may be delicate, flocculant scums easily brushed away, and some may be cobwebby filaments. By brushing away or peeling back the top layer of color, you may reveal other colors below. Finally, there may be thick, black sediment, underlying the more colorful layers and smelling richly of sulfide. Here (as a simple introduction) is a color-by-color and layer-by-layer guide to what you might be seeing in those colorful mudflat layers:

The top layer: a blue-green to black felt-like mat of photosynthesizing cyanobacteria (you may even see bubbles of oxygen being generated as their waste product).



The sulfur cycle is complicated, involving many different organisms. The focus of this lecture is the section to the left of the diagram, in which reduced sulfur (sulfide) cycles with sulfate in a marine mudflat.

The second layer: lovely purple or pink scum, which is a layer of purple sulfur bacteria also photosynthesizing but in dimmer light conditions since they are in the shadow of the cyanobacteria.

The third layer: a delicate dark-green film of green sulfur bacteria photosynthesizing in even dimmer light.

The last layer: a thick, deep-black sediment that smells like sulfide and which is full of sulfate-reducing heterotrophs, "sulfur oxidizers."

And back to the top layer: here and there you may see a delicate cobweb of white slime or filaments on top of the sediment, sometimes on top of some purple sulfur bacteria that have become exposed.

Five types of bacterial metabolism are represented in those layers. Three are photoautotrophies (introduced in the lecture on cyanobacteria), one is a chemoautotroph, and one is a sulfide-breathing heterotroph. Their relationships to each other are evident in the layering patterns.

Cyanobacteria (as well as all eukaryotic photosynthesizers) are photoautotrophs (or photosynthesizers) producing their own sugars (e.g., $C_6H_{12}O_6$) from two raw materials, water (H_2O) and carbon dioxide (CO_2), using energy from the sun. In this community, they are often the top layer, basking in the sun.

Purple sulfur bacteria and green sulfur bacteria are photoautotrophs, too, but they are using hydrogen sulfide (H_2S) instead of water. Notice the similarities of these simplified equations, showing just the molecules, not necessarily in their proportions:

Cyanobacteria: light + H_2O + CO_2 ⇨ $C_6H_{12}O_6$ + O_2 (oxygen, a waste product)

Purple sulfurs: light + H_2S + CO_2 ⇨ $C_6H_{12}O_6$ + S (sulfur, a waste product)

Green sulfurs: light + H_2S + CO_2 ⇨ $C_6H_{12}O_6$ + S (sulfur, a waste product)

Note, the sulfur in the last two equations will most likely be converted to sulfate, SO_4.

One other difference in these photoautotrophies (besides using H_2S and producing SO_4) is pigmentation. Cyanobacteria on the top have a full spectrum of wavelengths of sunlight reaching them. They tend to use reds and ultraviolets for their energy needs. Shorter wavelengths of light, such as at the blue end of a spectrum, penetrate deeper into sediments or water and are available to photoautotrophs in deeper layers. Those photoautotrophs use a different assemblage of pigments to capture those short wavelengths. You can see a similar phenomenon on a large scale with seaweeds growing on a rock. At the top may be bright-green seaweeds, next a zone of yellowish-brown ones and finally a zone of reddish-brown. Those pigments are indicative of the different light conditions available to the seaweeds when the rock is submerged. (By the way, all those seaweeds are eukaryotes and all are doing exactly the same kind of oxygen-generating photoautotrophy.) Finally, you may have noticed that blue light penetrates furthest into the water and reds all but disappear if you have seen the color of a red bathing suit turn gray the deeper you go.

Back to the mudflat, beneath all those colorful photosynthesizers are sulfate reducers that are heterotrophs, similar to ourselves except that they

are breathing sulfate instead of oxygen. Heterotrophs consume sugars and other food molecules in order to get energy. Compare these two simplified equations:

$$\underset{\text{(sugar)}}{C_6H_{12}O_6} + \underset{\text{(oxygen)}}{O_2} \rightarrow \underset{\text{(water)}}{H_2O} + \underset{\text{(carbon dioxide)}}{CO_2}$$

$$\underset{\text{(sugar)}}{C_6H_{12}O_6} + \underset{\text{(sulfate)}}{SO_4} \rightarrow \underset{\text{(hydrogen sulfide)}}{H_2S} + \underset{\text{(carbon dioxide)}}{CO_2}$$

As with the photoautotrophs, the major difference is the sulfate and sulfide (instead of oxygen and water). Notice the cycle-like interactions that may readily be formed between oxygen generators and oxygen users as well as hydrogen sulfide generators and hydrogen sulfide users. One bacterium's wastes are another's starting materials. No wonder the sulfuretum thrives. By the way, the black color of the sulfide-smelling sediments is (along with the sulfide smell) another field mark of the sulfate reducers. Iron in the sediment reacts with hydrogen sulfide to make black iron sulfide.

Finally are those cobwebby filaments of white, which are the sulfur oxidizers that are chemoautotrophs. They make their own sugars using energy from bonds that hold together certain molecules, in this case hydrogen sulfide bonds. Again compare these simplified equations for autotrophies:

Cyanobacteria: $\text{light} + H_2O + CO_2 \rightarrow C_6H_{12}O_6 + O_2$

Purple sulfurs: $\text{light} + H_2S + CO_2 \rightarrow C_6H_{12}O_6 + S$

Sulfur oxidizers: $\text{chemical bond energy} + H_2S + CO_2 \rightarrow C_6H_{12}O_6 + S$

Although these chemoautotrophs of the mudflat are living in the light, plenty of other chemoautotrophs live in total blackness, such as in the deepest parts of the ocean, where sulfide minerals are available. One example is the deep Pacific rift zone where there are underwater sulfur hot springs supporting chemoautotrophs, which in turn support a diverse community of other prokaryotes as well as eukaryotes, including large tube worms. Some of the animals of the community (including the tube worms *Riftia*) have symbiotic chemoautotrophs in their tissues and therefore a direct supply of the sugars being synthesized. These worms lack mouths, having no need to eat, and are bright red with hemoglobin. The hemoglobin binds oxygen and keeps it from interfering with the reactions of the chemoautotrophs. Otherwise, hydrogen sulfide would react spontaneously with oxygen and be used up without any autotrophy occurring.

Chemoautotrophs are favorites of astrobiologists who are examining planets and moons for evidence of life. Chemoautotrophy is appealing because it can occur in the dark, such as deep inside a planet, the surface of which might be uninhabitable. Furthermore, minerals of all sorts are the starting materials for chemoautotrophs. These include (along with hydrogen sulfide in the example) hydrogen carbon monoxide, ammonium, nitrite, and iron. Recall that ammonium and nitrite chemoautotrophs were already discussed in the context of aquarium filters.

Our own form of heterotrophy (and that of all animals) is this:

$$C_6H_{12}O_6 + O_2 \Rightarrow H_2O + CO_2$$
(sugar) (oxygen) (water) (carbon dioxide)

. . . and we share it with a host of bacteria, including some in the lineage of our own ancient eukaryotic ancestors. About 2.5 billion years ago our ancestors acquired as symbionts some heterotrophic bacteria that were able to respire oxygen. It was just around the time that significant amounts of oxygen were beginning to accumulate in the atmosphere from the wastes of cyanobacteria. Oxygen is actually a toxic molecule to most living systems. The origins of oxygen-using heterotrophy may have had roots in detoxifying oxygen. Eventually those respiring symbionts became well integrated with the host cell, carrying only a remnant of their former genomes. They are our mitochondria, without which we would have insufficient energy to operate.

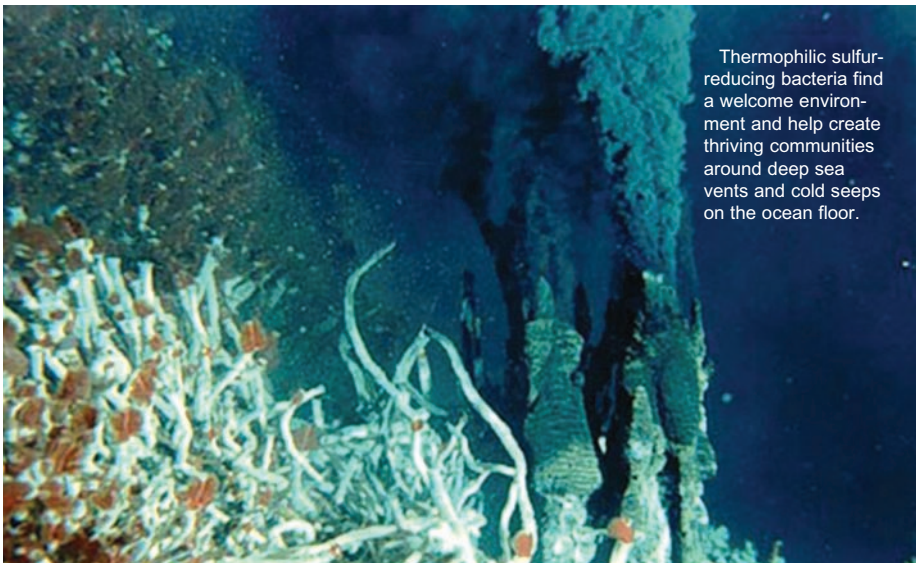
Here is why: The oxygen in the above equation has an intriguing function (as does the sulfate in the sulfate reducer's equation on the previous page). Consider this metaphor: The equation is a little like an assembly line with different activities along the way and products and wastes at the end. As long as those products and wastes are promptly removed, the assembly line moves forward unimpeded. However, if products and wastes begin to heap up at the end, eventually they may slow or stop the assembly. It may help to envision the scene from an *I Love Lucy* television show episode in which Lucy and Ethel are working at a conveyor belt wrapping chocolates. They fail to keep up with the task, the products pile up unprocessed (unwrapped), and the results are something like what happens if a cell fails to keep up with a flow of end products such as electrons and hydrogen ions.

Carbon dioxide is one such waste and it readily diffuses away. Hydrogen ions and electrons get generated along the assembly line and become part of the stored energy that will be used later to fuel all sorts of activities and syntheses. These are not shown in the simplified equation. Some hydrogen ions and electrons generated at the end get "hauled away" like waste by binding them to oxygen (of sulfate) to make water (or hydrogen sulfide). So the primary role of oxygen (or sulfate) in heterotrophy is to remove hydrogen ions and electrons from the end of the assembly line such that it runs more efficiently.

This use of oxygen may sound trivial but you know that it cannot be, because we cannot live for more than about six minutes without oxygen. It is because that assembly line is the means by which we store up most of the energy available to us for future use. Any backup of wastes whatsoever jeopardizes our supply of energy and apparently we are living somewhat on the edge with very little savings. We make lots of energy (thanks to the efficient assembly line of our mitochondria) but we are highly dependent on having lots of energy by being enormous, very active, multicellular creatures working to stay upright and mobile in spite of gravity. In contrast, aquatic creatures have it a little easier due to the buoyancy of water. Meanwhile, small eukaryotes such as yeast can even function fairly well with little or no oxygen and therefore with a slightly less efficient metabolism.

To conclude, let's go to a rather dramatic example of bacterial metabolism with global consequences that is nonetheless often overlooked: that is, herbivory (the eating of plants in whole or in part) as done by animals. It is an essential part of any food web, being a primary means by which the biomass of photosynthesizers gets broken down and passed along, some of it eventually to carnivores and much of it to decomposers. Nearly all herbivorous animals are entirely dependent on bacterial symbionts in order to get enough energy from their diet of plants[1]. This has had great consequences for the morphologies, physiologies, and behaviors of herbivores. Many (such as cattle, rabbits, and sauropods) are (or were) rotund, shaped like fermentation vats because that is what (from a microbial point of view) they are. Many can eat seemingly impossible diets such as wood (termites) or sugar water (aphids) as their sole source of nutrition. Behaviors of herbivores often include being sedentary (the better to slowly digest) and staying together in groups especially for extended care of young. One challenge of herbivory is to pass along the symbionts to offspring, which are born sterile. This typically is accomplished either by feeding the young regurgitated material or special feces. Herbivory with bacteria (which is almost the sole way that animals can be herbivores) is a major way by which carbon from plants, on a global scale, gets moved to the rest of the food web. Yet many discussions of herbivores as part of a food web and the global carbon cycle rarely acknowledge a role for bacteria. Furthermore, the various identifying traits of herbivores' morphologies and behaviors are not theirs alone, because animal herbivores are never independent of the powerful influence of having evolved as a container for a microbial community.

1. There is an alternative mechanism for animal herbivory. That is "fungus gardening" as done by some termites and ants that grow fungi in underground gardens in order to process their plant diet. Fungi are prodigious herbivores themselves and generally manage quite well without bacteria. Indeed, they are frequent competitors with bacteria.



Thermophilic sulfur-reducing bacteria find a welcome environment and help create thriving communities around deep sea vents and cold seeps on the ocean floor.

FOR GREATER UNDERSTANDING

Questions

1. What is one of the best places to observe a suite of interrelated autotrophies and heterotrophies?
2. How are the sulfate reducers in a mudflat similar to ourselves?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Professor Dyer suggests that students interested in pursuing further study on bacteria, archaea, and viruses should investigate any up-to-date college textbook on microbiology.

Websites to Visit

The *You Tube* website provides the *I Love Lucy* television show assembly line film clip — http://www.youtube.com/watch?v=4wp3m1vg06Q

Lecture 14:
Future Directions

The Suggested Reading for this lecture is Betsey Dexter Dyer's *A Field Guide to Bacteria*.

There is no resounding and definitive conclusion for a topic so enormous as all of the bacteria and archaea, and this been only an introduction. However, there is something that scientists call "future directions," often part of the conclusions of a scientific paper, where it is acknowledged that there are still lots more experiments, more analyses to be done and data and observations to be analyzed. You could come up with your own list of future directions for the study of bacteria and archaea. For example, you may wish to pursue some of these topics.

1. Include bacteria in your nature studies, wherever you are. These lectures are meant to be an encouraging beginning. Additional information may be found in *A Field Guide to Bacteria*.
2. Try looking at animals and plants from a bacterial point of view with all of their dependencies on bacteria.
3. Reread some favorite book or article about pathogenic bacteria, but perhaps with some new ideas about the nature of pathogenicity.
4. Say, "Where are the bacteria?" the next time you see some complicated model of the carbon cycle or food web or biosphere.
5. Live more comfortably with your normal bacteria, regardless of heightened concerns generated by some industries. Or perhaps generate some of your own concerns about cavalier misuses of antibiotics.
6. Take a course in microbiology. Depending on the professor, there will be a particular focus. Many microbiology courses, especially for persons going into health professions, place a heavy emphasis on the pathogens. Others give a broader overview, including some environmental microbiology courses.
7. Book a trip to Yellowstone to see the gorgeous thermophiles. (And plan other trips to extreme environments such as salinas, karstic caves, and sulfureta.)
8. Contemplate viruses, which are not organisms but are genetic entities, bits of DNA or RNA sometimes bundled in a little protein coat, sometimes not. They have no independent existence outside of a cell. As with bacteria, only a tiny minority of viruses (albeit well studied, and well publicized viruses) are pathogens. The vast majority are not. Instead, they seem to be benign, and some are beneficial. Our own genomes carry a load of viral DNA that comprises about one-third of our total DNA. This is extraordinary and not well understood. How could we contain so much of it? Where do all those viruses (most, presumably, benign) fit in to the proper functioning of our genomes, whatever that

might be? This is one of the amazing outcomes of human genome projects and definitely a frontier in genetics about which we know little. This is why I teach about viruses in conjunction with my genetics course, and I must say it is a very exciting time to be teaching genetics. I can hardly keep up with all the new developments. I believe that a genetics course is the appropriate place for viruses unless presented in their own dedicated course: "Virology." That said, a microbiology course for the health professions with a focus on pathogens will certainly include pathogenic viruses, mainly because such courses are about primarily "pathogens." They will also include something about pathogenic fungi and protists and our immune system responses to pathogens.

9. Contemplate fungi, an extraordinary taxonomic group, the feats of which were only hinted at in these lectures. That one is definitely on my own "future directions" list.
10. And finally, become a little more "bacteriocentric" in your contemplation of the biosphere (and our place in it) and even "bacteriophilic" in recognizing and appreciating archaea and bacteria.

FOR GREATER UNDERSTANDING

Questions

1. Has your view of bacteria changed after reviewing these lectures? If so, in what ways?
2. Will you change any of your day-to-day behaviors in light of what you now know about bacteria?

Suggested Reading

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Ithaca, NY: Cornell University Press, 2003.

Other Books of Interest

Brock, Thomas D., Michael T. Madigan, John M. Martinko, and Jack Parker. *Biology of Microorganisms*. 7th ed. Prentice Hall, 1994.

actinobacteria. One of two large subgroups of Gram-positive bacteria; often has a thread-like morphology.

agar. A favorite hardening agent for making bacteria medium so that bacteria can make distinct, well-separated colonies on a supportive surface; a substance derived from seaweed also used to give a firm consistency to ice cream.

animal. A group of heterotrophic, multicellular eukaryotes comprised predominantly of invertebrates (for example, arthropods and nematode worms). It is one of the four kingdoms of eukaryotes.

antibiotic. A compound that kills or disables bacteria, often produced by other bacteria or by fungi or more lately synthesized and modified in the lab.

archaea. An enormous, diverse group of microbes with simple cells with many morphological and functional similarities to bacteria; traditionally bacteria and archaea were considered one and the same; lately, thanks to DNA sequence evidence, the archaea are recognized as a unique group.

autotrophy. A type of metabolism by which an organism synthesizes its own food using simple starting materials such as carbon dioxide and water and energy (chemical bonds or light). *See also subgroups* **chemoautotrophy** *and* **photoautotrophy** *below*.

a. chemoautotrophy. Autotrophy that uses chemical bonds such as from minerals as a source of energy.

b. photoautotrophy (also called photosynthesis). Autotrophy that uses light as a source of energy.

bacteria. An enormous, diverse group of microbes with simple cells; sometimes used colloquially to refer to bacteria and archaea collectively, although archaea are now recognized as a separate group.

bacteriocentric. Placing bacteria (and archaea) in central, essential roles in the ecology and biodiversity of our planet.

bacteriophilic. Demonstrating not only an awareness of the bacterial (and archaeal) world, but also feeling an appreciation for it.

bioluminescent. Generating visible, detectable light as a product of one or another biological chemical reaction.

carbon dioxide. A simple gas of carbon commonly released by heterotrophs as a waste product and the starting material for most autotrophs.

chloroplasts. Compartments within plants in which photosynthesis occurs and which originated as symbiotic cyanobacteria. *See also* **autotrophy.**

colony. When referring to bacteria, it is a group of identical bacterial cells, having been begun by one individual that divided repeatedly.

cyanobacteria. Photosynthetic bacteria that synthesize food from carbon dioxide and water and that release oxygen as a waste; some in this lineage became the chloroplasts of all plants and photosynthetic protists. *See also* **autotrophy.**

DNA. A linear molecule comprised of four types of subunits (adenine, cytosine, guanine, and thymidine) that in their various orders are a source of information by which cells build, organize, and operate themselves.

extremophiles. Organisms (usually bacteria and archaea) able to grow in what humans would consider to be extreme environments: high temperature, high salinity, high alkalinity or acidity, etc. Note that most of Earth is “extreme” from the limited point-of-view of humans, because we are not aquatic or subterranean, the two largest living spaces on the planet.

eukaryote. A diverse group of organisms with complex cells that originated through symbiosis between bacteria and archaea about 2.5 billion years ago. Typically they have been organized in four kingdoms: animals, plants, fungi, and protists.

firmicutes. One of two large subgroups of Gram-positive bacteria; often has an especially firm cell wall.

flagellum. The whip-like motility appendage of bacteria. Note that this is one of many examples in biology of terminology lagging far behind science. The term flagellum is also used for the motility organelles of archaea and of eukaryotes, even though those are entirely different structures. There is no distinct term for the archaeal structure. Various terms are recommended for those of eukaryotes, such as cilia, undulopodia, and "eukaryotic motility organelle."

fungi. A group of heterotrophic, single, or multicellular eukaryotes comprised predominantly of molds, yeasts, and various mushrooms. It is one of the four kingdoms of eukaryotes.

genetic engineering. The various manipulations of DNA in laboratories, taking advantage of normal bacterial activities with DNA that include cutting it and inserting it into other genomes, often across species "boundaries." Actually, bacteria and archaea have very few boundaries and may be considered promiscuous with the DNA. See also **horizontal transfer**.

genome. The total of all of the DNA of an organism.

Gram-positive bacteria. A large and diverse group of bacteria that serendipitously all (or nearly all) stain blue with crystal violet due to the nature of their cell walls.

Gram stain. Crystal violet, one of the many products of the nineteenth-century German dye industry; Dr. Christian Gram found that it preferentially stained blue what came to be known as "Gram-positive bacteria." Note that Gram-negative bacteria do not stain blue, but their colorless cells are often counterstained pink to help them to show up. Archaea do not have any distinctive Gram stain properties.

halophile. Organisms (typically microbial) that thrive in high salinities.

heterotrophy. A type of metabolism by which an organism must take in food in order to get materials and energy.

horizontal transfer. The practice of bacteria and archaea (and some eukaryotes) to informally pass around bits of DNA, sometimes incorporating those into their genomes.

hyperthermophile. Archaea and bacteria that thrive in temperature from 80 degrees Celsius to over 100 degrees Celsius.

JIZZ. A term adopted by ornithologists from plane spotters in World War Two. It is a modified acronym of "General Impression of Shape and Size." For ornithologists (and field microbiologists) it represents the total of all of the information, including habitat and activities, that allow identifications to be efficiently made in the field.

magnetotactic. Orienting by detecting the magnetic poles of Earth.

medium. Refers to the nutrients and minerals that microbiologists offer to microbes in hopes that they might thrive. *See also* **defined medium** *and* **undefined medium** *below*.

a. **defined medium.** Made from a long list of chemicals, this type of medium is taken right off the stockroom shelf and mixed to approximate what beef broth, for example, might look like if it were broken down into its component parts.
b. **undefined medium.** Looks like food. It might be beef broth or some decoction of plant material.

mesophile. Organisms that live at moderate temperatures, from the point of view of ourselves and our preference for "room temperature" 25 degrees Celsius as the midpoint.

methane. A simple, flammable gas of carbon, often generated by methanogenic archaea; many bacteria consider methane to be a useful starting compound for their own metabolisms; therefore, healthy methane-oriented communities don't actually generate much methane; it gets used.

Microbe (microorganism). A catch-all term for any organism, including single-celled eukaryotes as well as bacteria and archaea, that cannot be seen easily or at all by a human eye.

microbiota. A collective term for microorganisms, analogous to flora (collective term for plants) and fauna (collective term for animals). For example, instead of "gut flora," the better term would be "gut microbiota."

microfossil. Fossils of microorganisms inside of rocks that must be sliced very thin with diamond-bladed saws, polished and then placed under microscopes in order to see the petrified cell remnants.

mitochondria. Compartments within eukaryotic cells (protists, fungi, plants, and animals) in which most of the processes of heterotrophy occur. Mitochondria originated as symbiotic proteobacteria.

myxobacteria. A group of proteobacteria capable of banding together to make enormous (up to 1 millimeter tall) structures that are often colorful and tree-like.

NCBI. National Center for Biotechnology Information, essentially the central public database for all DNA and protein sequences and their annotations. The database is extremely fast, well organized, constantly updated, and is a free source (from anywhere in the world) to almost any sequence except perhaps for some held as proprietary secrets by some companies. It might be considered a "Wonder of the Twenty-first Century World."

nitrogen. An element that comes in many molecular forms, such as nitrate, nitrite, and ammonia. Bacteria and archaea are instrumental in converting nitrogen molecules from one form to another, thus operating and facilitating a nitrogen cycle.

oxygen. A simple but highly reactive gas of two oxygen atoms. Oxygen readily oxidizes (and this often destroys) other molecules. Oxygen is a waste product of some photoautotrophies and in small quantities is useful in some heterotrophies. In general, though, it is toxic and corrosive.

Origin of life. Extrapolated to have occurred about 4 billion years ago, right after Earth was cool enough for liquid water and right before the first microfossils may be found. The earliest known life-forms are considered to be archaea and bacteria. (Eukaryotes begin evolving about 2.5 billion years ago.)

pathogen. Organisms (usually microorganisms) that have or develop relationships with hosts that are damaging or lethal for that host. The term is also used for some viruses that are not organisms.

Petri plate or dish. A good idea from Julius Petri, who realized that many colonies of bacteria growing on a firm agar surface needed only a shallow (stackable) dish and loose cover, which describes a Petri dish.

phylogenetic tree. A family tree of organisms by which relatedness is determined by some set of criteria. Those criteria used to be about morphology and functions. Now they are more likely to be based on analyses of entire genomes, which allows for more comprehensive and nuanced definitions of relatedness.

plant. A group of photoautotrophic, multicellular eukaryotes comprised predominantly of angiosperms (flowering plants), gymnosperms (non-flowering plants), and ferns and mosses. It is one of the four kingdoms of eukaryotes. The placement of seaweeds is somewhat controversial. I put them into the protists.

prebiotic. Food that you take in with the intent of nourishing not just yourself, but also your normal community of digestive-system bacteria (for example, a bowl of oatmeal).

probiotic. Fermented food containing bacteria that are similar to those of your digestive system (for example, yogurt). Now that this is becoming more recognized by the medical community, it is easy to get freeze-dried probiotic bacteria in tablets at pharmacies.

prokaryote. A collective term for bacteria and archaea together and used to contrast with eukaryotes.

proteobacteria. A large, diverse group of bacteria focused on in this course because many have distinctive field marks.

protist. A large, diverse group of heterotrophic or autotrophic, mostly single-celled eukaryotes. Many are featured in biology classes, including amoeba, paramecium, spirogyra, and euglena. It is one of the four kingdoms of eukaryotes.

species. A concept on inclusiveness that works best for defining those animals (especially mammals, birds, and insects) and those plants that are extremely conservative with their DNA, exchanging it sexually only with similar organisms to themselves. A "species" is a community of interbreeding organisms capable of producing offspring that can also interbreed. This definition falls apart for organisms that either are promiscuous with their DNA or which reproduce often or exclusively asexually (without any exchange of DNA). That would be the vast majority of organisms: archaea, bacteria, most protists, many plants, and many animals. The word "species" is often used loosely (without the stipulations for conservative exchanges of DNA) to mean any distinctive "type" or organism such as *Escherichia coli*. Note the italics of the binomial name by which "species" are known. The first word is a genus (a sort of surname) and the second word, never capitalized, is the species name.

sulfur. An element that comes in many molecular forms, such as sulfate and sulfide. Bacteria and archaea are instrumental in converting sulfur molecules from one form to another, thus operating and facilitating a sulfur cycle.

symbiosis. An intimate relationship between two or more species of organisms, such that the associated organisms are more fit (leave more offspring) in a particular environment than the solitary organisms would. A lichen is a classic example, consisting of a fungus and algae. Together they can live on the hard, dry surface of a gravestone, an environment that the individual algae and fungus would find impossible.

thermophile. An organism (typically bacteria and archaea) that thrive at temperatures between 60 and 80 degrees centigrade.

transposon (also called jumping genes). A bit of DNA in the genome of an organism that has the capability of functioning semi-independently of the rest of the genome. For example, it might replicate itself. It might also formerly have had some semi-independent function, now lost but is still recognizable (by its sequences) as a transposon. Transposons might seem exotic but they are far from rare. About half of the total DNA of the human genome is comprised of transposons; in contrast, less than 10 percent of our genomes is comprised of genes. We do not know what all those transposons are doing.

virus. It is not an organism, rather, it is a bit of DNA (or RNA) in the genome of an organism that has the capability of functioning semi-independently of the rest of the genome. For example, a virus might replicate itself. It might also formerly have had some semi-independent function, now lost, but it is still recognizable by its sequences as a virus. Viruses might seem exotic, but they are far from rare. About one-third of the total DNA of the human genome is comprised of viral DNA; in contrast, less than 10 percent of our genomes is comprised of genes. We do not know what all those genomic viruses are doing. Very few viruses are pathogens. That is an illusion of the older methods of detecting viruses, which consisted mostly of looking for pathogenic damage. Benign and beneficial viruses were not easily detected until genome sequencing and analysis became commonplace. Even though most pathogenic viruses have beautiful, geometric protein coats and therefore viruses are typically depicted that way, the majority of viruses never make protein coats.

Suggested Readings:

You will receive the greatest benefit from this course if you have the following text:

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Ithaca, NY: Cornell University Press, 2003.

Suggested Readings:

De Kruif, Paul. *Microbe Hunters.* 70th anniversery ed. Orlando, FL: Harvest Books, 2002.

Dobell, Clifford. *Anthony van Leeuwenhoek and His "Little Animals."* Mineola, NY: Dover Publications, 1960 (1932).

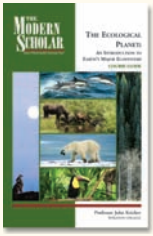
Ford, Brian. *The Leeuwenhoek Legacy.* Bristol, UK: Biopress, Ltd., 1991.

These books are available online through www.modernscholar.com or by calling Recorded Books at 1-800-636-3399.

If you enjoyed this course by Professor Dyer, you may also enjoy these other Modern Scholar science courses. They are available online at www.modernscholar.com or by calling Recorded Books at 1-800-636-3399.

Human Anatomy: The Beauty of Form and Function
Professor John K. Young—Howard University College of Medicine

Our bodies perform an amazing number and wide variety of tasks that we literally could not live without. Renowned scholar John K. Young provides a fascinating look at how the human body is constructed, how it employs its different parts to our advantage, and how it can malfunction if not properly maintained. Professor Young describes not only the basic anatomical bones and organs that constitute our physical form, but also the role each plays in the synchronized effort to keep us alive.



The Ecological Planet: An Introduction to Earth's Major Ecosystems
Professor John Kricher—Wheaton College

Renowned ornithologist John Kricher presents an absorbing analysis of the diverse ecosystems that exist on Planet Earth. He provides a factual study of the many fragile and threatened portions of our biosphere while giving a thorough description of the interaction between each system and the effect of man's presence in them. Professor Kricher explains the amazing variety of flora and fauna that inhabit the individual ecosystems and synthesizes current ecological issues facing mankind.