Introduction
Becoming Bacteriocentric

I love studying and thinking about bacteria, but this was not always the case. When I was growing up, I was a serious amateur naturalist. Insects were my favorite organisms, and I planned to become an entomologist. I was also very fond of rocks, shells, birds, bird nests, feathers, skeletons, leaves, and flowers. I had many collections and little experiments set up in my research lab in the basement. When I was eleven, I was given a microscope for Christmas and became quite enthusiastic about the world of microorganisms—but not about bacteria. Tiny crustaceans, ciliates, nematodes, and miniature annelids were abundant in drops of pond water. However, bacteria were below the limits of easy detection with my microscope, and even if I had seen them, I suspect that the tiny dots and dashes—the typical forms of bacteria—would not have held my interest.

In college, I took many biology courses and completed a project involving microscopy during my senior year. However, I did not appreciate bacteria then and, to be honest, I was even intimidated by them and preferred not to think about them at all. Somehow I avoided taking any course in microbiology as an undergraduate.

In graduate school, I became more comfortable with bacteria, since they live in abundance in the hindguts of termites—a subject of my graduate research. This continues to be my favorite microbial symbiosis. Top-quality microscopes and the unusually large and active bacteria characteristic of the termite microbial community all contributed to making my first real experiences with bacteria positive ones.

At that point in my career, I was already working at a professional or near-professional level in biology. However, I was still under the

impression that an appreciation for and understanding of bacteria could be achieved only after years of work. At no point did it seem to me that I could have studied bacteria as an amateur naturalist. Even as a professional biologist, I found them quite difficult.

All that changed for me in the summer of 1980 when I joined other scientists on a field trip to the microbial mat communities in Baja California, Mexico. There we marveled at felt-like blue-green bacterial mats, pink layers of scum, black sediments, red-tinted salt crystals, and bubbles rising to the surface of murky water, all accompanied by a distinct sulfur smell. I learned that many bacteria can be identified by *macroscopic* field marks—characteristics that anyone can see, smell, or sometimes even hear, without the use of a microscope. It occurred to me right then that it would be possible to write a field guide to the macroscopic characteristics of bacteria.

Although most people are aware that bacteria are all around us, few would guess that they produce such distinctive and accessible signs. Whether you're walking on the beach, visiting a zoo or aquarium, buying groceries, looking for fossils, drinking beer, traipsing through a swamp, or cleaning scum from a dripping outdoor faucet, you're surrounded by bacterial field marks. You don't need a laboratory or fancy equipment to find out what kind of bacteria are there—this guide will tell you how.

If you want to know what to look for at the zoo or on the seashore, turn to the next chapter, "Guide to Habitats," or look in the index. Or maybe you prefer to read about a particular group of bacteria and take off on a search for them; in that case, start with any chapter in the book. The following section of the introduction gives more detail about how the book is organized and how to start becoming bacteriocentric.

How to Use This Guide

The purpose of this guide is to add a bacterial dimension to nature studies and to make more obvious the most abundant and diverse organisms on Earth—bacteria. This guide presents all the major taxonomic groups of bacteria in a useable, accessible format for *amateur naturalists* who may or may not have access to a microscope. This approach to bacteria is untraditional but is one that should appeal to users of field guides for other unusual subjects: fungi, minerals, bird calls, and the like. The sorts of challenges encountered in guides to fungi, for example, are the same that I have dealt with here: the subjects are cryptic, easy to overlook, and challenging to interpret.

This field guide is intended to be carried into the field by serious amateur naturalists, biology teachers at all levels, and even some professional biologists who may appreciate the accessibility it affords to these otherwise obscure organisms. Indeed, many serious amateurs and professional biologists are aware of the abundance and diversity of bacteria yet have always considered them to be off limits for simple study or appreciation. Although microscopy is encouraged (and detailed instructions given for those who have access to microscopes), the major theme of this guide is the use of macroscopic field marks to identify nearly every major group of bacteria. Indeed, the macroscopic identifiers are especially valuable to those who take an ecological approach to nature studies. With the aid of this guide, for example, bacterial components of the carbon, nitrogen, and sulfur cycles may be identified in the field, along with their more obvious plant and animal cohorts.

This field guide is organized by chapter according to the major taxonomic groups of bacteria. A taxonomic, rather than ecological, organization was chosen because it better reflects the extraordinary diversity of bacteria, even within closely related groups. Each chapter covers a branch on the bacterial family tree that has been constructed based on changes in DNA sequences that have occurred over the last 4 billion years. Thus, the current classification system and the organization of this guide both reflect the evolution of bacterial groups.

Because many of these bacterial groups are intimately associated with specific environments such as hot springs or marine mud flats, each chapter includes an ecological/environmental focus to place the bacteria in context with their surroundings. Summary lists at the end of each chapter also organize the macroscopic field marks for quick identification. The next chapter gives ideas for planning field trips to explore assemblages of bacteria in their natural environments.

Sometimes you never see the actual organism you have set out to study. Instead, you see signs of its presence or its activities. Take birds, for example. Certainly they are among the most watchable and identifiable of subjects for study. However, different manifestations of birds can also serve as identifiers: bird nests, bird eggs, bird tracks, bird songs, and even a brief flash of a bird, seen at such a great distance or so fleetingly that it becomes a real challenge to make any identification.

Think of this field guide as a naturalist's approach to those tiny, cryptic organisms that are at once the most populous and the least visible of living things on Earth. There are approximately 5×10^{30} bacterial cells on Earth according to University of Georgia microbiologists William Whitman, David Coleman, and William Wiebe—who refer to bacteria as "the unseen majority." Another 10^{18} bacteria, probably dormant, are circulating in the atmosphere attached to the dust of airborne soils and sediments, according to Dale Griffin and his colleagues at the U.S. Geological Service. But even with the aid of a microscope, most bacteria appear as nearly featureless dots and dashes displaying only a few cryptic behaviors (if jiggling about can be considered a behavior).

What can be done to make bacteria more accessible? In this guide I suggest looking for the manifestations, or *field marks*, of bacteria, which can be surprisingly visible and obvious once you know the signs. In a sense, this field guide is about what birders call "jizz"—the collection of characteristics of a particular bird (along with its habitat) that in total allow a well-trained observer to identify a bird in a matter of seconds, even as the bird is disappearing into the underbrush. Bacterial jizz is for the most part about what bacteria are doing, often on a large, detectable scale: producing bubbles, slimes, and scums; exuding odors and flavors; showing a stunning array of pigmentations; and participating actively and often visibly in nutrient and mineral cycles. In fact, in some cases bacteria are the sole proprietors of whole sections of these cycles. Without bacteria, the ecological wheels would cease to turn. Because different types of metabolism are such a central part of what differentiates one group from another, I describe metabolic processes in individual chapters rather than in the introduction. For

example, you will find a description of photosynthesis in chapter 1 and chemoautotrophy in chapter 7 (consult the index to locate other topics).

Each chapter tells you where to go and what to look for (and smell, taste, or touch) to identify a particular bacterial group. In some cases (for example, cyanobacteria, methanogens, and intestinal microbes), one can even listen to the bacteria as they produce popping, fizzing bubbles of gas. Each chapter also has sections on what you will see if you look under a microscope, and, if it's possible to do so, how to culture (grow) bacteria in this group. All that is asked of the reader is to be open minded. Take a few trips to so-called extreme environments, such as hot springs or salt flats. Poke around and look closely. Everywhere bacteria are making their presence known, producing field marks that can be interpreted by naturalists at all levels. It is the aim of this guide to make those field marks accessible, interpretable, and less mysterious—and in so doing, to reveal the wonderful diversity of the bacterial world.

In Defense of Bacteriocentricity

Anthropocentrism—that tendency to see the universe in terms of human values and human experiences—is generally frowned on in modern nature writing. Indeed it is considered a rather unsophisticated point of view. Even more so in scientific research, anthropocentrism is thought to reflect a great error in logic, leading to false interpretations and a failure to see the whole picture. Most scientists (or at least their reviewers) are quite conscientious about editing out any traces of anthropocentrism from reports of field and lab studies.

Nevertheless, teachers of biology (and their students) know very well how useful a vivid analogy from personal experience can be. Some of us secretly appreciate it when "objective" science is put, even briefly, into human terms for the purpose of making the difficult a little more accessible. Thus, throughout this field guide I occasionally lapse into presumptuous anthropocentrism for the purpose of creating memorable examples of an otherwise cryptic

and often overlooked world. But what I really want to say here is that I've done something far more presumptuous—and it is something that I recommend you try yourself. My primary goal throughout has been "bacteriocentricity"—that is, to put myself in the place of bacteria, to try to experience the world as they experience it. I have tried to see myself as enormously large (as indeed I am—most organisms on Earth are microscopic) and strangely multicellular (most organisms on Earth are unicellular). My range of metabolism is quite limited—centering only around oxygen respiration—and it takes me years and years to reproduce, which after all is the sine qua non of all living things. How strange that I don't bud or divide with bacterial frequency and efficiency.

A bacteriocentric point of view is a useful one to cultivate, whether you are trying to observe and understand the bacterial world yourself or to teach about it to others. Teachers in particular might want to lead students through the mental exercise of "being a bacterium." For example: Why is that pink scum positioned just so beneath the green scum? What does it "want" or "need"? What are its interactions with the other bacteria of the community? In fact, such an exercise is similar to one used by professional microbiologists when they try to culture bacteria. It's called "thinking like a bacterium"—trying to guess what parts of the microenvironment are essential, what aspects might be combined in a test tube to create the right conditions. (It is the method described in appendix A, on culturing bacteria.) A microbiologist wonders if there exists a bacterium that prefers high temperatures, low-nutrient conditions, and an acidic environment—and accordingly tries to establish those conditions in the lab in the hopes of attracting and nurturing such a microbe. It works!

Therefore, reader, try seeing the microbial world from a microbial point of view. Risk the presumptions of bacteriocentricity for a better understanding of the most predominant and most invisible organisms on Earth. Can this approach be taken too far? Perhaps. While I was immersed in writing this guide, a friend asked me for advice concerning a mild case of food poisoning he was experiencing. I found that I was unable to properly sympathize with him (the human host) but instead came down quite strongly on the side of his intestinal bacteria, which, after all, were experiencing an invasion and were being dislodged from their habitat and deprived of their usual nutrients.

Taxonomy and Nomenclature

Attempts to classify bacteria have been fraught with difficulties. Most bacteria are tiny rods, indistinguishable from each other; many of the rest are tiny spheres. No wonder bacteriologists have turned to other characteristics more prominent than morphology! Metabolism—the means by which organisms get energy and food—is quite diverse among the bacteria and has traditionally been the primary characteristic for distinguishing them.

Since the 1980s, DNA sequences have become the basis for classification. Techniques for sequencing DNA have advanced so far that the process is now routine and widespread. Certain sequences that are highly conserved, meaning that they have undergone few mutations, are good candidates for constructing phylogenetic trees of organisms. One such sequence consists of DNA that codes for the 16S subunit of ribosomes, which are ubiquitous cell structures that are used to synthesize protein. The 16S sequence, so-called because its weight and size cause it to sediment in a centrifuge with particles designated as 16 Svedbergs, is one of the most trusted sequences for constructing family trees. Carl Woese, at the University of Illinois, and Mitch Sogin, at the Marine Biological Laboratory in Wood's Hole, as well as many scientists associated with their labs, have been among the most prolific of researchers producing the 16S ribosomal sequences for bacteria and other organisms.

As more and more bacterial genes were sequenced during the 1980s and 1990s, there came some big surprises. First, bacteria, or prokaryotes, could be divided into two large, very different groups, now named the archaea (or archaebacteria) and the bacteria (or eubacteria) (fig. I.1). Second, metabolism, once a trusted indicator of bacterial phylogeny, was found to be an unreliable trait for most groups of bacteria. Most of the major bacterial groups presented a seeming jumble of metabolic diversity. Third, some groups once considered "primitive" now became positioned on advanced branches, and vice versa. The new bacterial phylogeny that emerged was at first a challenge to explain and defend. However, this new phy-

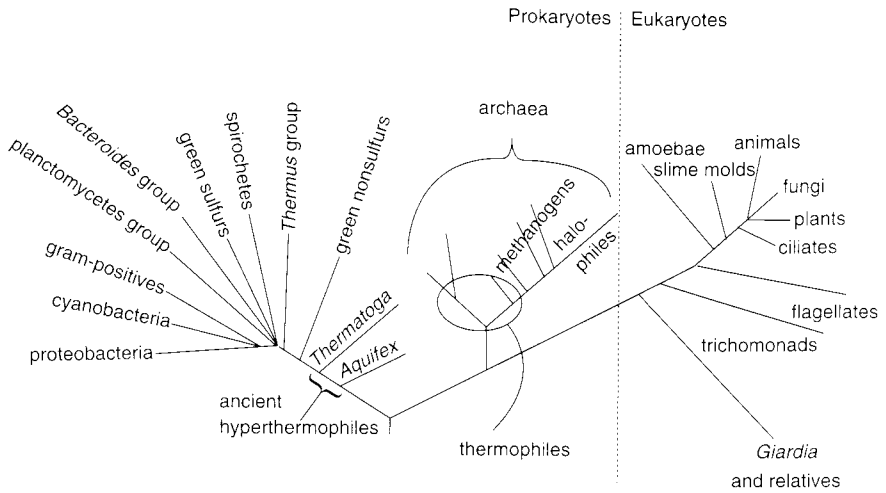

FIGURE I.1. This phylogenetic (family) tree shows the divergence of different groups of organisms from the beginning of life on Earth, about 4 billion years ago, as measured by differences in DNA sequences. The evolutionary distance between two groups is proportional to the total length from the tips of the two branches to the node that joins them. The thermophilic archaea include the hyperthermophilic species featured in chapter 3.

logeny also produced new ways of understanding bacterial evolution. Among other things, it appears that most of the great diversity of bacterial metabolism evolved early; recent branches merely display variations on several major themes.

Bacterial taxonomy based primarily on DNA sequences has now been incorporated into most publications concerning bacterial classification. In the long run, it may appear to historians of science that the breakthrough work of Woese and Sogin—and the almost complete rearrangement of our understanding of taxonomy and evolution—produced a true paradigm shift. The organization of this field guide is based on this new (and still shifting) taxonomy, allowing the reader to identify most major groups of bacteria, as well as many of the subgroups.

HOW MANY SPECIES OF BACTERIA ARE THERE?

The smaller or more inaccessible an organism is, the more difficult it is to answer the question, "How many species are there?" We know with considerable confidence how many mammals and birds

inhabit the Earth. It is much more difficult, however, to know the numbers of flies or deep-sea worms. Most challenging of all are the bacteria. How many bacterial species are there? More than 2,000 have been described, some with great precision. The best-known of these—such as the gram-positives and the proteobacteria, which occupy several chapters of this field guide—can be cultured readily in the lab and have significant medical and economic importance to humans. We know what these bacteria can and cannot "eat" and what conditions favor their growth. The 300 to 500 or more genera are divided into 2,000+ species, with many of them among the famous "lab rats" such as *Escherichia, Streptococcus, Staphylococcus,* and *Pseudomonas.* When microbiology students are given the task of identifying an "unknown" bacterium, they are almost invariably assigned an easily cultivated species from among the gram-positives or the proteobacteria.

Has the number of bacterial species been *under*estimated? Many field microbiologists would answer this question with an emphatic "Yes!" Every year, we learn a little more about the microbes living at great ocean depths, in polar ice and boiling springs, and encrusted within deep sediments. New species of bacteria are continually being discovered and named, with no end in sight. Perhaps there are many thousands more to be added to the list—some scientists have estimated that less than 1 percent of bacterial species have been cultured and characterized. Perhaps, too, the characterizations of new species will not be as readily organized in the neat identification tables found in microbiology lab manuals. The fact that there are many more recorded species of gram-positives and proteobacteria than of other groups may reflect not their real-world abundance but rather the ease with which these groups can be studied and categorized in the lab.

Has the number of bacterial species been *over*estimated? Strangely enough, this question can also be answered in the affirmative because of the difficulty in firmly assigning bacteria to the level of species. The modern concept of species was first developed to apply to large, sexual organisms for which producing fertile offspring when mating outside of the species is, by definition, not possible. Bacteria do not reproduce sexually and have therefore never been assigned to species according to this definition, but rather according to differences in their culture characteristics in the lab.

But here is the problem: Bacteria are extraordinarily promiscuous with their DNA. By a process called "horizontal transfer," DNA sequences can be exchanged among different species and even among different kingdoms. Horizontal transfer of DNA is not only possible, but it is apparently carried out readily by intermingling bacteria in the wild. As a result, then, bacteria can acquire DNA sequences not only from each other but from humans, for example, effecting a transfer of DNA from the animal kingdom to the bacterial kingdom. Some microbiologists (such as Sorin Sonea and Maurice Panisset) have suggested that there are really no bacterial species at all but rather a sort of continuum of flowing genes over a huge amount of space and time. At any given point we have a snapshot that gives us the illusion of taxonomic groups because exchanges occur most easily between similar bacteria and less easily between more distantly related groups. Similarly, bacterial groups that are not physically near to each other cannot easily exchange DNA, whereas those that inhabit the same space may benefit from having interdependent metabolisms: two partners in a tight consortium may not both need to maintain a full set of genes.

Thus we should—with caution—use the analysis of DNA sequences to assign bacteria to large taxonomic groups but also realize that fine differences in sequence, metabolism, and morphology may not be reliable differentiators of genus and species. Does this mean that the concept of species should be abandoned for bacteria? No. Identification methods continue to be very useful for the diagnosis and treatment of pathogens. Furthermore, many lab techniques remain our only way of sorting out and understanding the largely invisible world of bacteria.

Much of what we know about bacterial taxonomy has been hard won by clever, creative researchers using methods that are well worth learning and practicing. But taxonomists are also cautious about applying this information too simplistically when assigning phylogenetic groups, especially genus and species.

NAMING BACTERIA

Serious naturalists and professionals in biology take pride in knowing names of phyla, orders, families, genera, and species. This process of assigning names is, of course, much more than just

a mental exercise. It is essential because regional nicknames or common names for organisms are often ambiguous or limited in their usage; we need scientific terminology to communicate outside of our own locales. However, while this field guide is full of Latin- and Greek-derived names, it is also full of common names. This requires some explanation.

First, in ordinary conversations about their organisms, professional microbiologists often use nicknames (some, seemingly affectionate), and at the very least use truncations of much longer terminology—referring, for example, to the alpha proteobacteria as "alphas," the Chlorobiaceae as "green sulfurs," and the methanogens as "methanos." (This nicknaming is not obvious in the professional literature, which is almost always written in strictly formal style, but, in microbiology in particular, perhaps it helps researchers feel a little closer to their subjects, making the invisible less so.) I've attempted throughout to use the common names known to many professional microbiologists, in the hopes of letting the reader in on the sometimes casual nature of microbiological field and lab talk.

In spite of nicknaming, microbiologists take their scientific terminology very seriously. The seriousness takes two forms:

1. Considerable careful and persistent effort is made to identify bacteria to the level of genus and species. This task is not easy in most cases. A microbiology lab can look like a chemistry lab, with numerous test broths and multicolored stains. In fact, most lab training of microbiologists centers around the many techniques used for identifications. In most cases, however, it is well beyond the purview of this field guide to lead the reader through identifications to genus and species. The few exceptions are those bacteria that display extraordinarily specific field marks.
2. Bacterial phylogeny has been in a welcome state of flux, with even phylum-level classification being overturned based on new evidence from DNA. However, many bacterial groups still remain ciphers. Therefore, microbiologists generally try to show caution and restraint in coining new names. Until we know exactly what a bacterial group is and does and where it properly belongs on the family tree, we use relatively noncommittal names. A good example can be found in the proteobacteria, in which

the new subclassifications based on DNA evidence have simply been assigned the Greek letters alpha through epsilon instead of elaborate (and often multisyllabic) Greek or Latin names.

Most current bacterial terminology plunges from the level of phylum (or something like phylum) to genus and species, bypassing completely any mention of order. For example, *Bergey's Manual of Systematic Bacteriology* (1994) places green sulfurs in major category I (gram-negatives), group 10 (the anoxic phototrophic bacteria), and subgroup 4, whereas *The Prokaryotes* (1992) places them in the sole family in division E (both family and division called Chlorobiaceae). However, most bacterial family trees based on DNA sequencing assign the green sulfurs their own major branch, suggesting that they are, at the least, a phylum. Because of this lack of consensus, it is often not possible in this guide to identify what level (for example, phylum, class, or order) a bacterial group such as green nonsulfurs, spirochetes, or gamma proteobacteria corresponds to.

Therefore, I ask that you bear with the mixture of seemingly dignified Greek and Latin names interspersed with unmemorable temporary assignments, such as "alpha proteobacteria," seeing them instead, perhaps, as reflections of these exciting, unsettled times in bacterial taxonomy—for professionals and amateurs alike.

Balance and Focus

This guide presents all the major taxonomic groups and many of the subgroups of bacteria. With the exception of planctomycetes, which are inferred rather than identified, all the major groups are identifiable in the field without a microscope. Nevertheless, balance may be of concern to some readers. Have any important bacteria been left out by an emphasis on those bacteria with the most rewarding, most accessible macroscopic field marks? Indeed, this guide presents not a balanced treatment but rather one that is intended to be inviting to those who would not otherwise consider looking for or at bacteria. Many bacteria presented in traditional microbiology classes—many of these well-studied pathogens—are treated very briefly because they are identifiable only in professional labs.

Actually, although it is not always acknowledged, balance is a

problem for any book on bacteria. For example, many microbiology textbooks (and courses) are dominated by a few groups of medical importance, often leaving the impression that most bacteria are pathogens. Textbooks on environmental microbiology often manage a more even presentation, but nevertheless do not do as much as they could with macroscopic indicators. Much of the information on quick macroscopic field identification gets passed along through word of mouth (from expert to expert) or is published in technical papers. Even *Bergey's Manual of Systematic Microbiology*, long considered the bible of the microbiology lab, devotes much more space to well-studied groups—often those bacteria that proliferate like weeds in the lab—than to the more fastidious or slow-growing species that actually predominate in the environment.

Professional microbiologists will be quick to discover that the sections pertaining to their own particular bacteria of expertise are by no means exhaustive—nor are they intended to be in the usual professional context. Rather, this is a collection of tricks of the trade and rules of thumb by which professionals identify bacteria in the field. Many of the methods are communicated by word of mouth or in the medieval guild style of passing information from master to apprentice, still typical of many Ph.D. programs. Perhaps professionals will appreciate a written compilation of their "trade secrets" for macroscopic identification of bacteria.

So, yes, this book too has a slant. It tilts in the direction of those bacteria that are most likely to be identified in the field, without resort to the usual battery of laboratory tests and extensive microscopy. It is a happy coincidence for the organization of this guide that so many of the major taxonomic groups and subgroups are identifiable directly through the senses. Or it might be no coincidence; after all, bacteria are the most numerous and diverse organisms on Earth. Their apparent invisibility may be just an artifact of a human-centered point-of-view.

Ecology on a Miniature Scale

Intimacy with the Environment

"Microscopic" is the normal scale of the world. Most organisms on Earth are tiny and always have been and probably always will be.

The relatively huge, lumbering size of ourselves and our fellow animals is exceptional—albeit quite successful in its own way, especially if you use beetles as the representative animal. J. B. S. Haldane, a mathematical biologist, noting that there are 8,000 species of mammals but 400,000 species of beetles, commented that the creator must have had "an inordinate fondness for beetles."

What does it mean to be microscopically small? Smallness means to be in intimate contact with the environment. A little moisture or dryness, slightly more concentrated salt, an elevated temperature or pH are all sensed directly by single cells. The responses produced are rapid, usually involving some change in metabolism, even shutting down to dormancy if conditions are too stressful.

Like all organisms, bacteria need water to live; microbial ecology is aquatic ecology. On the microscopic scale, "water" could mean a droplet on a leaf or a moist soil particle, the interior of an animal's intestine, the open sea, or the hot sulfury waters of a thermal spring. Water—in some form—is needed for cells to be active and to reproduce.

While ecology on a larger scale is usually divided into terrestrial and aquatic habitats, microbes experience the environment as an aquatic continuum. The moist soil at the side of the river is continuous with the river water and the water that has splashed up on the rocks. The moist intestinal environment is a continuum with the moist feces and moist soil on which the feces are deposited. Similarly, the dry desert is merely an aquatic environment in which the dormant inhabitants are waiting for rain, at the dry end of a temporal continuum.

Rather than terrestrial and aquatic, the microbial world is divided according to what nutrients and energy sources are available and what chemical and temperature conditions are present. Such parameters may change dramatically (from a microbial point of view) over the length of just a few bacterial cells. The bacterial world can be thought of as a mosaic of tiny islands of discontinuous resources.

Life on a soil particle, for example, is quite different from life on the surface of a nearby rootlet of a plant. Life in the top layer of a microbial mat is different from life even a few millimeters down. Understanding what bacteria are taking from their environment

and depositing as waste is the key to understanding microbial ecology and the distribution of microbial species, as well as their identification in the field.

MOVEMENT

Being tiny means experiencing almost any aquatic environment as extremely viscous. This phenomenon was discovered and quantified in the early 20th century by an engineer named Osborne Reynolds. He came up with a measure of fluid flow called the Reynolds number, which helps to understand the relationship between size and the ability to glide through a watery environment of a particular viscosity. A whale in the ocean has a huge Reynolds number (about 300,000,000), which means that with a few strokes of its fins it can glide a great distance. Humans glide less efficiently because they are smaller and have a Reynolds number of about 10,000. Applying this engineering concept to bacteria was the brilliant idea of Harvard biologist Howard Berg. A bacterium in the ocean does not glide at all. If it stops twirling its flagellum, it comes to an immediate stop. It has a Reynolds number of about 0.00001. You can experience a similar phenomenon by trying to swim in a pool full of gelatin (or perhaps just use your imagination). Even if you push off strongly from the side of the pool, you (bacteria-like) will fail to glide through the viscous gel. That's why so many bacteria don't even "try" to glide. Instead they attach to a surface and allow the watery environment to flow by. Motion is relative—flow brings nutrients and removes waste, and that is often all a bacterium needs. Rapid flow can be really inconvenient for a tiny bacterium, however, if it wants to maintain its position; that's why the bacteria in rivers and intestines so often live firmly attached to surfaces. Attachment can also mean clinging to surface films of still water, to minute particles, and of course to other organisms. Another strategy is to float, or to at least maintain some approximate position in the water by means of gas-filled vacuoles.

Motile bacteria that use tufts of flagella or gliding mechanisms rarely move far. Usually they jockey for position in a community, finding optimal positions in the sun or in a gradient of sulfide or oxygen or temperature. A few millimeters is a long way. The major exception to this rule can be found in the spirochetes (chapter 16)—

long, skinny organisms so efficient at motility that they seem to defy the restraints of the Reynolds numbers. They can move through the most viscous of environments, such as thick mud or full intestines. This means of motility is one reason why some of them can be such insidious pathogens, corkscrewing through our tissues.

SIZE

There must be something especially advantageous about being tiny. The logic is simple: Most organisms are tiny bacteria, and the greatest organismal diversity is found among these tiny bacteria. Furthermore, most of the evolutionary history of Earth has been dominated by (tiny!) bacteria. What is so wonderful about that size? Bacteria have a certain intimacy or immediacy with the environment such that slight changes in a local area can produce rapid and specific responses. In addition, reproduction (cell division) in bacteria is faster than it is for larger cells or for multicellular organisms. Perhaps that kind of efficiency counts for a lot. There seems to have been little deviation through evolutionary history from the fast-responding, rapidly dividing body plan typical of most bacteria. Therefore, the truly large bacteria are worth speculating about: Why big?

Only a few bacterial groups have gigantic representatives: the gram-positives, the proteobacteria, and the spirochetes. These are also three of the most thoroughly studied groups of bacteria. Cyanobacteria should also be mentioned here, although they are for the most part large and conspicuous due to multicellularity.

How gigantic is "gigantic"? The behemoth bacterium *Epulopiscium fishelsoni*, a gram-positive species that inhabits surgeonfish guts, is a strong candidate for the largest. It is a rod about 80 × 600 micrometers in size—in other words, 0.08 × 0.6 millimeters, or about half a millimeter long, bigger than most animal cells. If you are willing to squint at specks, you can actually see this cell with your naked eye. Which leads us to ask, is there anything special about surgeonfish guts? These fish eat algae, which puts them in the same league with terrestrial herbivores such as cows. Plant and algal material can be difficult to digest, and all known herbivores cultivate symbiotic bacterial communities in some part of their digestive system to help with that process. Guts are safe,

nutrient-rich places, extremely popular as habitats especially among fermenters such as gram-positives. Some of the largest bacteria I have ever seen—long gram-positive rods—were in the symbiotic community of termite guts. The abundance of nutrients in such a habitat might allow bacteria the luxury of time and growth. Therefore, while we might not expect to find truly gigantic bacteria in every herbivore gut, we might expect to find many large ones.

Speaking of guts, that is where we might expect to find some of the largest spirochetes too (see chapter 16). Again, it has been my experience in looking at termite digestive symbionts that the spirochetes are especially large (up to 100 micrometers long) and active. Also, the spirochete *Cristispira*, found in some molluscan digestive systems, can be up to 150 micrometers long (but only about $1^1/_2$ micrometers wide). You still need a microscope to see them. The biggest of the spirochetes—*Spirochete plicatilis*—may be free living, enjoying the rich environment in and around strands of *Beggiatoa* (see chapter 9) in some sulfur-rich environments. If you are looking at *Beggiatoa* (highly recommended) you may find this spirochete too. It can be up to 250 micrometers long (a quarter of a millimeter) but less than a micrometer thick.

Among the proteobacteria, the large bacteria, such as *Beggiatoa*, are those that perform a fairly delicate and unusual metabolic procedure: sulfide oxidation. Their size may be related to their metabolism. This process requires having a source of unoxidized (reduced) sulfur, such as hydrogen sulfide (the smelly gas of salt marshes), along with a supply of oxygen. Oxygen, however, readily oxidizes hydrogen sulfide without any assistance from or benefit to the bacteria. Perhaps in some of these bacteria, large cells provide a way of keeping reactive compounds segregated. *Beggiatoa gigantea*, for example, can have cells up to 25 to 55 micrometers wide and about 10 micrometers long, arranged in long multicellular filaments. Two species of *Beggiatoa* from deep-sea thermal and cold-seep communities have cell diameters of 120 to 200 micrometers. These cells actually have relatively little cytoplasm (cell contents), as most of the space is a large storage vacuole. In these bacteria, nitrate is probably used to oxidize sulfide rather than oxygen, and it may be that nitrate is stored in the vacuoles.

Another strong contender for the "biggest bacterium" prize—

Thiomargarita namibiensis (sulfur pearl of Namibia)—is also known to store nitrate. This species consists of a balloon-like cell typically 100 to 300 micrometers but sometimes as much as 750 micrometers, or $^3/_4$ of a millimeter, in diameter. Look at a metric ruler and imagine a pearly droplet that just fits between two of the millimeter lines. Whether it truly deserves to take the prize from *Epulopiscium* (the species found in surgeonfish guts) depends on how you want to count the enormous nitrate-filled vacuole of *Thiomargarita* that comprises 98% of the cell. *Thiomargarita* has been described by microbial ecologist Bo Jørgensen as "holding its breath" by stockpiling so much nitrate.

The cyanobacteria get lots of credit for being very long, albeit very skinny (less than a micrometer). If you gently pull apart the top blue-green layer of a microbial mat and look closely, you will see the tiny lint-like strands that hold the layer together, like felt. These minute strands are multicellular clumps of long filamentous cyanobacteria, not individual cells. Long filaments may consist of hundreds of cells. Thus, most cyanobacteria are not truly among the largest bacteria. However, *Oscillatoria princeps* is of interest because its cells are quite thick (up to 60 micrometers).

ACTIVE VERSUS DORMANT SPECIES

A critical problem in microbial ecology is knowing which bacteria are normally active in a particular habitat and which are primarily dormant. The distinction can be subtle. Active bacteria use whatever nutrients and other conditions are supplied by the surrounding habitat. If resources are typically scarce, then the active population is likely to have a slow growth rate and may be difficult to detect. In contrast, the dormant population is inactive, either in a resistant spore form or some other quiescent state, until there is an influx of unusual nutrients. A new and transient source of rich food, not typical of the usual available resources, such as a dead beetle, may allow some dormant bacteria to flourish temporarily. It matters little where the beetle died, as a variety of dormant bacteria are almost always present in nearly all environments, resting until an opportunity occurs. The microbiologist M. J. Carr called this "watchful waiting."

Many dormant bacteria are also called "opportunists"; opportunists are among the easiest bacteria to detect, and they include a number of pathogens. Simply put a soil particle on some rich nutrient medium, and opportunists will be the first to grow. The more typical active population of the soil, in contrast, may never get a chance to demonstrate its slow reproductive rate. Therefore, routine microbiological culture methods are not always the most accurate way to detect and evaluate typical (often low-level) bacterial activity in an environment.

PATHOGENS

The pathogenic bacteria (sometimes referred to as germs) get plenty of publicity in spite of being in the minority among microbes. This is perhaps no surprise: we are human-centered creatures, concerned and even obsessed with any organisms that might do harm to us, our pets, or our food supply. Relatively forgotten or ignored are the myriad microscopic populations of soils and waters, most of which have no interest whatsoever in inhabiting or injuring the human body. Many bacteria are photosynthesizers (makers of their own food using light energy) or chemosynthesizers (makers of their own food using chemical energy). Other bacteria would never survive in the temperate environment of the human body, preferring instead much greater extremes of temperature and salinity. Others are quite conservative in the range of nutrients that they use, and would find the human body's rich and diverse mixture of chemicals a hostile environment. And for the bacteria that *do* live in and on us, there is, in most cases, no particular advantage to gravely injuring or killing their host. Much better is a coexistence of host and bacterium.

Furthermore, the human body (like any animal, plant, or fungus) is extremely well defended. We have a tough, dry, inhospitable skin and a vicious immune system which, when functioning properly, defends against the majority of potential invaders. Not only is it vicious, but it is also sometimes too easily aroused. In *Lives of a Cell*, Lewis Thomas described having an immune system as being surrounded by a minefield—one of danger to ourselves as well as to bacteria. Overstimulation of the system can result in fever, tissue damage, and shock. Mild stimulation brings on allergic reactions of all sorts as well as autoimmune diseases. Indeed, bacteria are not solely responsible for all of the detrimental or even lethal outcomes

that can occur from infections; a massive inflammatory response can be just as harmful.

We also have our own indigenous—and often helpful—population of bacteria covering our skin and lining our digestive system, making it difficult for other bacteria to enter the body. A moderate exposure to bacteria of all kinds may prime the immune system to withstand later invasions. This is not to say we should be overly casual. Some bacteria arrive like the Trojan horse, tricking the immune system with chemical disguises (although this is, fortunately, relatively rare). Others get through breaches (cuts, scratches, bites) in the skin or digestive lining and reproduce quickly in places not easily monitored by the immune system. Some of our own indigenous bacteria, with which we normally have a symbiotic relationship, can turn nasty if there is a change in our usual defenses—such as a compromised immune system. Symbiosis is often not so much about mutual altruism as about mutual control. As Lewis Thomas put it in *Lives of a Cell*, "Disease usually results from inconclusive negotiations for symbiosis, an overstepping of the line by one side or the other, a biological misinterpretation of borders."

Who are the pathogens? They are a minority of bacteria. They tend to be opportunists, capable of reduced activity and even dormancy until a rich source of nutrients (such as ourselves) should suddenly appear undefended. Then they escalate their activities, consuming a variety of nutrients and multiplying quickly. Most of the best-known pathogens are members of the gram-positives and the proteobacteria, and others are spirochetes. *Chlamydia* species (relatives of planctomycetes) are all obligate inhabitants of other organisms' cells. The virulent members of these groups are well studied, well publicized, and, at least in industrial countries, fairly well controlled; several, however, are top killers in developing countries (see table I.1). Most of the pathogenic bacteria are not covered in this field guide—which would otherwise become in part a medical manual—but especially well-known pathogens are mentioned in passing when nonpathogenic bacteria of that group are discussed. Indeed, one of the more noteworthy characteristics of the pathogens, especially from the point of view of this guide, is just how limited they are to certain taxonomic groups and how rare their activities are within those groups.

TABLE I.1. Diseases of Humans and Other Animals, and the Bacteria That Cause Them

Disease or condition	Causal group and genus			
	Proteobacteria*	Gram-positives	Spirochetes	*Chlamydia*
Anthrax		*Bacillus*		
Cholera	*Vibrio* (γ)			
Diphtheria		*Corynebacterium*		
Ear infections	*Haemophilus* (γ)	*Streptococcus*		
Gastrointestinal infections** (including food poisoning)	*Salmonella* (γ) *Shigella* (γ) *Campylobacter* (ε) *Escherichia (E. coli)* (γ)	*Clostridium* (botulism) *Staphylococcus*		
Leprosy		*Mycobacterium*		
Lyme disease			*Borrelia*	
Meningitis**	*Neisseria* (β)	*Streptococcus*		
Plague	*Yersinia* (γ)			
Pneumonia**	*Legionella* (γ)	*Streptococcus* *Mycoplasma*		
Rocky Mountain spotted fever	*Rickettsia* (α)			
Sinus infections	*Hemophilus* (γ)	*Streptococcus*		
"Staph" infections		*Staphylococcus*		
Stomach ulcers	*Helicobacter* (ε)			
"Strep" throat (scarlet fever)		*Streptococcus*		
Tetanus		*Clostridium*		
Tuberculosis		*Mycobacterium*		
Typhoid	*Salmonella* (γ)			
Typhus	*Rickettsia* (δ)			
Venereal diseases	*Neisseria* (gonorrhea) (β)		*Treponema* (syphilis)	*Chlamydia*
Whooping cough	*Bordetella* (β)			

* Greek letter indicates alpha (α), beta (β), gamma (γ), delta (δ), or epsilon (ε) group.
** There are also viral versions of this disease.

Observing Bacteria

How extreme is your backyard?

How many different bacterial groups can you see from your backyard? The answer to this question depends on how extreme your backyard is. Most people do their landscaping with an emphasis on eukaryotes (mainly plants and animals). A rule of thumb is, the more visible the eukaryotes, the more invisible the prokaryotes (although the latter are present by the trillions, far outnumbering animals and plants). Bacterial field marks are not so obvious against an overwhelming background of eukaryotes, especially as observed from the point of view of a fellow eukaryote.

Another rule of thumb is, the greater the diversity of species—whether prokaryotic or eukaryotic—supported by a particular environment, the less likely it is that any individual prokaryotic species will predominate. In other words, the more nonspecific and moderate the environment, the more likely there are to be many different types of prokaryotes, with each type in relatively low numbers. Moderate environments include ordinary marine and fresh waters and any soil supporting a diversity of plants. Environments featuring some extreme condition, such as high or low temperatures, acidity or alkalinity, high salinity, or a lack of oxygen, are likely to appeal to only a few types of organisms—and typically these organisms are prokaryotes. It is often in such extreme environments that prokaryotes predominate to the point of displaying prominent field marks.

Viewing a wide variety of prokaryotes, then, involves traveling to a wide variety of habitats. Because most prokaryotic groups are distributed worldwide, the same types may be found wherever similar habitats exist. Salt flats in Mexico have most of the same bacterial types as salt flats in Utah or in Israel. Hot springs in Japan have most of the same bacterial forms as hot springs in Wyoming and Iceland. Thus, you will have to travel out of your backyard, but not necessarily out of your country, to see all of the bacterial groups.

What will you miss by not looking through a microscope?

Most bacteria are tiny rods or spheres, almost indistinguishable from each other. Usually what makes a bacterial species taxonomically distinct is what it *does*, not what it looks like. Bacteria take in various chemicals from their environments and put out other chemicals as products or waste products. In large enough numbers, they can produce substances that can be seen (or smelled or touched or tasted or even heard) on a macroscopic level. These field marks, along with characteristics of the habitat, are the basis for bacterial identification in this field guide.

I love microscopy, though, and I do not wish to make light of how exciting and important a tool it is for bacteriology. Some bacteria are quite distinctive in their morphology. Many photosynthesizers, for example, are large and colorful; helical spirochetes spiral easily through dense substrates. If you do not use a microscope, you are indeed missing out on the experience of seeing these bacteria directly. Therefore, consider using this guide as a starting point in your search for bacteria. This book will tell you how to find abundant, identifiable populations of bacteria that you can later scrutinize up close with a microscope.

Keep in mind that you will need a fairly powerful microscope, one with a capability of magnifying 400 to 1,000 times. Bacteria are about 1/10 to 1/100 the size of the more common microorganisms such as *Paramecium* and *Amoeba* that you may have encountered in an introductory biology lab. Without proper lighting and good lenses, bacteria can look much less distinct than tiny dots and dashes—they can appear to be nothing at all!

Throughout this guide, you will find sections on viewing a particular group under the microscope; these provide hints on what to look for if you have the opportunity to collect a sample and observe it. Appendix C also gives specific instructions on how to use a microscope to observe bacteria.