



BEGUILING BACTERIA

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I will admit that this title might be considered on the cute side, but it's too accurate to reject. To me, bacteria are enthralling. As a case in point, they weren't supposed to be the subject of this month's column. I was going through my folder of recent items of interest with the aim of focusing on some molecular rather than microbiological topic. But those bacteria kept coming to the fore, and there were just too many good items to ignore. By "good" I mean recent findings revealing new aspects to the microbial world, surprises indicating that the terrain is less well-charted than we might have thought. It's probably because I like good surprises that I've always been interested in bacteria, and my recent foray into the literature just deepened my conviction that there's nothing better to gladden the heart of biologist than a peak into this small world.

The first article to catch my eye was "Bacteria's New Bones" (Callaway, 2008). No, bacteria aren't that amazing—they don't have real bones, but rather a molecular skeleton that gives them shape. In other words, bacteria are more than just sacks of interesting molecules and can now be considered worthy of study in cell biology. A number of cytoskeletal proteins have been identified. FtsZ, a distant relative of the eukaryotic protein tubulin, creates a belt around the middle of most bacteria and cinches the dividing cell closed. Without FtsZ, rod-shaped bacilli grow longer and longer, never splitting. These bacteria also need MreB, which forms a helical pattern inside the cell wall, and probably directs the activity of wall-building enzymes. Without MreB, bacilli become spherical. For more complicated bacterial shapes, other proteins are required. The crescent-shaped *Caulobacter* needs crescentin or it will straighten out. Work is still being done on how spirochetes retain their spiral form; some have an internal tail or filament that gives them their twist.

These skeletal structures in bacteria are now coming to light for two reasons. One is the identification of a number of misshapen mutants. As with many biological functions, it's easier to figure them out when things go wrong. Also, the relatively new field of cryo-electron microscopy (cryo-EM) provides enhanced pictures of microbial structures. It gives three-dimensional images without the use of harsh chemicals needed in traditional EM. Among the amazing pictures generated by this technique is one of an actin-like filament, MamK, decorated with a chain of iron-containing magnetosomes in *Magnetospirillum*, a bacterium that can

orient itself in relation to the earth's magnetic field. Cryo-EM is likely to reveal much more about bacterial "bones," features that were invisible with traditional EM. This is an important point to make to students: Biologists study what they can find to study. Often, new fields open up because of new techniques that detect the previously undetectable. It's difficult to keep this very basic limitation in mind, that is why we so frequently make the mistake of thinking that we understand the whole picture when we only comprehend what our senses and our technologies make available to us.

Bacterial Diversity

Another example of this involves bacterial diversity: Just how many different kinds of bacteria are there? If you go by the number of bacteria that have been cultured and studied in some depth, then the number is a few thousand. However, some estimate that 99.9% of microbes will not grow on nutrient rich agar, the microbiologist's medium of choice. Difficulties in growing bacteria are hardly a new problem. Even today, the bacterium that causes leprosy can only be grown in the footpads of animals like rabbits or in the armadillo but what is becoming more and more apparent is just how many species are included in that 99.9%. One reason for this greater awareness is the increasing use of genomic analysis on microbial samples (Glausiusz, 2007). Last year, ocean water samples yielded 6 million new genes and thousands of new protein families. This doesn't give a hint as to how many different bacterial species there may be in seawater, but it suggests that the number is much higher than microbiologists had suspected. Other environments host totally different bacterial populations, but it takes ingenuity to discover ways to culture them. In her work on soil bacteria, the Australian microbiologist Belinda Ferrari finds that a mud slurry is an ideal medium for many of these microbes that can't survive in sugar-rich agar.

Another initiative is called SLIME (Subsurface Life in Mineral Environments), where researchers investigate caves to identify bacteria responsible for the deposition of oxidized iron and manganese crusts on cavern walls. Getting to some of these sites in the Southwest U.S. can take days, and then researchers have to put on clean suits to avoid contaminating specimens. Since these microbes live in strange environments—at least strange to humans—it's not surprising that they have unusual growth requirements. Because so little is known about them, the only way to grow them at the moment is *in situ*, in glass tubes exposed to the environment they like best: darkness, high humidity, and low temperatures.

Traveling for days to visit research specimens may seem extreme, but these bacteria, and others that seem equally fussy, are so intriguing and have such interesting chemistry, that they are worth getting to know. Bacteria originally found in another alluring environment—a canal

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contaminated with agricultural runoff—yielded anaerobic ammonium-oxidizing (anammox) microbes. They were difficult to grow in culture, and it took researchers a year to discover the right mix of carbon dioxide, nitrate, and methane—in very limited amounts—for good growth. Now these bacteria are used to clean waste water on a large scale. So there are economic rewards from bending culture techniques to bacterial needs rather than trying to force the microbes to grow in what researchers think a healthy microbial diet entails. What I've mentioned here are only a few examples of the kinds of efforts underway to both identify interesting bacterial genomes and culture these diverse organisms. The two approaches obviously go together, and for one fascinated by these creatures, the implications are almost dizzying. It's like being back in the time of Pasteur and Koch all over again, back when each new research foray led to the discovery of new organisms and new chemical activities.

Diversity Within

Another terrain that's proving to have a more diverse microbial population than was suspected is very different from remote caves and polluted water: it's the human gut. Since classic culture techniques were patterned somewhat after this milieu, it might seem that most of these organisms would have been identified by now. But again, genomic analysis is yielding surprises, thanks in part to the Human Microbiome Project (HMP). In a recent article on this endeavor, the authors make a bold claim for the significance of microbes to human life: "If humans are thought of as a composite of microbial and human cells, the human genetic landscape is an aggregate of the genes in the human genome and the microbiome, and human metabolic features as a blend of human and microbial traits, then the picture that emerges is one of a human 'supraorganism'" (Turnbaugh et al., 2007, p. 804). In other words, they are contending that you can't understand human genetics without understanding bacterial genetics as well. Now this argument is obviously being made by a bunch of microbiologists, but still, even those less bacterially-obsessed than myself have to admit that bacteria—for better or worse—are a fact of human life, and really of any life.

Like the analysis of ocean water mentioned earlier, the HMP is an example of metagenomics: studying the genomes of entire communities. This approach makes me a little queasy both because of its scope (all life forms within the body) and its sloppiness. It's one of those broad-stroke endeavors, and it's not going to reveal much about any one member of the community, though it gives some sense of how large and diverse the community is. This is like using satellite imaging to study an ecosystem; you miss a lot of detail yet it does give a great deal of information rapidly. For example, metagenomics is a way to study the microbial colonization of the infant gut, and even in what would seem to be relatively simple system—the gut is sterile at birth—the emerging picture is complex (Comstock, 2007). Sampling began with the first stool after birth, and in all, 25 samples were taken over the first year from each of the 14 infants in the study. While there are 22 broad taxonomic categories of bacteria, only three are abundant in the infant intestines: Gram-positive bacteria (Firmicutes and Actinobacteria), the Bacteroidetes, and the Proteobacteria. These three are found in adults as well, though there are few Proteobacteria which are facultative anaerobes; their ability to grow with or without oxygen may give them an edge as early colonists.

By the time the infants had reached their first birthdays, their gut flora were becoming similar to those of adults; this might

relate to the introduction of solid food which leads to significant change in the bacterial profile. Other results were less clear-cut. While antibiotic treatments led to reduced microbial loads, there didn't seem to be any long-term differences as a result of their presence. All the babies in the study were breast fed to some extent, so no comparisons with strictly bottle-fed infants were possible, but researchers were surprised to see little similarity between microbes in breast milk and feces. Bifidobacteria were only minor fecal components even though they are thought to be abundant in breast-fed infants. But again, this is a small study using a relatively new technique. Like any good study, it provides clues to the next questions that need to be asked.

Being Germ-Free

One way to explore the significance of bacteria to the mammalian supraorganism is to look at germ-free animals since they have to go it alone without a microbial community to support them. The first time I learned about the plight of germ-free animals, I was intrigued. Here was another biological surprise: being without bacteria is not a good thing, despite the bad rap that 'germs' get. Basically, germ-free animals are weak runts. For example, they have small hearts. No one is sure why, but it's intriguing that something normally going on in the gut spurs heart muscle development. Another puzzle is that germ-free mice are more active, perhaps because of differences in neurochemicals that could result from the lack of microbial processing of nutrients in the gut. Other differences are more understandable. Immune system development is reduced because of lack of antigen stimulation, and obviously, digestion of food and metabolism of drugs are impaired.

While most of this has been known for some time, what the HMP provides is information on just how rich the microbial life is that these animals are missing. Estimates vary depending on the sampling techniques used, but data from a number of studies indicate that there are over 50 bacterial phylotypes in the mouth and almost 200 in the colon. The term "phylotype" is approximately equivalent in meaning to "species," and is based on 16S ribosomal RNA gene sequence analysis. Depending on the location of origin, up to 80% of human associated phylotypes haven't been cultivated. So while some microbiologists are going to distant caves to work on hard-to-cultivate species, there are many elusive microbes much closer to home. Learning about these human symbionts is important because of the close association between the characteristics that allow a microbe to survive in the body and those that make it a pathogen: "The biochemical mechanisms for sensing host environments, interacting with host surfaces and even communicating with the host are often the same in human pathogens, commensal microorganisms and mutualistic microorganisms" (Dethlefsen et al., 2007, p. 814).

While metagenomics makes strides, analysis of individual genomes of pathogens continues to bear fruit. In 1995, the first bacterial genome sequence was completed, that of *Haemophilus influenzae*. This was a massive effort, but now bacterial genomics has yielded the sequences of dozens of organisms. Even many strains of the same species, *E. coli*, have been analyzed. What luxury—to have this richness of data, to be able to find small differences at the same time others are studying the genomic opulence of the entire ocean. Of course, new knowledge brings new puzzles. Two strains of *E. coli* can vary by as much as a quarter of their genomes, and there are many similar genetic puzzles among other pathogenic microbes. For example, the organisms responsible for leprosy, typhus, and plague have hundreds or

even thousands of pseudogenes. All these species have adapted to their host niches and as a result, may have lost the need for many proteins, so: “Every genome should be viewed as a work in progress, burdened with some non-functional ‘baggage of history,’” despite the fact that in the past bacteria were thought to be free of the pseudogene “baggage” found so commonly in eukaryotes (Pallen & Wren, 2007, p. 837).

It's thanks to their relatively small genomes that we've learned so much about the genetics of bacteria, and it's difficult to comprehend how quickly all this has happened. I am writing this column in February, and a few days ago, Joshua Lederberg died (Broad, 2008). He received the Nobel Prize in 1958 for his work on bacterial recombination, demonstrating that genetic material is transferred between bacteria. I was explaining this to a non-biologist friend of mine this week, after she said she thought we always knew this! Admittedly it is 60 years since Lederberg's discovery, but still it's amazing how the wonders of one age become the common knowledge of another. This is what makes teaching about the history of science so difficult, and also so necessary. It's important for students to understand that science it is not just a matter of new knowledge accumulating over time, but attitudes and mind-sets change as well. When Lederberg published, his work was startling to many because bacteria were seen as questionable living things, so different from “real” life, from organisms with nucleated cells, that their right to be called full-fledged forms of life was debatable (Lederberg & Tatum, 1946). Some biologists saw them as more closely related to viruses—and we all know how difficult they are to define as living or not. This bacterial ambivalence is considered one reason why Oswald Avery's research on DNA as the carrier of genetic information in bacteria was not given much attention when it was published in 1944 (Judson, 1979). Even those who considered his findings valid thought they might not extend to the eukaryotic world, because bacteria were just so different, so simple.

Webs & Trees

As many of the studies I've cited here indicate, those simple days are long over. There are complexities to be faced everywhere in bacterial research today. One of the most intriguing areas is attempting to work out the evolutionary relationships among bacteria. What makes this difficult is just what Joshua Lederberg discovered: genes can be transferred horizontally from one bacterial species to another, as opposed to just vertically from ancestors. In fact, a recent article on horizontal or lateral gene transfer cites Lederberg and Tatum's 1946 paper as its first reference (McInerney & Pisani, 2007). It reports on new experiments in which attempts were made to transfer a wide variety of genes into *E. coli*. While some genes were easier to transfer than others, no gene family was completely resistant to such manipulation. Though this was obviously an artificial situation, it lends support to the increasingly popular idea that at the base of the tree of life lies a web of lateral genetic transfers. This would mean that the taxonomic ideal of eventually working out what lies at the base of the tree would be impossible, because there is no such base; the horizontal movement of genes over billions of years has obliterated the genetic beginnings of life.

Among other things, this research illustrates the power of metaphors in scientific thinking. The tree of life is an image, both literal and metaphorical, that is closely tied to evolutionary theory. As is commonly mentioned, the only illustration in *Origin of Species* is a branched diagram of species relationships, and Darwin's “red notebook” in which he tackled the species

question has a simple tree diagram, as he seems to be thinking visually about how species are related to each other and arise from each other like the branching of a tree. This diagram is likely to be reproduced many times as Darwin's 200th birthday in 2009 nears. In fact, the issue of *Nature* which arrived yesterday has an article in anticipation of this event, or perhaps in celebration of his 199th birthday on February 12 (Padian, 2008). In any case, included is a photo of the tree sketch from his notebook, and for the first time (I am slow) I noticed that right above the tree are the words “I think.”

A further indication of the strength of the tree metaphor is another article in the same issue which reports on a study of the properties of ancient bacteria (Gouy & Chaussidon, 2008). Using several modeling techniques, researchers estimated the sequence of an early form of EF-Tu, a transcription factor found in all organisms and thus assumed to be present in early life. They discovered that the proteins produced from these genetic forms were heat resistant, suggesting that the early environment was warm, from 65° C to 73° C, a fact also suggested by the geological record. Used to illustrate this review of the research is not a web but a colorful tree with three main branches representing bacteria, archaea, and eukaryotes. The base of the tree is a gnarled stump labeled “LUCA” for Last Universal Common Ancestor, paleomicrobiologists' term for the bacterium-like organism that represents earliest life. While this stump has two colors to show that it's related both to the bacterial branch and the branch that later bifurcated into archaea and eukaryotes, it doesn't have a web-like structure at all, and there is absolutely not webbing of the branches above.

I think it's helpful to make students aware of such divergent imagery. As Stephen Jay Gould (1991) wrote years ago, scientists choose their words carefully but their images belie what they are really thinking. In this case, most biologists are still wedded to the tree of life as an evolutionary symbol. A web, on the other hand, connotes ecology. Webs are how individuals interact in an ecosystem, but now some mixing of metaphors might be called for. It may take some time, and a lot more data, for the web idea to penetrate phylogenetic imagery.

To tie this web metaphor back to the genomic research with which I began, I want to mention work on microbial genome exchange being done at the J. Craig Venter Institute in Maryland. Venter was one of the masterminds of the Human Genome Project (HGP), and he continues to be an important force in genomics; the work on ocean bacteria discussed above was also funded by this institute. In the exchange work, microbiologists took the genome of one bacterium, *Mycoplasma mycoides*, and transferred it into another, *Mycoplasma capricolum* using a clever technique to eliminate *M. capricolum* genes (Ball, 2007b). This is really the ultimate in horizontal gene transfer, and is seen as a prelude to incorporating an entirely synthetic genome into a *Mycoplasma* shell. This bacterium was chosen because it has a very small genome, and Venter's group is attempting to discover the minimum number of genes needed to maintain bacterial life. They hope to then use this “minimal” organism as a foundation on which to build new organisms with useful functions, such as ones having cellulase and hydrogenase enzymes that could digest plant material and generate hydrogen. The question researchers were trying to answer with the *Mycoplasma* transfer was how much gene change could be tolerated. The two species differ in a quarter of their genes, so at least that much difference still results in viable cells. This work is a reminder of how much we already rely on bacteria for industrial reactions, and what the possibilities are for the future.

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There may be other bacterial webs on the horizon as well, as Philip Ball (2007a) describes in an article on recent research which suggests that “bacteria can sprout webs of electrical wiring that transform the soil into a geological battery” (p. 388). First, microbiologists found that the soil bacterium *Shewanella oneidensis* can grow long filaments that conduct electricity. They argue that the bacteria use the filaments, which they term “nanowires,” to shunt electrons, resulting from metabolic activity, on to the surface of mineral grains in the soil. Now the same group has found evidence that the “wires” link up to form a network that can shuttle electrons to the soil surface. Others are not yet convinced of the results. They think the electrons may not be moving through the “wire” filaments, but are shed on the surface of other molecules. As is so often the case, only more research will settle the issue. But however the matter is resolved there’s some interesting bacterial chemistry at work here, even if the physics doesn’t turned out to be as intriguing as this research suggests.

While we’re waiting for these results and for researchers to create “new” bacteria, it seems to me that there is more than enough going on in the world of microbiology research to keep even the most blasé biologist interested. There are obviously so many bacteria we haven’t yet discovered, with all kinds of clever abilities, that it seems hopeless to try to imitate their ingenuity. Whatever Venter’s colleagues come up with will probably seem tame compared to bacteria that have novel genetic coding (Atkins & Baranov, 2007), cause their kin to commit suicide (Kolter, 2007), or become more virulent when exposed to light (Kennis & Crosson, 2007).

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