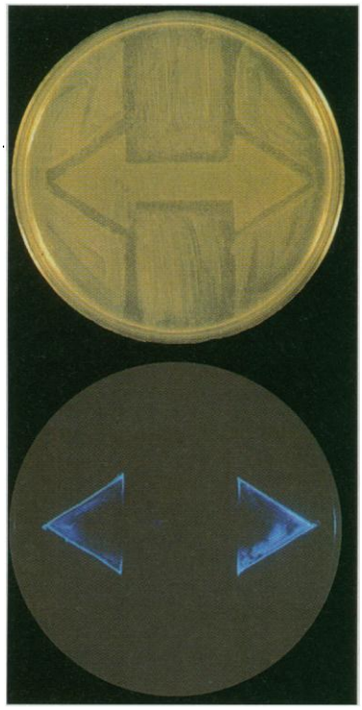
A Symphony of Bacterial Voices

Bacteria can communicate with members of both their species and others, thereby allowing them to coordinate their activities

Hum by yourself in an auditorium and the room will swallow the sound. But wait until the rest of the chorus shows up, and together you can fill the hall with music. Some activities require a group to make an impact. Indeed, even microbes behave as if they understand that there's power in numbers.

Over the past 5 years, scientists have found that a wide variety of bacteria gather a crowd before acting in unison—a phenomenon that's been dubbed quorum sensing. "Bacteria want to do some things when they're alone and others when they're in a community," says Bonnie Bassler, a microbial geneticist at Princeton University. "Sensing and responding to high population density allows them to switch to a new set of tasks."



Lighting up. In this culture, photographed by incident light (top) and by bioluminescence (bottom), the arrow contains *V. harveyi* bacteria. *E. coli* and *S. typhimurium* bacteria applied around the arrowheads produce signals that induce *V. harveyi* bioluminescence, while a non–signal-producing *E. coli* strain applied at the crossbar has no effect.

In some cases, doing so benefits not just the microbes but also the creatures they inhabit. The classic example is light emission by the luminescent bacterium *Vibrio fischeri*, which lives in a specialized organ in host animals such as the Hawaiian bobtail squid. The light that is turned on when the organ is sufficiently filled with bacteria eliminates the squid's shadow as it swims in the moonlight, making it hard for its predators to see it. Thus, the animals live longer, and the bacteria get a home.

In many other cases, though, the results of quorum sensing render bacteria harmful to their hosts. Some bacteria, when their population is large enough, congregate in slimy mats called biofilms, which have been implicated as a cause of many human infections (see Review by Costerton, Stewart, and Greenberg on p. 1318); others produce virulence factors, proteins that allow pathogenic bacteria to exploit their host (see Review by Galán and Collmer on p. 1322).

As researchers tease out the molecular details of how bacteria sense and respond to their neighbors, they may learn how to foul up the counting systems of specific strains. This could lead to ways to thwart the ability of pathogenic bacteria to cause illness without generating side effects by wiping out indigenous flora, as current broad-spectrum antibiotics usually do. "Instead of sterilizing the human host, you're trying to make it unfit for the particular bacteria to survive," says microbiologist Peter Greenberg of the University of Iowa, Iowa City. Conversely, research on quorum sensing could spawn methods to enhance desired activities, such as the production by plant bacteria of antibiotics that protect their host plants from disease.

Because recent research suggests that bacteria can take a census of other species as well as their own, understanding quorum sensing will likely also reveal insights into microbial ecology. "In most natural environments, bacteria live in complex mixed communities," says Greenberg. "Understanding how these develop and organize is going to be critical in attempts to control and manipulate them."

The light at the end of the tunnel

Microbiologists got their first inklings that bacteria might be counting their neighbors in the late 1960s when J. Woodland Hastings, currently at Harvard University, and his colleagues noticed a perplexing pattern of bioluminescence in *V. fischeri* and various other marine bacteria. During early growth, these bacteria remained dim. But when their cells reached a certain density, their luminescence shot up.

Kenneth Nealson and Terry Platt, working with Hastings at Harvard, soon traced the effect to some unknown signaling substance that the bacteria secrete into their growth medium. But that substance remained elusive until the early 1980s. Then, using as an indicator a natural *V. fischeri* strain that can't make the signal but does respond to it, Anatol Eberhard of Ithaca College in New York and his colleagues purified the bacterium's quorum-sensing signal, a small molecule called an acylated homoserine lactone (acyl-HSL).

Over the next several years, researchers learned that *V. fischeri*'s quorum-sensing system has two key components: an enzyme that makes the acyl-HSL plus a protein that detects the chemical and responds to it by activating specific genes. As the bacteria multiply within a confined space, the enzyme, called LuxI, produces ever-increasing amounts of acyl-HSL. Eventually the chemical, which diffuses freely into and out of the bacterial cells, reaches a critical concentration at which it activates the second component, a protein called LuxR. The activated LuxR in turn binds to specific stretches of regulatory DNA adjacent to the *V. fischeri* genes needed for bioluminescence and turns them on. This system ensures that the bacteria produce light only when large numbers are present, keeping them from wasting energy when their population is too small to emit a visible glow.

By the mid-1990s, microbiologists had found that many other bacteria that are, like *V. fischeri*, members of the gram-negative group use the same general acyl-HSL scheme for quorum sensing. (Gram-negative bacteria are so called because they don't retain the purple Gram stain.) And bacteria use other signals as well. "It was originally thought that this weird communication system found in fish light organs was just some kind of fluky thing," says Gary Dunny, a microbiologist at the University of Minnesota, Minneapolis. "But it turns out that it's probably quite the norm for bacteria."

About a dozen human pathogens are among the bacteria so far known to possess an acyl-HSL quorum-sensing system. Take, for example, *Pseudomonas aeruginosa*, a common cause of hospital-acquired infections and of lung infections in people with cystic fibrosis. *P. aeruginosa* is hard to eradicate because it forms biofilms, which shelter the bacteria from antibiotics, detergents, and the host's immune system. Greenberg's team has shown that acyl-HSL production by the bacteria is what triggers their congregation in biofilms.

In addition to infecting humans, many gram-negative bacteria live on plants and in the soil, where they have such beneficial effects as protecting plants from infections or helping fix nitrogen, as well as harmful ones such as causing wilting diseases or crown gall tumors. Some of these bacteria, too, use acyl-HSLs for quorum sensing. In work described

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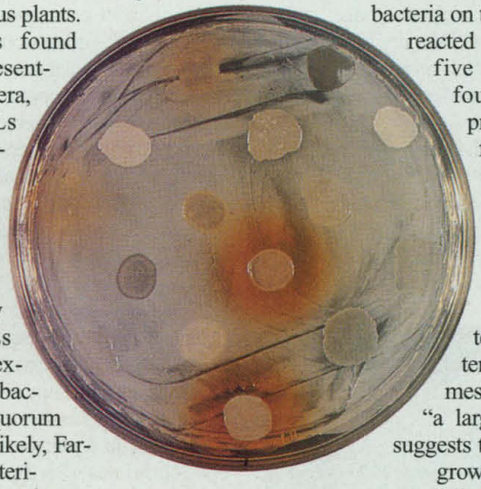
in the November 1998 issue of *Molecular Plant-Microbe Interactions*, for example, microbiologist Stephen Farrand of the University of Illinois, Urbana-Champaign, and his colleagues surveyed a total of 106 bacterial strains, representing seven different genera that live on various plants.

The researchers found that about half, representing all seven genera, produce acyl-HSLs and have the biochemical machinery to activate gene expression in response to acyl-HSL signals. They haven't yet demonstrated directly that the acyl-HSLs activate the gene expression or that the bacteria use them for quorum sensing. But that is likely, Farrand says: "If the bacterium goes to the trouble of making an acyl-HSL, my guess is it has—or had [sometime in its evolutionary past]—a quorum-sensing system that uses it as a signaling molecule."



Cross talk. The orange halos indicate that the *P. aureofaciens* bacteria growing on this petri dish produce the antibiotic phenazine in response to signals from some bacteria collected from wheat roots.

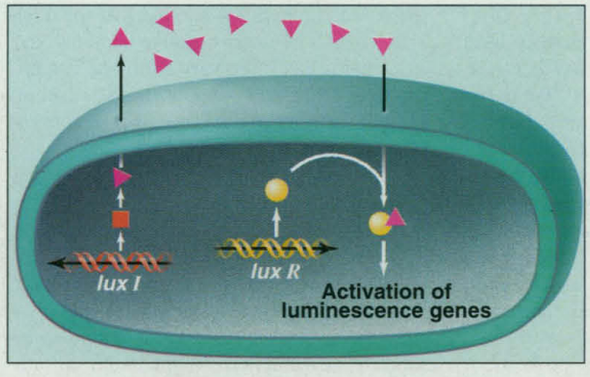
The discovery that multiple strains of plant bacteria make similar signaling molecules raised the possibility that the bacteria can hear members of other species as well as their own. To find out if such eavesdropping occurs, Leland S. Pierson III, a microbiologist at the University of Arizona, Tucson, and his colleagues turned to a plant-associated bacterium, *P. aureofaciens*. This microbe uses its own acyl-HSL quorum-sensing signal to determine when to make an antibiotic that helps it suppress its microbial competitors and also provides a service for its wheat plant hosts—protecting them against the fungal "take-all" disease.

In work reported in last November's *Molecular Plant-Microbe Interactions*, Pierson and his colleagues showed that production of the antibiotic, called phenazine, can also be turned on by signals from dozens of other bacterial strains. They isolated 700 strains of bacteria from wheat roots, let them grow in culture, and exposed a *P. aureofaciens* strain that doesn't itself make an acyl-HSL to the culture fluid in the lab. In 8% of the cases, signals in the fluid—which the group has since shown to be acyl-HSLs—stimulated the *P. aureofaciens* to make phenazine.

Pierson then went on to show that the same kind of cross talk can take place in the wild. His group prepared five sets of seeds, each coated with one of five different bacterial strains that had activated the phenazine biosynthetic gene in the lab plus a strain of *P. aureofaciens* equipped with a "reporter" gene that would produce an easily detectable product—it raises the freezing temperature of water—in response to acyl-HSLs. The researchers then planted the seeds and 10 days later looked to see whether the reporter bacteria on the seedling roots had reacted to signals from the five test strains. They found that all had produced positive effects. "There's a potential for communication between unrelated populations of bacteria in the real world," says Pierson.

One reason bacteria might want to intercept their neighbors' messages, he says, is that "a large amount of signal suggests that other bacteria are growing and happy. That tells the bacteria that this is a great place to be." Another is competition. As Pierson puts it, "a plant root is not Club Med. There are limited nutrients, and a bacterium needs to know who else is there so it can make decisions about how to expend energy and succeed in that environment." In *P. aureofaciens*'s case, this means making antibiotics to inhibit the growth of competing organisms.

With all this chatter, it might be hard for one species to hear its own members count. Work by Princeton's Bassler and Michael Silverman of the now defunct Agouron Institute in La Jolla, California, suggests that one way bacteria solve this problem is by using multiple quorum-sensing systems. The researchers have shown that the luminescent genes of the marine bacterium *Vibrio harveyi* are regulated by an elaborate mechanism that involves two discrete quorum-sensing systems. One of these uses an acyl-HSL signal, although both its synthesis and the responding molecule differ from those of more conventional acyl-HSL quorum-sensing systems. But the second uses a different, as yet unidentified signal.



Out and in. In *V. fischeri*, an enzyme (red square) produced by the *LuxI* gene makes an acyl-HSL (triangles). It freely diffuses through the bacterial membrane and, on reaching a sufficiently high concentration, activates LuxR, a protein that turns on gene expression.

Activation of either system is enough to turn on the light-producing genes, so Bassler wondered why there should be such redundancy. Experiments she has since performed with Iowa's Greenberg provided an answer. They found that a strain of *V. harveyi* that can sense the acyl-HSL but not the second signaling substance turns on light production only in response to signals from *V. harveyi* and a few other bacteria. Conversely, a strain of *V. harveyi* that can't sense the acyl-HSL of quorum system one but can pick up the second type of signal responds to a broad range of bacteria. "I think *V. harveyi* has system one for intraspecies communication and system two for interspecies communication," says Bassler. "Maybe the combination provides it with a way to know its proportion of the total bacterial population. It could then adapt appropriately by turning on different sets of genes, depending on whether it's alone or in a consortium."

E. coli and *S. typhimurium* catch up

The discovery that *V. harveyi* employs unconventional molecules—non–acyl-HSL signalers and non–LuxI/LuxR proteins for making and responding to the signaling compounds—may also help resolve a paradox: *Escherichia coli*, a gram-negative bacterium, has served as a prototype for understanding much about bacterial life, but even though quorum-sensing systems have been turning up all over the place, this organism didn't appear to have one—at least not one involving acyl-HSLs. New findings from Bassler's group now suggest that *E. coli* and its close relative *Salmonella typhimurium* may have quorum-sensing systems similar to *V. harveyi*'s second system.

Last year, the researchers showed that *E. coli* and *S. typhimurium* make a substance that activates luminescence gene expression in a *V. harveyi* mutant that can sense signals only through its second system. Since then, Bassler's team identified a gene involved in producing the substance in *V. harveyi*, *E. coli*, and *S. typhimurium*. [The results are in the 16 February issue of *Proceedings of the National Academy of Sciences* (*PNAS*).] Similar

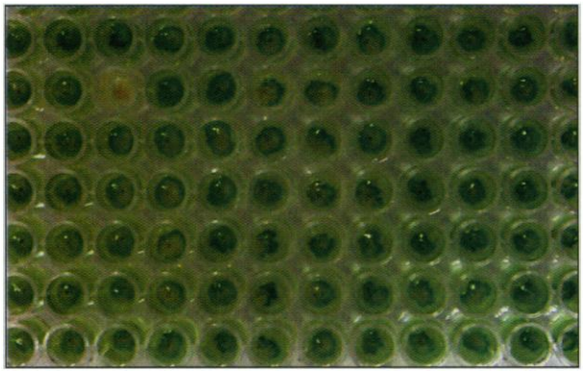
CREDITS: (TOP) L. S. PIERSON; (BOTTOM) ADAPTED FROM PETER GREENBERG

genes—and presumably similar signaling systems—seem to be widespread. By surveying the databases, Bassler and her colleagues have discovered related genes in over a dozen other species of bacteria.

The signaling substance this gene helps produce may tell *E. coli* to go on the attack once its population reaches a critical level. Working with the O157:H7 strain of *E. coli*, which has gained a justifiably bad reputation as a dangerous food-borne pathogen, James Kaper's team at the University of Maryland School of Medicine in Baltimore has shown that the gene Bassler identified produces a substance that induces the expression of this organism's type III secretion system. This system produces a "molecular syringe," which injects bacterial factors critical for successful infection into animal host cells.

And *E. coli*'s linguistic abilities may not stop there. In work reported in the 13 April issue of *PNAS*, microbiologist Philip Rather of Case Western Reserve University in Cleveland and his colleagues have uncovered what appears to be another quorum-sensing system in *E. coli*.

Like other quorum-sensing bacteria, *E. coli* may use its multiple systems to sense both its own numbers and those of other species. These pathogens might use other microbial "noise" to figure out when they've reached their destination. "Maybe they're picking up signals from normal bacteria in the intestine," says Kaper. "They then recognize it as an environment in which they want to make this type III secretion system."



Turn-off. As indicated by the loss of the green color in one well, certain natural compounds can inhibit production of *P. aeruginosa*'s quorum-sensing signal.

The gift of tongues

Still more quorum-sensing languages have turned up in the gram-positive bacteria, which do take up the Gram stain. "We're beginning to discover that bacteria have a whole language, and acyl-homoserine lactone is just one word. *E. coli* has other words it can use. And gram-positives use another dialect," Greenberg says. One case in point is *Staphylococcus aureus*, which causes infections ranging from small skin abscesses to life-threatening conditions such as endocarditis and toxic shock.

When this pathogen infects its hosts, it activates a set of genes that encode a variety of protein-degrading enzymes and toxins that help it disseminate in the host and wreak havoc on the immune system. There has been some disagreement about the identity of the signaling molecule that activates these genes, but the latest evidence, published in the 15 February issue of *PNAS* by microbiologist Richard Novick's group at New York University Medical Center in Manhattan, shows that a short peptide can do the job.

In that work, the Novick team synthesized peptides they had previously implicated in quorum sensing by *S. aureus* and showed that they activate the microbe's virulence genes. The fact that the researchers could duplicate the effect with completely synthetic peptides confirms that the molecules are the quorum-sensing signals, Novick says.

The Novick team has also found that although the peptides from different *S. aureus* strains share basic chemical features, they divide into four groups based on variations in amino acid sequences and length. And because peptides from one group can inhibit virulence-gene expression in *S. aureus* strains of another, the finding opens the door to developing new drugs for treating *S. aureus* infections.

Indeed, exploiting such dialects may turn out to be a broader strategy for turning bacteria's quorum-sensing systems against them. Researchers have found that the acyl-HSL signals of gram-negative bacteria have side chains that often vary from one bacterium to another, and some acyl-HSLs can inhibit the activity of the LuxR-like proteins of other species.

New results from Greenberg's team suggest another potential way of inhibiting quorum-sensing signals in these organisms. The enzymes that synthesize the acyl-HSL signals use a substrate that organisms from bacteria to humans exploit to construct a variety of molecules. As a result, trying to inhibit those enzymes might seem an unpromising strategy for shutting down a quorum-sensing system. But Greenberg and his colleagues found a specific molecular feature that distinguishes the acyl-HSL–synthesizing enzyme in *P. aeruginosa*, the biofilm-forming pathogen, as the group reports in the 13 April issue of *PNAS*. "This leads to the idea that you could find analogs that don't bind to proteins in humans but do bind specifically to this family of [microbial] proteins," says Greenberg. Such compounds might thwart the production of the signaling molecule and thus of *P. aeruginosa* biofilms.

Techniques for scrambling or shutting down quorum-sensing systems could be valuable against some nonbacterial pathogens, too. Quorum-sensing systems are now turning up in more complex, nucleated cells. "Often ideas are discovered in bacteria and if they're great, eukaryotes use them too," says Greenberg. Next week at the American Society for Microbiology meeting, for example, William Goldman, a microbiologist at Washington University School of Medicine in St. Louis, will present evidence that the fungus *Histoplasma capsulatum*, which causes histoplasmosis, a flulike respiratory disease in humans, can sense its own numbers.

Goldman and his colleagues have found that the fungal cells in dense cultures are adorned with a particular sugar whose presence correlates with the virulence of the fungus. But when they diluted those cells in fresh culture medium, newly budding cells did not display the sugar. "It continues to stay off until they reach a certain cell density and then—wham—everyone turns it on," says Goldman. If, however, the researchers add fluid from dense cultures to the diluted fungi, the buds express the sugar right away. "Here's a trait that's clearly related to virulence, and it's turning on and off in response to cell density," he adds.

At the same time that researchers are nailing down the biochemical details of the quorum-sensing systems, the many new findings are opening unexplored areas. Given bacteria's knack for languages, Greenberg wants to know, for example, whether they speak to animals or plants, and if so, whether the microbe or the host benefits. If the host can understand or even hear the bacteria, it might "get a leg up in responding to the impending infection," he says. Alternatively, the bacterium may trick the host into responding inappropriately.

And now that it seems clear that different bacterial species can hear each other, "we need to figure out the role of this cross communication in the normal ecology of the strains," says Arizona's Pierson. For example, he says, it might help the organisms coordinate tasks, with one strain producing an enzyme that inactivates an antibiotic while another secretes a substance that prevents the community from drying out. "Different species of bacteria have different capabilities, but they work together," says Bassler. "They're behaving like multicellular organisms. That's really smart"—but perhaps no smarter than might be expected of organisms that orchestrate their activities by performing in concert. —EVELYN STRAUSS

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