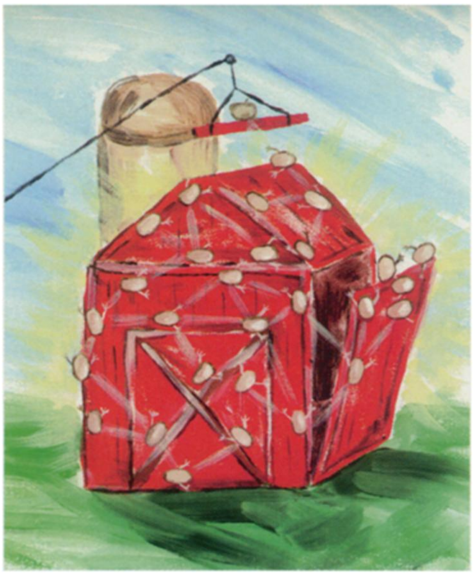

Mob Action

Peer pressure in the bacterial world

By EVELYN STRAUSS

"Team player" is not a phrase that usually comes to mind when thinking about bacteria. Rather, these single-celled creatures seem to excel at independence. Each individual can survive in a changing and often harsh world, where the difference between life and death is one cell.

These rugged individualists live a streamlined life, in which the drive to reproduce appears to rule. As soon as one cell divides into two, each strikes off on its own, foraging for food, seeking safety, and trying to become two once more.

Bacteria often seem so single-minded and self-reliant, they hardly merit consideration as potential members of community organizations. Yet scientists have gradually realized that diverse bacteria form coalitions to accomplish feats that individuals cannot perform.

Once considered an oddity, this organizational ability appears to be a common occurrence. Evolution seems to have distributed a standard kit of two genes to a variety of unrelated bacteria. Armed with these tools, the various species exploit a similar tactic for mobilizing mass production of useful molecules. Each member of an assembly is counted, and the bacteria wait until the crowd has gathered enough members—a quorum—to render success likely. Then each bacterium begins to work toward the common goal.

"This is a radical concept," says Kendall M. Gray of the University of South Florida in Tampa. "They're single-celled organisms, but they exhibit complex behavior. They're not only communicating, they're coordinating their behavior so that they behave in a uniform manner."

Scientists first glimpsed this process, called quorum sensing, in the late 1960s. J. Woodland Hastings, then at the University of Illinois at Urbana-Champaign, and his colleagues observed an odd characteristic of *Vibrio fischeri*, a luminescent bacterium that inhabits squid. While growing *V. fischeri* in the laboratory, the scientists noticed that although the number of cells increased during the first few hours, the sample remained dark. Luminescence shot up when the population reached a relatively high density and the microorganisms were about to run out of nutrients.

"One bacterium can devote all its energy to making light, but it's still essentially invisible," says Gray. "If it waits until there are a billion cells before it turns on the power, it has something useful."

Similarly, many bacteria secrete enzymes that help them survive. Each bacterium transports a relatively small amount of an enzyme to the outside world. It can no more change the concentration of that enzyme in the environment than a person could dye the ocean red with a drop of food coloring. If there are many bacteria—particularly in a confined area—the enzyme would be available in a high enough concentration to do some good.

During the past 30 years, scientists have figured out the fundamentals of quorum sensing, using *V. fischeri* as their primary subject. Each bacterium produces a small, diffusible molecule that passes freely out of and into cells, explains microbiologist E. Peter Greenberg of the University of Iowa in Iowa City, who has spearheaded this work.

As the population of bacteria increases, so does the concentration of this signaling molecule. Once the concentration in a bacterial cell has reached a threshold, the signaling molecules can grab onto proteins called LuxR. This embrace enables LuxR to bind to specific stretches of DNA and turn on the adjacent genes. In *V. fischeri*, some of these genes provide blueprints for the molecular apparatus that produces light.

In the early 1990s, scientists found that other bacteria possess communication systems similar to that of *V. fischeri*. In each species, the signaling molecules are constructed from a common module by an enzyme called an inducer protein. Inducer proteins customize this module by adding different chemical groups to it. The signaling molecules bind to a responsive protein, such as LuxR, and together they turn on a gene. Scientists have now identified genes for pairs of responsive-inducer proteins in over a dozen species of bacteria.

"It's a very general phenomenon, and it's likely to be a key to the life of microorganisms in many settings," says Barbara H. Iglewski of the University of Rochester (N.Y.) School of Medicine and Dentistry. "If the bacteria can't talk amongst themselves, they're not able to survive."

The discovery of quorum sensing tuned biologists in to the world of bacterial communication. They have since found a variety of ways in which bacteria coordinate their activities.

Most bacteria that use quorum sensing systems inhabit an animal or plant. The microorganisms benefit from the process, but the host organism may or may not. *V. fischeri*, for example, lives symbiotically in squid. It generates light that prevents the squid from casting a predator-alerting shadow while searching for food in the moonlight, and in return, the squid provides it with a home (SN: 9/14/96, p. 167).

Other bacteria produce molecules that damage their host. *Pseudomonas aeruginosa*—which causes lung disease in people with impaired immune systems or cystic fibrosis—produces enzymes that degrade tissue and allow the bacterium to escape from the lungs into the bloodstream. The bacterium waits in the lungs until its population reaches a critical density before beginning to churn out these agents of destruction. Luciano Passador, also at Rochester, likens *P. aeruginosa*'s strategy to that of an army that amasses its troops quietly and invades only when it can overpower its opponent.

"So many bacteria get into the bloodstream simultaneously that the immune system doesn't stand a chance of fighting them off," he says. Furthermore, if the bacteria produced molecules early in infection, they might stimulate the host to make antibodies that would inactivate the enzymes when the bacteria tried to use them later, Greenberg reasons.

P. aeruginosa has many homes in addition to the human lung. It lives in the soil, water, "everywhere," says Passador. Presumably, it needs different proteins in different environments.

"A soil bacterium that also infects people wouldn't necessarily want to increase production of its tissue-degrading enzymes when it is reaching high population densities in dirt," he says.

According to this theory, bacteria are doing more than just counting themselves. They sense particular chemical and physical cues that help them respond to different environments.

For instance, outside the lung, *P. aeruginosa* can grow as a film on many surfaces, including implanted medical devices. These sources of infection are hard to eradicate because the films resist antibiotics and detergents that would kill individual bacteria. Greenberg and his collaborators have recently shown that *P. aeruginosa* strains with defective quorum sensing systems form abnormally thin films that succumb to detergents, he says.

Scientists have found that particular compounds in the environment stimulate quorum sensing. *Agrobacterium tumefaciens*, an organism that causes tumors in plants, directs its host to make certain substances. In addition to providing nutrition for the bacterium, the host chemicals spur *A. tumefaciens* to produce a responsive protein. This, in turn, enables individual bacteria to detect the presence of their neighbors.

Scientists say other bacteria probably use a similar scheme for figuring out where they are. This tactic ensures that they invest in the equipment with which to sense population density only under specific conditions.

For the most part, scientists have focused on the detailed mechanics of quorum sensing. Only recently have they begun to move their studies from the laboratory into the field. Leland S. Pierson III of the University of Arizona in Tucson has developed a system for listening in on bacterial conversations in a natural setting—the roots of a wheat plant.

Pierson studies *Pseudomonas aureofaciens*, a bacterium that infects and benefits wheat plants. In large numbers, it makes an antibiotic that inhibits the growth of a fungus that causes take-all disease, which can ruin entire wheat harvests.

Pierson engineered a strain of *P. aureofaciens* that was incapable of making its own signaling molecule but could respond to messages from other bacteria. He inserted into this strain a gene that makes an easily detectable protein rather than the usual machinery for antibiotic production. When the bacterium experiences conditions that would normally trigger antibiotic output, it manufactures this protein instead, thus alerting the researchers that it has received a signal.

When Pierson added a strain that produces the signaling molecule to roots that had been infected with the strain that lacks the signal, production of the protein increased dramatically. These results indicate that distinct bacterial populations can talk in the "real world," he says.

Now Pierson wants to find out whether bacteria of one species can hear unrelated organisms. Most bacteria in the water, soil, plants, and people live in neighborhoods composed of diverse members, raising the possibility that there are conversations between different species. If so, this might allow bacteria to find out who else is present and modify their behavior accordingly.

Pierson

Wheat grown from a seed planted in fungus-infested soil contracts take-all disease (left). Wheat grown from a seed coated with fungicide-producing P. aureofaciens *remains healthy, even when grown in fungus-infested soil (right).*

An organism could eavesdrop on its neighbors in several ways. Signaling molecules from distinct species are not always unique, so a bacterium has an opportunity to sense the presence of strains that mimic its own dialect. Furthermore, while some responsive molecules are very picky, others are promiscuous. They can recognize a variety of signaling molecules, although they tend to function most effectively when supplied with the type made by their own inducer gene.

Although scientists have not yet examined communication between species in the natural environment, they have gathered laboratory evidence suggesting that it occurs. *Burkholderia cepacia* is associated with fatal lung infections in people with cystic fibrosis. It infects human lungs—but almost always after they've been colonized by *P. aeruginosa*.

Researchers have found that *B. cepacia* apparently has a way of intercepting messages from *P. aeruginosa*. First, scientists grew *P. aeruginosa* in laboratory broth, then disposed of the bacteria. When the investigators added this broth to *B. cepacia*, the bacterium increased its manufacture of molecules thought to be necessary for it to survive and spread within a person.

In a second experiment, the scientists added broth from a *P. aeruginosa* strain that makes only small amounts of its signaling molecule. This broth triggered a much weaker response by *B. cepacia*, indicating that *B. cepacia* is responding to *P. aeruginosa* signals, the researchers argue.

The ability of different species to communicate with each other appears widespread. In 1979, Greenberg showed that 19 of 28 marine bacteria he tested produced factors that induced *V. harveyi*, a luminescent marine bacterium, to generate light. Last year, Pierson tested about 40 wheat root bacteria and found that approximately one-third sent a recognizable signal to *P. aureofaciens*.

Bacterial messages may reach organisms more complex than bacteria. One of the *P. aeruginosa* signaling molecules suppresses key activities of the mammalian immune response, according to an unpublished report by David I. Pritchard and his colleagues at the University of Nottingham in England.

Pierson wonders whether the conversations are always friendly, even among bacteria. In principle, these microorganisms could manipulate each other by interfering with one another's quorum sensing systems.

"What a great way to compete—control the other organism's genes," he says.

No one knows yet whether this happens in nature, but it is possible. In the laboratory, scientists have found ways to confuse bacteria. In *V. fischeri*, foreign signaling molecules can bind to LuxR and prevent it from functioning normally, even in the presence of its usual signaling molecule.

Furthermore, scientists have found hints that a species of algae fouls up communication among the microbes. These algae, which are particularly successful at defending themselves against bacterial colonization, produce natural inhibitors of bacterial quorum sensing, reported Staffan Kjelleberg of the University of New South Wales in Sydney, Australia, and his colleagues in the November 1996 JOURNAL OF BACTERIOLOGY.

These findings reveal a possible chink in the bacterial armor. Scientists would like to crack the code, as these algae seem to have done, and use the information for therapeutic and agricultural purposes.

"Once we've figured out how to scramble messages, we'll know a lot more about how bacteria communicate," says Passador. "Then we should be able to infiltrate bacterial organizations and undermine their activities." □