Bugging the

e Bugs

BONNIE BASSLER

By Alisa Zapp Machalek

Bonnie Bassler is a spy.

Like a secret agent, she gathers intelligence by bugging conversations. But Bassler is not after people. She is eavesdropping on much tinier beings: bacteria. Her goal—the cornerstone of espionage—is to decode a secret language, then possibly scramble or redirect the discussions.

Bassler, 42, a microbial geneticist at Princeton University, leads a research team investigating bacterial communications that underlie a long list of ailments,including cholera, tuberculosis, typhoid, pneumonia, and food poisoning. By revealing how to disrupt key bacterial exchanges, Bassler's work might help vanquish these ailments.

Let It Glow

The bacteria Bassler investigates do not cause illness in humans, but float free in the world's oceans, live in sand, and coat fish, coral, and debris. Most remarkably, they glow in the dark.

Such glowing, or "bioluminescent," marine bacteria are extremely common. People dining by candlelight or peering into refrigerators with burned-out bulbs sometimes see their seafood sparkle. For Bassler, this blue-green glow shines a light on bacterial communication.

"Bacteriologists have had it wrong for a couple hundred years," sighs Bassler. "We always thought free-living bacteria lived asocial, reclusive, individual lives, but that's not the case. They could never do all they do without communication systems."

Bassler studies *Vibrio harveyi* (abbreviated *V. harveyi*) bacteria, which can only glow when they are in a sufficiently large group known as a quorum. Using a chemical strategy dubbed quorum sensing, the bacteria manage to assess their own population size and distinguish themselves from all other kinds of bacteria.

"Science is an incredibly creative endeavor. If you're good at it, it's art."

DENISE APPLEWHITE

Bonnie Bassler is a bacterial geneticist at Princeton University. Bassler studies how bacteria, or "bugs," communicate with each other.

Besides its role in bioluminescence, quorum sensing is used by many kinds of bacteria to mastermind behaviors ranging from mating to releasing toxins and triggering disease, an activity known as virulence. Most notably, quorum sensing is used by many harmful bacteria to avoid a full-blown response from our immune systems—until it's too late for us. The bacteria appear relatively innocuous as they quietly grow in number. Then, when their population reaches a certain level, their behavior,

Luminescent bacteria can make seafood glimmer blue-green. Next time you see raw fish, turn out the lights and check out theglow!

appearance, and metabolism instantly change, culminating in an infection that can ambush and overwhelm our immune system defenses. It's akin to thousands of pedestrians in a city intersection simultaneously walking into the street,locking arms, and paralyzing traffic.

We Have a Quorum

Quorum-sensing bacteria leak into their surroundings a chemical that, like body odor, indicates their presence. As the bacterial population increases, so does the concentration of this chemical, called an autoinducer. When the amount of autoinducer reaches a certain level, the whole bacterial neighborhood is transformed. The once-independent, solitary bacterial cells suddenly become part of a large, multicellular organism.

Communication is key to the formation of biofilms, the slimy bacterial communities that can cause infections and are often stubbornly resistant to antibiotics.

An everyday example of such bacterial transfiguration is the tenacious slime that can accumulate on teeth,ships, bathtubs, and kitchen drains. These slippery coatings are actually sophisticated microbial communities called biofilms in which bacteria either become part of protective coatings,walls, or nutrient-filled channels, or they swim around inside these structures as highly mobile, independent cells.

Tapping into the chemical communiqués needed to make biofilms has many applications, according to Bassler. Biofilm-blocking substances could reduce illness caused by infected catheters and other implanted medical devices. They could prevent bacterial buildup in industrial cooling towers and swimming pools. And, if added to toothpaste or mouthwash, they could battle tooth decay.

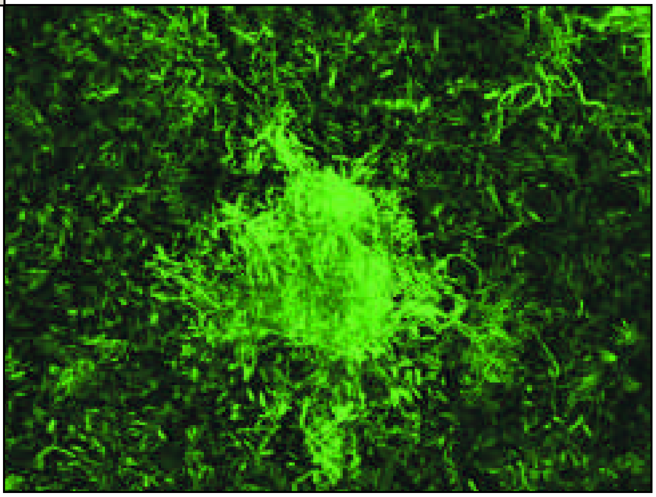
By studying the way bacteria join together into organized groups,scientists also learn how humans and other higher organisms developed, Bassler says. After all, our bodies are essentially collections of genetically identical cells that, based on chemical cues, specialized into different tissues (skin, bones, a heart, brain, and so forth). According to Bassler, these tissues probably interact with each other using chemical crosstalk similar to that in bacteria.

Knock Their Lights Out

The idea that bacteria communicate was considered "fringe science" for decades after the early 1970s, when researchers first showed that some marine bacteria not only gabble, but turn the lights on when the party gets big enough. Bassler says many scientists originally dismissed the work as "quirky results in non-interesting, non-[disease-causing] bacteria."

Bassler's fascination with bacterial communication, quorum sensing, and luminescence began in 1990, while she was a graduate student. She heard a visiting scientist describe his discovery, made years earlier, of the first genes for quorum sensing. Instantly excited about the topic, she charged up afterwards and persuaded the speaker to hire her onto his research team.

That's how Bassler's spy work started. She began her investigation trying to "knock the lights out" of the mysterious, glowing bacteria. In other words, she was trying to use molecular techniques to find the genes needed for bioluminescence by disabling or "knocking out" these genes. If she succeeded, the result would be a "dark" strain of bacteria, incapable of glittering.



P. SINGH AND E. PETER GREENBERG

Despite repeated efforts, Bassler's bacteria kept shining. Then suddenly, a light went on in her own head.

"Maybe these bacteria have two communication systems and knocking out one is not enough!"

She was correct. Bassler determined that *V. harveyi* contains not only the original quorum-sensing system that had been discovered a decade earlier, but another, even more complicated system. In the original system, quorum-sensing behaviors are sparked by a molecule called AI-1, for autoinducer 1. Each species of quorum-sensing bacteria makes and detects its own version of AI-1 and doesn't recognize the molecules made by other bacteria. In effect, each strain of bacteria is speaking its own language.

In contrast, the system Bassler discovered—one she likens to "bacterial Esperanto"—is a universal language that can be recognized and used by many, if not all, quorum-sensing bacterial species.

Bassler concluded that quorum-sensing bacteria are bilingual. They use a special code to talk to members of their own species and a common language to converse with all others.

Having two different communication systems offers clear advantages for bacteria, Bassler explains. They can covertly track their own numbers, spy on their competition, and monitor their environment, while also building alliances with other microbes.

Esperanto Espionage

Upon finding the new communication system, Bassler's next mission was to unmask the main Esperanto message molecule. Using several laboratory techniques, she decoded each step in the biochemical pathway that bacteria use to make the universal signal molecule. But all her genetic tools failed to reveal the molecule's true identity.

Bassler decided to switch tactics. She recruited an X-ray crystallographer, a scientist who blasts high-energy X-rays at microscopic crystals of molecules to illuminate their detailed structures and chemical properties. The idea was to get at the Esperanto molecule by examining its cellular alliances, specifically LuxP, the molecule to which Esperanto attaches in order to initiate cellular communication. In essence, they moved from gathering intelligence on the main suspect to snooping around the phone booths the suspect uses to talk with contacts.

The plan worked—the image the scientists captured of LuxP had 13 too many atoms, meaning they had photographed not only the phone booth, but the suspect inside it! Bassler could finally identify the Esperanto message molecule. She called it AI-2, for autoinducer 2.

Then the plot thickened again. Something didn't look right about AI-2. Most molecules in living creatures contain large amounts of the elements carbon, hydrogen, oxygen, and nitrogen, and a sprinkling of phosphorus and sulfur. AI-2 appeared to contain the element boron.

Boron is used in a range of industrial products,including insulation fiberglass,laundry detergent, and pyrotechnic flares (the green ones). Although boron is found in plants and in some bacteria, until Bassler's investigation, no one could pinpoint a biological role for it.

Boron's mystique also helped Bassler recruit a chemist to make AI-2, and similar molecules,from scratch. She reasoned that such molecules could serve as decoys to subvert, rather than incite, virulence and other quorum-sensing behaviors. If researchers can get this "trick" to

Boron is found in or used to make a variety of industrial and household products. Bassler was the first to reveal a biological role for it.

work in *V. harveyi*, the same strategy might help topple the bacteria that cause diseases in people.

"If all bacteria use AI-2, you can fantasize about one anti-AI-2 molecule that could serve as a broad-spectrum antibiotic," muses Bassler.

Most current antibiotic drugs are designed to kill bacteria. The "bugs" that survive this treatment go on to breed generations of microbes that are invulnerable to the antibiotics. According to Bassler, targeting quorum sensing would be a radical new antibiotic approach that might be less likely to foster this sort of drug resistance.

"The hope is that, instead of killing the bacteria, we could try behavior modification drugs. It would be like putting earmuffs on the bacteria so they couldn't hear the quorum-sensing signals." This would tip the balance in favor of our immune systems, which would then be better able to combat the bacteria, she explains.

Case Closed, Doors Open

Bassler and others have now exposed a network of molecules that *V. harveyi* uses to make, send, or detect quorum-sensing messages. All these signals eventually reach the kingpin—a master regulator protein called LuxR, which turns on the 100 or so genes that launch quorum-sensing behavior.

But until recently, a critical player in this network eluded Bassler and her team. What molecule serves as "command and control," switching LuxR on and off? Her recent studies divulged the answer—or actually, answers. It turns out that there are four molecules responsible for controlling LuxR. And, even more surprising, they are not proteins, as are all the other molecules in the communication chain. Instead, they are small RNA molecules (sRNAs), chemical cousins of DNA in genes.

EDITH WIDDER

Deep-sea angler fish make good use of luminescent bacteria. Female anglers lure prey using a long spine as a fishing rod, the tip of which is lit up by millions of the bacteria.

Bassler named these newly discovered molecules Qrr 1 through Qrr 4, for quorum regulatory RNA 1 through 4. Independently, each can trigger quorum sensing in an instant, but to turn it off, you need all four.

Bassler is now focused on trying to understand how each sRNA works and how the four operate together. Small RNAs were only detected in the past few years and are associated with processes ranging from the development of embryos and the formation of the nervous system to cancer. If the sRNAs that Bassler uncovered prove similar to these medically important sRNAs, they may offer tantalizing possibilities for new ways to study, detect, prevent, or cure illness.

If that happens, Bassler won't be surprised. She's continually aware of the broader implications of her research.

"I think every day that what I do is valuable. We're asking fundamental questions about how nature works—how information is transferred from outside to inside an organism," Bassler says.

"I'm working on a glow-in-the-dark bacterium because that's absolutely the best system to use to understand this question," she continues. "But everyone in my lab knows that we're not just working on *V. harveyi*, we're working on you and me."

Bassler says her research in the past few years has gone in directions she never dreamed. In addition to her collaboration with a crystallographer and a chemist, she's invited a theoretical physicist to join her team. He is using computer modeling to help understand bacterial chatter.

"What's most exciting to me is that this work has really stretched me as a scientist," she remarks.

Taking her work into areas outside her own expertise is not only thrilling, it is also helping Bassler train a new generation of interdisciplinary scientists.

"As a bacterial geneticist, I never imagined I'd be making molecules in my lab or doing crystallography. But now the postdocs and grad students in my lab understand these subjects. They are developing into scientists of the future, limited only by their imaginations."

Secret Life

Hundreds of scientists now study bacterial communication, and practical applications of this work multiply almost as fast as the microbes themselves. Bassler's pioneering work in the field was recognized in 2002, when she was one of 24 scientists, artists, scholars, and activists who won prestigious MacArthur Fellowships.

This honor, commonly called the "genius award," recognizes not only intelligence but also exceptional creativity and promise. Bassler was cited for work that "reveals new insights into the basic biology and ecology of bacteria, findings that may have direct application in the future treatment of disease." She was awarded $500,000 over 5 years to spend any way she would like.

Bassler's enthusiasm and creativity extend beyond her time in the laboratory. Early in the morning, for 5 days a week over the past 20 or so years, Bassler has taught aerobics classes at the local YMCA. She says it's a way to ensure she gets her own exercise. "If I didn't teach the class, I wouldn't go," she chuckles.

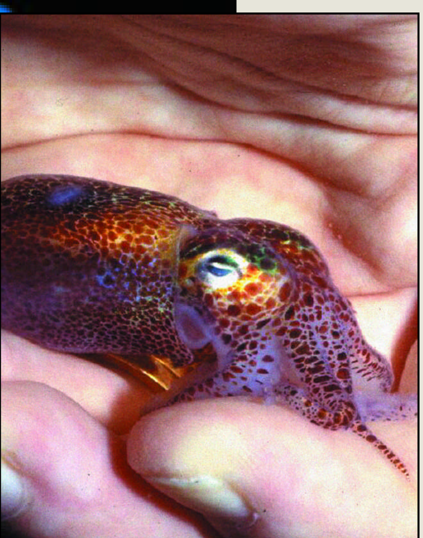
She also takes singing (jazz and opera) and acting lessons. "I am a curious person, so I wonder about these other things," she says. "I'm just trying them for fun. It's my other secret life."

Yet Bassler is quick to point out that science is really what drives her. "I'm glad I was lucky enough to find the career that's right for me," she says. "I think about science much more than I think about singing. I think about it in the shower, while going to sleep, all the time."

Science itself is a means of creative self-expression for Bassler. "We scientists discover things about nature the same way that an artist or choreographer or dancer creates new work," she says.

"I think science is an incredibly creative endeavor. If you're good at it, it's art." ■

Squid Row



JOHN FORSYTHE

On land, only a handful of creatures shimmer in the dark—fireflies and some earthworms, mushrooms, and algae. But in parts of the world's oceans, up to 90 percent of creatures glow, or luminesce. Light helps these organisms find food, mates, homes, and—if delivered as a blinding flash—repel predators.

Take bobtail squid, which use light not only to illuminate the night, but also to hide. Living in knee-deep waters around Hawaii, these miniature squid nurture large populations of glittering Vibrio fischeri bacteria, close cousins to the V. harveyi that Bassler studies (see main story). The V. fischeri live happily in the light organ on the squid's underside, where they dine on a rich fare of amino acids and other nutrients. In return, the bacteria provide light that the squid deftly control in order to camouflage themselves from predators.

During the day, the strawberry-sized squid bury themselves in the sand. At night, they wriggle out to hunt for shrimp, worms, and small fish. The squid keep a coat of sand on their backs so that when seen from above, they blend in with the sea floor. But on bright moonlit or starry nights, the squid have a problem since they cast a shadow on the sand, advertising a bite-sized calamari snack for roving eels, barracuda, or seals. With light sensors on their backs, reflective proteins, and a shutter-like opening in their light organ, the squid tune their bacterial glow to precisely match their surroundings. Cloaked in ambient light, they and their shadows essentially disappear.

At sunrise, the squid squirt out about 95 percent of the bacteria in their light organs. Depleted of their shining guests, the squid grow dim and retire to their sandy burrows. As they sleep, the remaining 5 percent of the bacteria multiply. By dusk, the bacterial population reaches a quorum. Flip! The squid begin to glisten, and it's time to hunt. —A.Z.M.