Denise Juppé

Strength in Numbers

Bacteria use "quorum sensing" to work together

Imagine finding yourself alone in a dark and unfamiliar place. Maybe this is a World War II story, and you've just parachuted down behind enemy lines. You're by yourself, but you know your comrades will soon be gathering here from somewhere out in the darkness. You don't have a flashlight, but you do—each one of you— have a transceiver that can send and receive radio signals. You set your device to the correct frequency and begin broadcasting. And you wait. Eventually you receive an incoming signal, then another and another. Finally, enough of the group have found their way to the site to begin the operation. Then, as one, you attack.

This is an analogy, albeit an imperfect one, for how certain species of bacteria communicate with one another. If you've ever given any thought to what happens when bacteria establish a new colony in the body, you may have envisioned them as being a bunch of lone micro-organisms, multiplying without regard to the others around them. They replicate with wild abandon until your immune system eventually catches on and sets out to evict them.

But this is not actually the case. Bacteria are single-cell organisms; alone, they don't have much chance of making an impact on their environment. Remarkably, though, we now know that they wait until they've sensed that there are enough of them—a quorum—to suit their purposes. Only then do they act, in a way that very much resembles a multicellular organism.

A "quorum" usually refers to the minimum number of members that must be present for an organization to conduct its business. But in this instance, it could be thought of as the minimum density of cells in a population of bacteria that results in altered gene expression. In other words, bacteria use quorum sensing to coordinate the expression of a particular gene when their numbers are sufficiently high. To condense a complex process into a few words, “gene expression” means that, through biochemical signaling, a specific gene is directed to manufacture a protein that produces some desired effect.

Bacteria aren’t equipped with transceivers, of course, instead they produce and detect signaling molecules called “autoinducers.” And while the analogy holds true for how microbes can communicate, the purpose of their communication is not always invasion. Researchers have shown that bacterja and other microbes often work together to accomplish a wide range of tasks. A few of the many processes that have been found to involve quorum sensing include bioluminescence, antibiotic synthesis, sporulation (the formation of spores), and even —because brewers yeast also sense their population density—the fermentation of beer. The variety of ways in which bacteria employ quorum sensing is impressive; the implications for our understanding of biological processes are exciting. Medical research is currently being conducted to determine whether we can leverage knowledge of bacterial communication to formulate new classes of antibiotics.

The notion of bacteria communicating was first proposed in 1970 by a group of researchers at Woods Hole Marine Laboratory, who had observed the behavior in a very benign bacteria called *vibrio fischerii.* In the wild, *v. fischerii* lives in a symbiotic relationship with the Hawaiian bobtail squid, whose light organ they inhabit. The squid hide during the day, but as night falls, they emerge. Their bacteria begin to luminesce, providing “counter- illumination camouflage” that matches the intensity of the moonlight at the sea surface. So the squid will not be perceived as dark silhouettes against the moonlit water when seen from below, but be invisible to predators and prey alike. Each squid will pump out most of its bacteria at dawn; the colony regenerates itself during the day.

The Woods Hole researchers had cultured *v. fischerii* in the lab; they noticed that the bacteria did not luminesce in culture at first, but only when the colony had grown to a certain size. They referred to the process as "autoinduction," because the bacteria themselves were inducing the expression of their light-emitting gene. In 1994 Dr. Steven Winans introduced the term "quorum sensing," as a kind of shorthand for this process. The phrase quickly caught on and replaced the older terminology in subsequent papers.

Bioluminescence is beneficial to the squid. A less innocuous example of how bacteria coordinate their behavior once they've reached a quorum is biofilm. A number of bacterial species produce biofilm, a tough protective layer that encases their population and helps transport nutrients between colonies. The plaque that can form on teeth is a biofilm. But some bacteria construct a biofilm as a prelude to more pernicious activities. Bacteria such as *pseudomonas aeruginosa,* a nasty microorganism that causes a range of infections, will grow within a host without harming it until they reach a threshold concentration. When these bacteria sense their numbers are sufficiently high to overwhelm the host's immune system, they become aggressive. Colonizing some bodily surface, like your middle ear, they begin creating biofilm, Once the biofilm is complete, the colony begins to secrete its disease-causing toxins. Researchers believe that biofilm formation is involved in some 80% of bacterial infections.

How does quorum sensing actually work? To be capable of quorum sensing, each bacterium must not only be able to secrete its own signaling molecules (autoinducers) but to detect, through receptors on its surface membrane, the concentration of signaling molecules in their environment. When a sufficiently high concentration, the "quorum" of autoinducers has been reached, the receptors begin secreting a substance that triggers gene expression, literally turning “on” (and sometimes “off”) a gene that controls processes such as bioluminescence and biofilm creation.

This sequence of bacterial events has significant implications for another area of medicine: antibiotic therapies. Because if you can have quorum sensing, you can also have “quorum quenching.” Quenching occurs when some action interferes with one or more mechanisms that bacteria rely on to assess their population density. Inhibit quorum sensing by disrupting the chain of communication and you might be able to halt or slow activities that depend on it, such as the infection process.

Dr. Bonnie Bassler, a molecular biologist at Princeton University, is among those researchers investigating whether quorum quenching might be a strategy that would yield the next generation of antibiotics, drugs that would circumvent the currently accelerating arms race between multi-drug-resistant bacteria and increasingly stronger antibiotics.

In discussing how anti-quorum sensing antibiotics work, it’s helpful to recall that an autoinducer is just a molecule. Those receptors on bacterial cell surfaces fit the shape of these molecules and so lock together with them. The newer types of antibiotics might work to disrupt quorum sensing at any phase in the process. Thus far researchers using molecular engineering techniques have been able to inhibit enzymes that trigger the production of autoinducers; introduce molecules that mimic autoinducer shapes and block receptors, reduce the number of receptors; overload receptors, and biochemically degrade the autoinducers themselves. Each of these strategies has its specific use, depending on the mechanisms a particular species of bacteria uses to regulate its quorum sensing sequence.

Back to biofilm. If bacteria produce it, but there must also be a feedback loop that tells them when they’ve made enough. It turns out that when a population of bacteria has released enough autoinducers to sense a quorum, it becomes difficult, on a biochemical level, for each individual bacterium to release more. Each bacterium's receptors become supersaturated with its own autoinducers, signaling the bacteria to stop making biofilm and start releasing toxins. The switch could also be thought of as a kind of quorum sensing since it triggers new gene expression. The cholera-causing bacteria, *vibrio cholerae,* operate in just this way.

Why the switch to secreting toxins? In a paper entitled "Why do Microbes Have Toxins?", Williams and Clark assert that bacteria release them for the simple reason that "our bodies are particularly abundant sources of nutrients, and toxin production is just one of several highly sophisticated adaptive mechanisms that have evolved to combat the body's defenses in order to acquire them."

Molecular biologists at Princeton, including Bassler, have been able to halt the production of biofilm by overloading the bacterial receptors with their own autoinducers. If biofilm production is prematurely halted, the immune system may be able to cope with the bacterial infection when it is in a less virulent stage. This class of antibiotics might not be quite as powerful as the ones currently prescribed but would also not lead to the bacterial resistance that develops from their use.

Every type of bacteria makes a signaling molecule (the autoinducer) that is specific to its own species and that binds only to its cell receptors. The signaling molecules they produce are all variations on the same basic structure. Essentially, bacteria have private, "intra-species" conversations in a dialect that no other bacterial species understands. But consider the jumble of bacteria that can coexist in the human gut. There are thousands. Bacteria must also constantly assess their population densities relative to their neighbors to determine what tasks they should be performing. Scientists have recently discovered that all bacteria produce a second signaling molecule that has its own receptor, and that these are identical across all species. Bacteria don't just talk to the members of their clan, they also talk to the neighbors in a kind of bacterial *lingua franca*, a universal language understood by all.

The discovery that bacteria "talk" to one another was made fifty years ago, but researchers continue to recognize more behaviors that depend on their chatter. If we learn to decipher their conversations, we will have cracked the code on how numerous biological processes happen. In a TED talk she gave in 2017, Bassler conjectures that bacteria "made the rules for how multicellular organization works." By studying them, we can learn more about our own multicellular, multi-organ bodies and the diseases that affect them. Our models will change, and with them our own ways of interacting with the microbes that surround us.

List of Sources

1. Britton, Scott, Hedwig Neven & Dawn Maskell. (2020, December 3). Microbial Small-Talk: Does Quorum Sensing Play a Role in Beer Fermentation? *Journal of American Society of Brewing Chemists.* Retrieved from https://www.tandfonline.com/doi/citedby/10.1080/03610470.2020.1843928?scroll=top&needAccess=true

2. Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., Greenberg, E.P. (1998 April 10). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*. Retrieved from http://www.sciencemag.org/content/280/5361/295.short.

3. Grandclément C, Tannières M, Moréra S, Dessaux Y, Faure D. Quorum quenching: role in nature and applied developments. FEMS Microbiol Rev. 2016 Jan;40(1):86-116. doi: 10.1093/femsre/fuv038. Epub 2015 Oct 1. PMID: 26432822.

4. Kristensen, Mikkel R. (2018, June 20). Quorum sensing and how it could resolve antibiotic resistance. *Medium*. Retrieved from https://medium.com/@mikkelreinckekristensen/quorum-sensing-a-bacterial-super-power-a10b786c9743

5. Lu et. al. (2015, April 8). Modulating *Vibrio Cholerae* Quorum-Sensing-Controlled Communication Using Autoinducer-Loaded Nanoparticles. *Nano Lett*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4390484/

6. TED. (2017, February 13). *Quorum sensing: Bacteria talks / Bonnie Bassler* [Video]. YouTube. https://www.youtube.com/watch?v=q2nWNZ-gixI

7. Windsor, W. Jon. (2020, June 12). How Quorum Sensing Works. *American Society for Microbiology*. Retrieved from https://asm.org/Articles/2020/June/How-Quorum-Sensing-Works

8. Yong YC., Zhong JJ. (2012) Impacts of Quorum Sensing on Microbial Metabolism and Human Health. In: Zhong JJ. (eds) Future Trends in Biotechnology. Advances in Biochemical Engineering/Biotechnology, vol 131. Springer, Berlin, Heidelberg. https://doi.org/10.1007/10_2012_138

9. Williams, P.H. & S.C. Clark. (1998) Why do Microbes Have Toxins? *Journal of Applied Microbiology Symposium Supplement.* (84) 1S-6S.