THE LANGUAGE OF BACTERIA

Bacteria, too, are talking to each other, using molecules as their words. Biologists call this bacterial communication ’quorum sensing‘; interfering with it could be the key to discovering new antibiotics.

They are microscopically small single-celled organisms and yet the secret rulers of the biosphere: Thanks to their enormous adaptability, bacteria populate even the earth’s most extreme habitats, from the deep sea to the stratosphere – and they do so in almost unimaginable numbers. Biologists estimate that they make up 15 percent of the earth’s entire biomass – only plants, which together comprise about 80 percent, represent a larger contingent. In this list, animals come in far behind, somewhere in the decimal regions.

The great variety of bacterial habitats also includes us human beings. For every one of the roughly one trillion human body cells, there are ten bacterial cells living on or inside us. And that is a good thing. For most of our tiny roommates are part of the healthy microbiome, without which neither our skin nor our intestines could accomplish their tasks. These ’good‘ bacteria also help to keep the bacterial baddies at bay. For they exist, too, of course – tuberculosis, whooping cough, or cholera are only some examples of bacteria-induced infectious diseases.

Social Single-Celled Organisms

At first glance, the life of a typical bacterium seems simple: As a single cell, it absorbs from its environment all kinds of usable molecules as nutrients. Once it has grown big enough, it splits in the middle. “A bacterium’s sole ambition is to produce two bacteria,” the pioneering geneticist François Jacob once summarized this process.

But there were doubts about this perspective early on. “If bacteria are really so simple and anti-social, how can they achieve anything? Bacteria simply seemed too small to have any kind of impact on their environment as individuals,” says the American microbiologist Bonnie Bassler. For the last roughly twenty-five years, Bassler’s lab at Princeton University has been a leader in a research field that paints a completely different, more complex picture of these presumably dim-witted organisms. As it turns out, they are surprisingly sophisticated and social. They coordinate their behaviors for mutual benefit, and to do so, they communicate with each other in the language of biochemistry, including across species boundaries. ’Quorum sensing‘ is what experts call this bacterial gabfest. Of course, their conversations do not revolve around the weather but have a clear goal: They make it possible for entire bacterial populations to coordinate their behavior and thus secure for themselves crucial survivalist advantages. Today we know that quorum sensing is not the exception but the rule in the bacterial realm.

More Light!

And yet, the bioluminescent bacterium *Vibrio fischeri*, in which the phenomenon of quorum sensing was first described in the 1970s, is in fact a unique creature. It can produce bluish-green light biochemically – biologists call this nocturnal light show, which can also be created by some mushrooms and animals such as fireflies, “bioluminescence.” In small numbers, *Vibrio fischeri* can also be found as free-living single-celled organisms in the ocean – in which case the bacterium does not glow. It feels most comfortable in the light organs of certain fish and cephalopods. There, in dense populations, it emits light and in return gets room and board from its host that cannot produce light itself. A classic case of symbiosis.

And there was light...

The most detailed studies of the symbiosis between marine animal and bioluminescent bacterium have been conducted with the Hawaiian bobtail squid (Euprymna scolopes). The cephalopod, which is only a few centimeters long, has two complex light organs in his underbelly where it harbors large numbers of *Vibrio fischeri* and feeds them amino acids. The glow of the bacteria protects the squid from attacks during his nocturnal hunting forays into shallow water: When viewed from the ocean floor, the silhouette of the animal is masked against the brighter surface of the water. The ’stealth bomber of the ocean‘ can adjust the light produced by the bacteria such that it matches the moonlight falling in from above. During the day, when it hides in the sand, the squid rinses its light organs with sea water. The few remaining bacteria stop glowing until they achieve high density again towards the evening, when the light show begins anew – thanks to quorum sensing.

Fig. 1: Hawaiian bobtail squid (*Euprymna scolopes*)
Photo: Chris Frazee and Margaret McFall-Ngai [CC BY 4.0], via Wikimedia Commons

In the early 1970s, Woody Hastings, a pioneer in bioluminescence research at Harvard University, was the first to notice a strange behavior in *Vibrio fischeri*, when he cultivated the bacterium in bottles with nutrient solution: Initially, a fresh culture did not glow at all. It was only when the bacterial cells had reached a critical density that the light went on as if a switch had been flicked.

Bacterial Democracy

The individual bacterium thus is apparently able to sense if it is surrounded by a sufficient number of siblings to collectively emit a visible glow, or else it saves itself the effort. The particulars of how this light switch of *Vibrio fischeri* works were discovered in the 1980s by microbiologist Mike Silverman, who later became Bassler's mentor. In 1994, the term 'quorum sensing' was coined.

In politics, quorum describes the necessary minimum number of votes for an election to be valid. In the democracy of classical Athens, for example, at least 6,000 citizens had to be present at a popular assembly to decide whether a fellow citizen should be banished from the city. To determine whether the quorum had been achieved, the pottery shards used as ballots were counted. But how does a *Vibrio fischeri* bacterium know how many of its siblings it is surrounded by and whether the necessary quorum has been achieved to start glowing?

After lengthy, microbiological detective work, Woody Hastings and other pioneers of the research field in the 1970s shed light on the molecular mechanisms of this bacterial democracy. *Vibrio fischeri* uses a special signaling molecule, which researchers initially called 'autoinducer' since the molecule secreted by the bacterium did not only affect other bacteria of the same species but also the bacterium itself. It was only later that the chemical structure of the autoinducer of *Vibrio fischeri* could be identified. It was a small organic molecule with a big name: N-(3-oxohexanoyl)-homoserine-lactone.

Every single bacterium is constantly releasing this molecule into its environment. At the same time, it uses a special receptor protein to measure the concentration of these molecules in its vicinity. This protein, which was called LuxR in *Vibrio fischeri*, can bind the N-(3-oxohexanoyl)-homoserine-lactone when the autoinducer molecule is in abundance. If population density is low, the autoinducer quickly diffuses away. With rising cell numbers, the concentration of the signaling molecule also goes up. If it exceeds a certain threshold value, LuxR activates the luciferase metabolic pathway, and all bacteria in the vicinity start to glow simultaneously (see Figure 2).

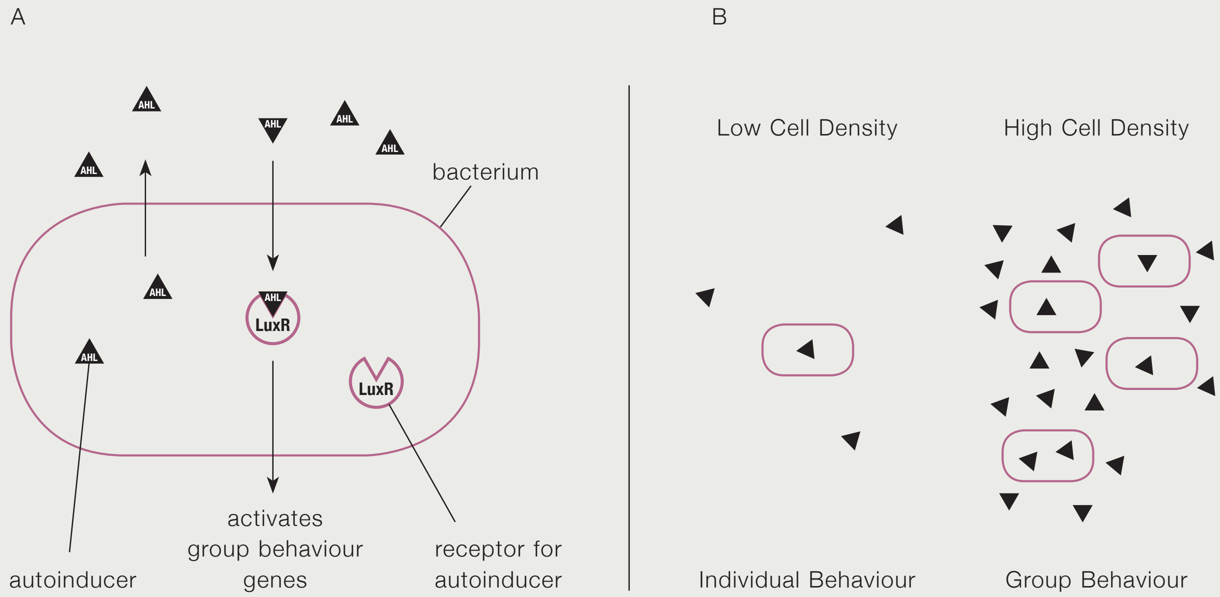

Fig. 2: Greatly simplified representation of the principle of quorum sensing in *Vibrio fischeri*: Every single bacterium releases a signaling molecule, the autoinducer, into the environment and at the same time a special receptor, LuxR, is used to measure the concentration of these molecules (A). If population density is low, the autoinducer quickly diffuses away. Only at high cell density, its concentration (B) rises above the threshold value, and the receptor triggers the particular response (such as bioluminescence).

Strategy: Sit and Wait

As it turned out, the principle of quorum sensing does not only control the light switch of bioluminescent bacteria. Most types of bacteria seem to use similar biochemical communication processes to coordinate the behavior of many individual cells. These include pathogens that use quorum sensing to orchestrate an attack on their host. “When it comes to an infection, it is not as though a couple of bacteria enter your body and immediately start producing toxins. We are much too big for that; they would have no effect,” explains Bassler. Moreover, the immune system could easily identify and kill invaders that misbehave from the start. “Today we know that many pathogens initially do not behave in a conspicuous way after infection. They wait and proliferate. And the whole time, they count each other with the aid of these small molecules. Once the right cell density has been reached, they collectively mount an attack. This way, they can overcome even the biggest host.”

In pathogenic bacteria, quorum sensing activates production of toxins and virulence factors. For example, the notorious hospital bug Pseudomonas aeruginosa uses quorum sensing to control the transition from a solitary lifestyle to aggregation and biofilm production. Biofilm is a slimy matrix that is collectively produced by bacteria and that protects them against the host’s immune response and the effect of antibiotics.

Circuits from the Construction Kit of Evolution

In the bacterial realm, the principle of quorum sensing is everywhere. In the 1990s, it became clear that a lot of bacteria have tools similar to those of *Vibrio fischeri*. But like in an electronics kit, the molecular switch can not only turn on the light but also control a whole set of other functions.

Many other species, too, use signaling molecules of the N-acyl-homoserine-lactone variety (AHLs) as autoinducers. All of them have the same basic chemical structure, but differ when it comes to the kind and length of their attached hydrocarbon chains (see Figure 3).

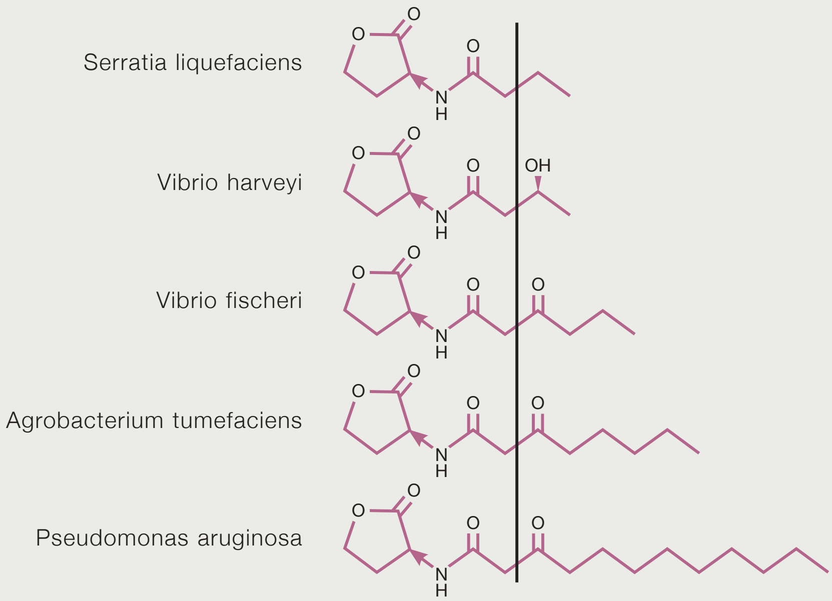

Fig. 3: Autoinducer molecules for the intra-species communication of some bacterial species. The basic structure to the left of the black line is always the same.

This attached chain makes sure that the signaling molecule fits the receptor protein of the particular bacterial species (in the case of *Vibrio fischeri*, LuxR) like a key fits a lock – and only this particular lock. “Every species uses its own molecule-receptor pair – it’s like a language that is understood only by this particular species and that allows it to have a private conversation to determine its numbers of kin,” explains Bassler.

Communication is everything – also among bacteria

But those who talk only with their peers are missing out on a lot of things. Bacteria rarely live in a pure culture; rather, our skin or our intestines are populated by countless different species. Wouldn't it be useful to also communicate with them to find out who lives where, and in what numbers?

In fact, Bassler and her team could show that approximately half of all bacteria that have been studied to this purpose have an additional signaling molecule, which she called, for simplicity's sake, autoinducer-2 (AI-2). Chemically speaking, however, AI-2 has nothing in common with the original autoinducer (now called AI-1). Its function, too, is different: Instead of intra-species communication, it enables the communication across species boundaries. If quorum sensing is the language of bacteria, this second system is a kind of bacterial Esperanto. It works because the AI-2 signaling molecules are chemically identical in all species that talk this lingua franca.

Over the years, the phenomenon of quorum sensing has shown itself to be more and more complex. For example, Bassler's lab discovered another quorum sensing system that is only 'understood' within the *Vibrio* genus. It enables the genus to have a conversation within the extended family, so to speak. Evidently all these systems interact with each other and allow the bacteria to optimally adapt their behavior to their environment.

Disrupting Communication as a Therapeutic Option

What is good for bacteria spells disease and death for a host infected with pathogenic species. But what if we could stop the bacteria from speaking to each other or make them close their biochemical ears? In the last decades, many bacterial pathogens have developed resistance to a whole range of common antibiotics. It might just be possible that an ancient war tactic – disrupting the communication behind enemy lines – could help in the fight against tuberculosis and other scourges of mankind.

This is where Bassler sees the great practical promise of the basic research into the mechanisms of quorum sensing. She sets her hopes on an entirely new generation of antibiotics that do not kill bacteria nor inhibit their growth, as antibiotics have done traditionally, but rather subtly interfere with their communication. Such quorum sensing inhibitors would perhaps not have as dramatic an effect as classical antibiotics, but they could give the body's immune defense the decisive advantage to fight off an infection. In fact, there are already very promising approaches out there. "We have a whole series of candidate molecules that disrupt quorum sensing and thus can block the creation of biofilms in Pseudomonas or the cholera pathogen. If you were a worm or a human cell in a Petri dish, we would already be able to save your life. In mice, too, some of these molecules have shown to have an effect," says Bassler.

New Antibiotics: A Long Way Off or Just Around the Corner?

Whether first antibiotics based on quorum sensing will be available soon or are still light years away – this is where scientific opinions differ. Bassler is optimistic that quorum sensing inhibitors will be the next big thing in the search for new antibiotics. And she is used to fighting headwinds. In fact, it took almost a decade to persuade people of the relevance of her alleged niche topic, before she managed to win, in 2003, her first big biomedical research grant from the U.S. government-funded National Institutes of Health. Currently, the molecules still lack potency and some necessary properties for use as medications. “But they are beginning to work! And we keep discovering new bacterial communication molecules that have the potential for drug development almost every year.”

For Bassler, it would be the realization of a dream that has motivated her since her mother died of cancer when Bassler was a young student. It is a dream that, after many years of basic research, now seems to be within reach: making a real contribution to medical progress.

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