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Interrupting Cholera's Conversation

Researchers have deciphered the molecular language that cholera bacteria use to coordinate their infectivity. The bacteria use this chemical communication to signal their presence to one another, so that they can plan as a group when to be most virulent and when to escape their host to find new victims.

Although cholera is rare in the U.S., it is epidemic in parts of Africa, Asia and Latin America, and the severe diarrhea it causes can lead to death if not treated. The researchers say that by interrupting the bacterium's chemical conversation, they may be able to stop cholera virulence. Their findings also offer hope that similar approaches may form the basis of effective treatments for a wide range of other bacterial diseases.

Howard Hughes Medical Institute investigator Bonnie Bassler and her colleagues at Princeton University reported their findings in the November 15, 2007, issue of the journal *Nature*.

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- Bonnie L. Bassler

Bassler and her colleagues have long studied a type of bacterial chemical conversation known as quorum sensing. This process depends on the bacteria releasing signaling chemicals called autoinducers into their environment, and subsequently detecting and responding to the build up of these molecules to coordinate with one another to ensure maximum infectivity and other group behaviors.

"We had shown that cholera had quorum sensing, and we had produced a mutant form of cholera that couldn't perform quorum sensing properly, which affected virulence," said Bassler. "This finding told us that there must be an autoinducer molecule that this mutant couldn't make that had a role in

virulence, but we had no idea what that molecule was.”

Bassler explained that the way the cholera bacteria use that molecule suggested it could make a useful treatment. “When people first get cholera, the bacteria immediately stick to the intestine in a structure called a biofilm and they release toxins,” she said. “During this time, they are multiplying rapidly and also releasing the autoinducer molecule. When the bacteria reach high cell numbers, the high concentration of the autoinducer molecule represses virulence and stops biofilm formation, enabling the bacteria to escape into the environment to spread to other people. So, if we could isolate and purify this molecule, and supply it to the bacteria to get them to prematurely terminate virulence, we thought it could be used as a treatment approach.”

Through their mutational studies, the researchers had identified the gene that codes for the enzyme that makes the unknown molecule. They inserted that gene into the gut bacterium *E. coli*, transforming the bacterium into a biological factory for large amounts of the chemical. That strategy allowed them to purify the chemical, which they called CAI-1, and analyze its molecular structure.

“This structure produced a real surprise,” said Bassler. “CAI-1 turned out to be a molecule brand new to biology. What's more, it was a simple molecule, almost like one you could buy at a chemical supply house. Because there was no precedent for this molecule, we felt we had to go to a lot of effort to demonstrate that this really was the correct molecule.” To do so, the researchers created synthetic CAI-1 and introduced it into cultures of cholera bacteria. The synthetic CAI-1 repressed virulence in those cells exactly like the natural molecule did. Carrying their studies further, the researchers are now exploring how CAI-1 is made by analyzing the function of the enzyme that produces it.

CAI-1's success in terminating virulence in cultures of cholera has encouraged Bassler and her colleagues to test the chemical as a treatment. “Next, we want to see whether we can cure a mouse of cholera using CAI-1,” she said. “These experiments also will enable us to answer some important questions about the properties of the molecule. For example, does it last in the gut? Is it stable? What should be the dose? Do we have to adjust the structure to make it more potent or less potent?”

The discovery of CAI-1 may also inspire efforts to control quorum sensing to treat other bacterial diseases, said Bassler. “Cholera uses quorum sensing in a different way than most other bacteria,” she said. “Cholera causes an acute infection; it gets into the host and then has to get out, so its strategy is to use quorum sensing to repress virulence when the bacterial cells reach high numbers. But other bacteria that cause persistent infections use quorum sensing to turn on virulence only when they reach high numbers—which makes biological sense because they want to hide from the immune system until they have successfully reproduced and then launch their attack en

masse. Thus, treatments for other bacteria that target quorum sensing are focused on developing drugs that block autoinducers. These drugs are very hard to make, and such efforts have not yet been very successful.

“If our studies with cholera demonstrate that it is possible to trick bacteria into reducing virulence, they constitute the first demonstration that manipulating such bacterial conversations can be a useful treatment. It will give the field solid evidence that quorum sensing is a viable new therapeutic target, which is especially important given the failure of so many traditional antibiotics.”

Also, emphasized Bassler, whose team has solved the structures of other quorum sensing molecules, the discovery of diverse quorum-sensing molecules such as CAI-1 represents another step in a promising and productive effort to decipher and manipulate the chemical language of bacteria.

“We know that there are molecules analogous to CAI-1 that are very species-specific, and we also understand that there are molecules that are generic and enable inter-species communication. Together, they give bacteria a multicellular character. And the fact that we are coming to understand this communication and even learn how to manipulate it both for medical and industrial purposes makes this a very exciting time for this research field.”