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A $5 fix for a nasty parasite: Team shows calcium-mediated attachment of C. parvum oocysts to environmental biofilms

December 12 2016

Kristen Jellison and Sabrina Jedlicka have a grant from NSF to develop a biomimetic surface that selectively attaches to *Cryptosporidium* oocysts. Credit: Ryan Hulvat

The life of a watershed is complex. The watershed is the area of land separating the smaller water flows that feed into a larger, common outlet—like a river, lake or ocean. As such, it is often home to a variety of wildlife, as well as subject to agricultural and recreational uses. From this complicated ecosystem, bacteria, viruses and parasites emerge—sometimes making their way into the water supply.

One reason that drinking water and public swimming pools are treated with chlorine is to kill such disease-causing organisms. Chlorine successfully eliminates a number of harmful pathogens—but not all of them.

Cryptosporidium parvum is one such chlorine-resistant pathogen. A protozoan parasite that is transmitted through either direct contact or ingestion of contaminated food or water, *C. parvum* is found in animal and human fecal matter.

According to the Centers for Disease Control and Prevention, *C. parvum* is a leading cause of waterborne disease among humans in the United States. Infection can lead to cryptosporidiosis which, in healthy people, causes severe diarrheal disease that can last for weeks. For the elderly, infants, or the immunocompromised, the infection can be deadly.

Because contaminated drinking water has been linked to major outbreaks of cryptosporidiosis, detecting and removing *C. parvum* in source waters is very important to protecting public health.

Now two Lehigh University engineers are investigating how *C. parvum* oocysts— the development stage in which the parasite exists in the environment—attach to environmental biofilms, which are the microbial communities that grow on underwater surfaces, like rocks. Their ultimate goal is the development of an improved detection method that can identify a contamination source quicker and more cheaply than current methods, preventing widespread infection.

"We think we can develop a surface that can be commercially produced for around $5," says Kristen L. Jellison, associate professor of civil and environmental engineering. "This would allow utilities and water resource managers to better protect the public from exposure by sampling more locations with greater frequency and obtaining more reliable and comprehensive data about the sources of the parasite in the watershed."

Jellison and her Lehigh colleague Sabrina S. Jedlicka, associate professor of materials science and engineering, are the first to demonstrate that the attachment of oocysts to environmental biofilms is a calcium-mediated process—a crucial step toward their goal. Jellison and Jedlicka describe their results in an article in *Applied and Environmental Microbiology* called: "Pseudo-second-order calcium-mediated *Cryptosporidium parvum* oocyst attachment to environmental biofilms."

Understanding the mechanism that enables oocysts to attach to biofilms is important for calculating the attachment efficiency, or the rate at which the parasite binds to the material. Understanding the rate is key for two reasons: One, it may enable calculation of oocyst concentration in the water based on numbers of oocysts attached to a material; and, two, it is needed to design materials that maximize oocyst attachment. Such attachment-enabling material will make it possible to detect the source of the infestation faster and more inexpensively than current methods allow.

"The number of oocysts on a given biofilm sample reveal how much of the parasite is present in the water," explains Jellison. "For example—simply for illustration's sake - say that five oocysts attach to a biofilm downstream of the contamination source. That may indicate the presence of 100 oocysts further upstream. If we see ten oocysts attaching, it might mean there are 200 at the main source."

Jellison's and Jedlicka's study provides new insights into the impact of calcium on the attachment of *C. parvum* oocysts to environmental biofilms. In addition, their modeling method could be used to elucidate the behavior of oocysts in commonly encountered complex aquatic systems—an important step in enabling future innovations in parasite detection and treatment technologies to protect public health.

Comparable data, much cheaper

The idea for using environmental biofilms to identify a *C. parvum* contamination source first occurred to Jellison during her work with the Philadelphia Water Department on the detection of *Cryptosporidium* in the Wissahickon and Schuykill watersheds.

The EPA-approved filtration-based methods used to test the water sources were expensive and the results were inconsistent. According to Jellison, one filter could cost as much as $120.00. That may not sound like a large sum, but considering the limited resources of many municipal water departments—and the number of filters that might be needed to accurately detect the source of a *C. parvum* contamination—it represents a serious obstacle.

"Recovery using current methods depends on variables such as the cleanliness of water and the skill of the person doing the testing," says Jellison. "*C. parvum* can be present in the water in very small amounts. You can check a 10 Liter sample and not detect the parasite. But it could be present in the water."

In her work testing the watersheds near Philadelphia, Jellison would scrape biofilms off of rocks both upstream and downstream of a suspected *C. parvum* contamination location and then test the samples in her lab at Lehigh. Over time, the biofilm samples provided comparable data to the approved filtration method - but at much lower cost.

"Being able to test the water accurately and in more places increases the likelihood of identifying the contamination source and decreases the chance that thousands of people will become infected," says Jellison.

Jellison's downstream biofilm samples contained more oocysts than the samples taken from rocks upstream of suspected point sources. However, there was no way to identify when the oocysts attached. The clues provided by the biofilms were important for confirming that *C. parvum* was entering the water at particular locations, but not much help in revealing when the contamination events occurred.

If Jellison could figure out when, precisely, the oocysts attached, she would be better able to identify patterns of oocyst contamination which would enable more effective source water protection strategies.

Designing materials to maximize attachment

That is where Jedlicka comes in. Jedlicka—a materials scientist who says she "comes from a field of designing surfaces to illicit behavior in cells"—does the mathematical modeling that enables the duo to calculate the attachment efficiency of *C. parvum* to biofilms.

"We need an understanding of the chemistry behind the binding process to know what designs will work best," says Jedlicka.

Jedlicka's ability to calculate the binding force is what led to the demonstration of a calcium-mediated binding process.

"When we took the calcium out, the binding efficiency went down significantly, which is one way we knew that calcium is important to binding," says Jedlicka.

In addition to contributing to improved detection methods, creating a surface design that maximizes the attachment of oocysts has other implications as well.

"If we can identify the binding mechanism that enables *C. parvum* to 'stick' to biofilms, we can create an improved means to remove it," says Jellison. "Understanding the mechanism could even open the door to preventing the parasite from binding in the intestine and making people sick."

Among the team's next steps is investigating the other chemical processes involved in the binding, in addition to calcium. They also plan to test their model under a number of conditions, including hydrodynamic sheer stresses.

Their ultimate goal, however, remains clear.

"If we can help utilities find a way to monitor multiple points in the watershed instead of just a few, they can put their limited resources to more effective use," says Jellison.

"Until then," says Jedlicka, "we'll just keep asking ridiculously hard science questions."

More information: Xia Luo et al, Pseudo-second-order calcium-mediatedoocyst attachment to environmental biofilms, *Applied and Environmental Microbiology* (2016). DOI: 10.1128/AEM.02339-16

Provided by Lehigh University