July 24, 2018

Microbiology

Killing *C. difficile* with targeted strikes

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This Break was edited by Max Caine, *Editor-in-chief* - TheScienceBreaker

ABSTRACT

C. difficile poses a huge threat to human health, but the friendly bacteria of the gut provide us with a natural resistance to infection. By reprogramming naturally occurring nano-weapons, we can specifically target C. diffcile without hurting these friendly bacteria. However, C. difficile can become resistant to these weapons, but do these resistant mutants pose a threat

Clostridium difficile is a bacterium that causes hundreds of thousands of antibiotic-associated diarrhoea cases every year, and these infections often prove fatal. Usually, *C. difficile* is unable to cause disease as the bacterium is kept in check by the friendly bacteria in the gut; the microbiota. However, antibiotic use, while often necessary, damages the microbiota and leaves *C. difficile* free to reproduce, release toxins, and cause disease.

C. difficile produces super hardy spores that are resistant to a myriad of stresses including high temperature, antibiotics, and even the chemicals in many cleaning products. These tough spores are also how the bacterium spreads from person to person, posing a real problem for health care professionals trying to limit its spread. This is also a problem when it comes to treating *C. difficile* infections. These infections are normally treated using additional antibiotics, however these also damage the microbiota further. As spores are unaffected by antibiotics, when a patient completes a course of medication they are susceptible to a relapse of infection when the spores that survive in the gut start to germinate. This highlights the need for more specific *C. difficile* treatments that don't harm the microbiota. This would allow time for the friendly bacteria to replenish, restoring natural resistance to *C. difficile*.

Recently, it was discovered that *C. difficile* makes nano killing-machines that kill competing strains of *C. difficile.* It seems *C. difficile* has a bad case of sibling rivalry. These nano-machines, called Diffocins, are shaped like oil rigs. Six legs support a body that houses a hollow drill. Upon contact with a bacterial cell, the hollow drill is pushed through the cell wall forming a hole in the bacterium, through which the bacterium's insides leak out, leading to rapid death. So why not use *C. difficile's* own weapon against it?

At the foot of the Diffocins' legs there are receptor binding proteins that recognise the surface of the bacterial cell. We discovered that if we modify these proteins we can alter the strains of *C. difficile* that the Diffocins target. These reprogrammed weapons were named Avidocin-CDs. We produced Avidocin-CDs that target all the major disease-causing strains of *C. difficile.* However, there was a problem. When we exposed *C. difficile* to Avidocins, we isolated strains that were completely resistant to killing. If *C. difficile* could become resistant to Avidocins, were they going to be useful as medicines?

We decided to probe further and look at the cell surface of the Avidocin-resistant *C. difficile* mutants, to see how they had become resistant. To our surprise, the mutants had lost their S-layer: a protein coat that completely encases the bacterial cell in a crystal armour. S-layers are common features of bacterial physiology although very little is known about them. We sequenced the genomes of these mutants and found a mutation in the gene encoding the major S-layer protein, essentially turning it off.

This gave us an exciting opportunity to answer two questions. What does the S-layer do, and are mutants without S-layers a threat to our health?
We found that these mutants grew poorly and formed strange, short, bent cells, and also showed a significant decrease in their ability to produce spores. More importantly however, we showed that these mutants could not produce toxins and showed increased sensitivity to two components of the human immune system, lysozyme and LL37.

This tells us that Avidocin-CDs target the *C. difficile* S-layer and that resistance can be conferred though loss of the S-layer. However, the resistant mutants pose little threat to human health as they are incapable of producing the toxins. *C. difficile's* toxins cause the symptoms of disease, so no toxins, no symptoms. Avidocin-CDs force *C. difficile* to make a difficult choice; lose the ability to cause disease or die. It is also likely that the mutants will be less able to pass from person to person as they produce fewer spores. Studying these mutants has also taught us a lot more about the roles of the S-layer, showing it is involved in cell shape maintenance, sporulation, and protection from the immune system. The S-layer is therefore a great target for the development of anti-*C. difficile* treatments.

Avidocin-CDs show great potential as anti-*C. difficile* therapeutics. Importantly, the S-layer of *C. difficile* is unique so these nano-machines will kill *C. difficile* without damaging the protective microbiota, lowering the risk of recurrent infection.