Tuning natural antimicrobials to improve their effectiveness at battling superbugs

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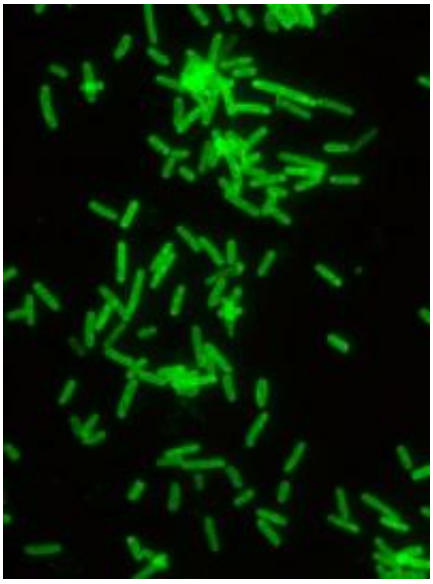

The truncated endolysin, tagged with Green fluorescent protein, bound to *C. difficile.* Credit: mage by Kathryn Cross, Imaging Partnership at IFR

Ongoing research at the Institute of Food Research, which is strategically funded by BBSRC, is exploring the use of virus-produced proteins that destroy bacterial cells to combat potentially dangerous microbial infections. Bacteriophages produce endolysin proteins that specifically target certain bacteria, and IFR has been studying one that destroys *Clostridium difficile*, a common and dangerous source of hospital-acquired infections. New research is showing that it is possible to 'tune' these endolysin properties to increase their effectiveness and aid their

development as a new weapon in the battle against superbugs.

*Clostridium difficil*e infection (CDI) is a common and growing problem as a cause of infections, especially in hospitals where the characteristics of the bacteria make it difficult to clear. At the moment, antibiotics are used to treat infections, but *C. difficile* is adept at acquiring resistance, meaning the number of effective antibiotics is ever decreasing.

This has driven the search for new antimicrobials, and at IFR Melinda Mayer and Arjan Narbad have been focussing on bacteriophage endolysins. These are relatively short proteins produced by viruses that specifically target certain species of bacteria and then break open the cell walls. They had previously isolated an endolysin, CD27L, which is active against *C. difficile* when applied externally, but does not affect a large range of other bacteria. This is important as any potential treatment must not affect the native gut bacteria in patients, whose gut microbiota may already have been disturbed.

However, although CD27L works in the laboratory, its activity would probably not be high enough to cope with the vast numbers of *C. difficile* cells in a growing population in the harsh gut environment to be used as an effective treatment. This prompted the researchers to look more closely at the endolysin.

Endolysins commonly have two domains, one at each end. One domain is thought to be responsible for the specificity of the endolysin, allowing it to bind specifically to wall molecules unique to the bacterial species. This is what was thought to give the endolysin its specific host range. The other catalytic domain attacks the cell wall, causing lysis.

They produced shortened versions of the endolysin containing only one of these domains. The truncated CD27L containing only the catalytic domain showed a much higher activity against the *C. difficile* cells.

Surprisingly, however, the truncated endolysin was still inactive on a range of other bacteria, even though the domain thought to make it specific had been removed.

Working with colleagues at the European Molecular Biology Laboratory (EMBL) in Hamburg, the structure of the catalytic domain was solved and used to design mutants to investigate what controls the specificity and activity of the endolysins. The researchers propose that the catalytic domain contributes to the specificity of the endolysin.

In the case of CD27L, binding to the cell wall is not a critical part of the activity of the endolysin, and from these results seems to reduce the activity. This fundamental science on the mode of action of endolysins establishes that in the development of valuable novel therapeutics it may be more appropriate to use truncated versions of endolysins.

More information: Structure-based modification of a Clostridium difficile targeting endolysin affects activity and host range Mayer, M.J. et al *Journal of Bacteriology* doi:10.1128/JB.00439-11

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