Study: Odd biochemistry yields lethal bacterial protein

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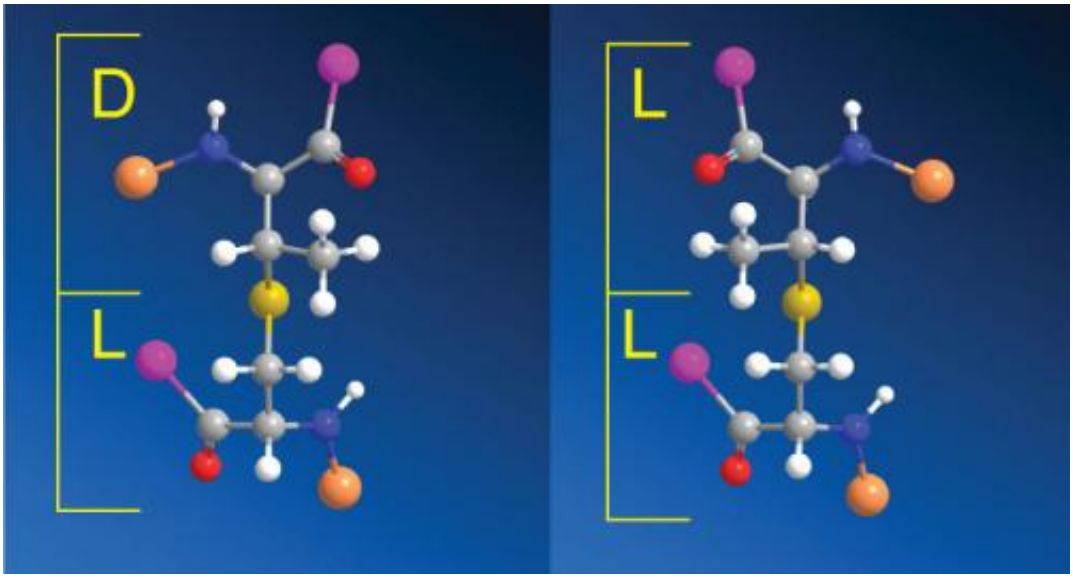


One enzyme shapes the components of a bacterial protein into rings with right-handed (D) and left-handed (L) stereochemistries. Credit: Graphic by Weixin Tang

While working out the structure of a cell-killing protein produced by some strains of the bacterium *Enterococcus faecalis*, researchers stumbled on a bit of unusual biochemistry. They found that a single enzyme helps form distinctly different, three-dimensional ring structures in the protein, one of which had never been observed before.

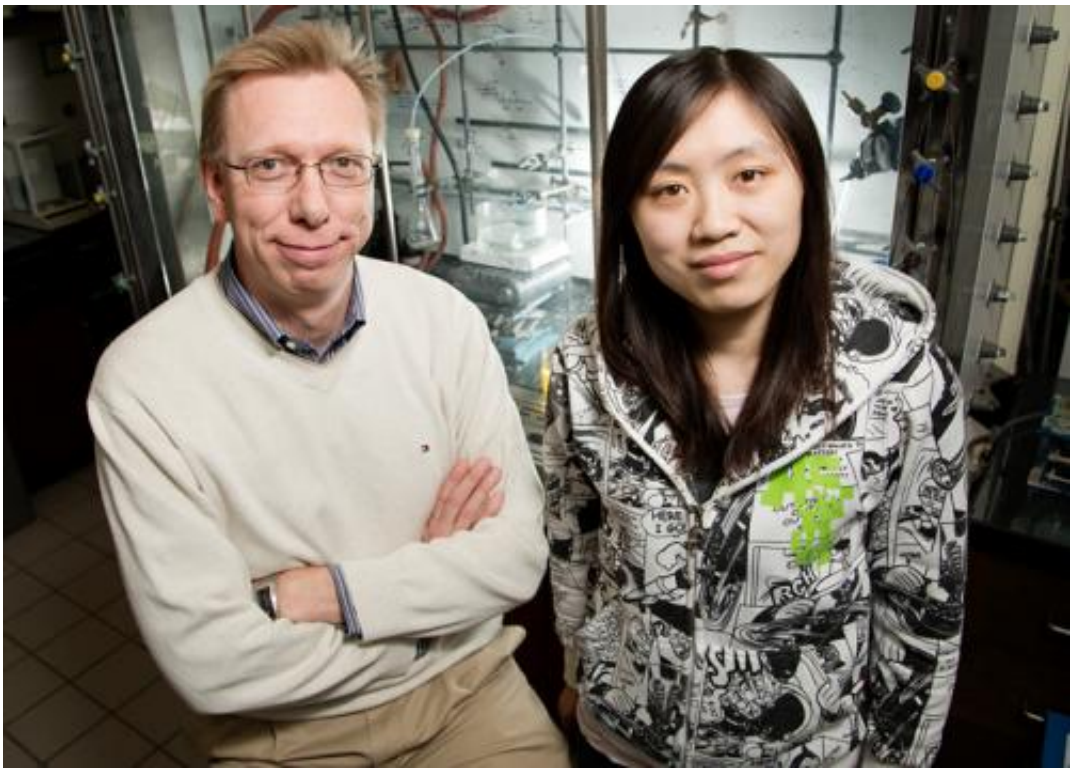
The new findings, reported in *Nature* Chemical Biology, should help

scientists find new ways to target the enterococcal cytolysin protein, a "virulence factor that is associated with acute infection in humans," said University of Illinois chemistry and Institute for Genomic Biology professor Wilfred van der Donk, who conducted the study with graduate student Weixin Tang.

Enterococcus faecalis (EN-ter-oh-cock-us faye-KAY-liss) is a normal microbial inhabitant of the gastrointestinal tracts of humans and other mammals and generally does not harm its host. Some virulent strains, however, produce cytolysin (sigh-toe-LIE-sin), a protein that, once assembled, attacks other microbes and kills mammalian cells.

"The cytolysin protein made by *Enterococcus faecalis* consists of two compounds that have no activity by themselves but when combined kill human cells," van der Donk said. "We know from epidemiological studies that if you are infected with a strain of *E. faecalis* that has the genes to make cytolysin, you have a significantly higher chance of dying from your infection." *E. faecalis* contributes to root canal infections, urinary tract infections, endocarditis, meningitis, bacteremia and other infections.

Enterococcal cytolysin belongs to a class of antibiotic proteins, called lantibiotics, which have two or more sulfur-containing ring structures. Scientists had been unable to determine the three-dimensional structure of this cytolysin because the bacterium produces it at very low concentrations. Another problem that has stymied researchers is that the two protein components of cytolysin tend to clump together when put in a lab dish.



Chemistry professor Wilfred van der Donk (left) and graduate student Weixin Tang, University of Illinois, determined the unusual structure of a bacterial toxin. Credit: L. Brian Stauffer

Van der Donk and Tang got around these problems by producing the two cytolysin components separately in another bacterium, *Escherichia coli* (esh-uh-REE-kee-uh KOH-lie), and analyzing them separately.

"The two components are both cyclic peptides, one with three rings and the other with two rings," van der Donk said. "Curiously, a single enzyme makes both compounds."

In a series of experiments, the researchers found that one ring on each of the proteins adopted a (D-L) stereochemistry that is common in lantibiotics (see image, above). But the other rings all had an unusual (L-L) configuration, something van der Donk had never seen before.

Scientists had assumed that the enzyme that shaped enterococcal cytolysin, a lantibiotic synthetase, acted like a three-dimensional mold that gave the ring structures of cytolysin the exact same stereochemistry, van der Donk said.

"But we found that the enzyme, enterococcal cytolysin synthetase, makes the rings with different stereochemistry," he said. "I don't know of any other examples where one enzyme can make very similar products but with different stereochemistries."

The researchers don't know how the enzyme accomplishes this feat, but found a clue in the sequence of amino acids that make up the protein rings. The chemical characteristics of the three amino acids in the middle of the ring structure and their proximity to another amino acid, a cysteine, determined whether the rings took on a D-L or L-L stereochemistry.

The researchers tested the idea that the amino acid sequence of the cytolysin protein was guiding the stereochemistry by looking at other lantibiotic proteins with similar sequences. So far, every protein they've tested that has the same sequence characteristics conforms to the pattern they discovered, van der Donk said.

Further tests showed that the cytolysin produced in *E. coli* had the same anti-microbial and cell-killing potency as the *E. faecalis* variety.

"Knowing the structure of enterococcal cytolysin and having a method to produce it in relatively large quantities will allow scientists to find out how it kills human cells and, in turn, how we might fight against it," van der Donk said.

More information: "The Sequence of the Enterococcal Cytolysin Imparts Unusual Lanthionine Stereochemistry," *Nature Chemical*

Biology, online Jan. 13, 2013.