Researchers discover how microbes build a powerful antibiotic

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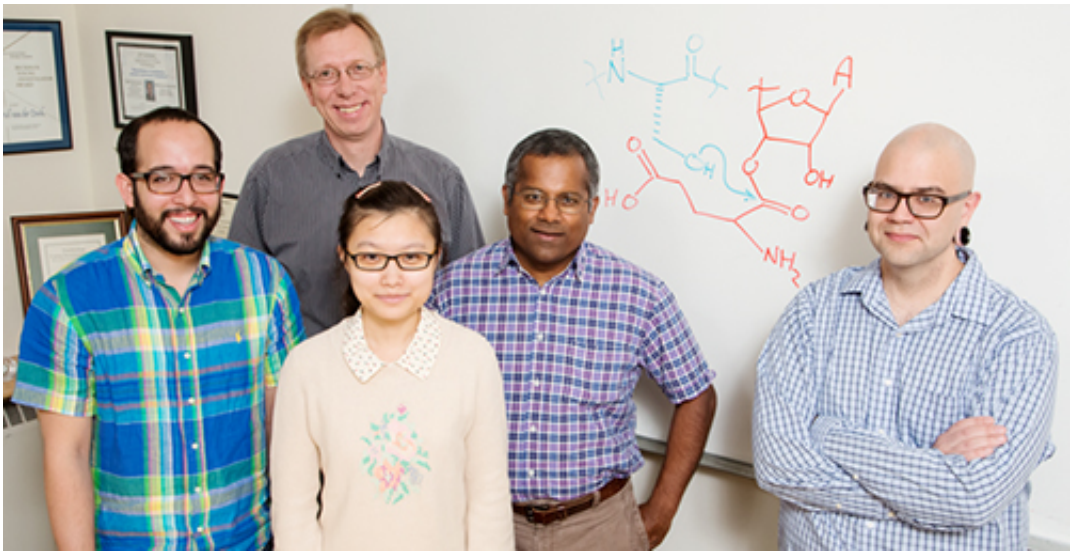

University of Illinois graduate research assistant Manuel A. Ortega, chemistry professor Wilfred van der Donk, graduate student Yue Hao, biochemistry professor Satish Nair and postdoctoral researcher Mark Walker solved a decades-old mystery into how a broad class of natural antibiotics are made. Credit: L. Brian Stauffer

Researchers report in the journal *Nature* that they have made a breakthrough in understanding how a powerful antibiotic agent is made in nature. Their discovery solves a decades-old mystery, and opens up new avenues of research into thousands of similar molecules, many of which are likely to be medically useful.

The team focused on a class of compounds that includes dozens with antibiotic properties. The most famous of these is nisin, a natural product in milk that can be synthesized in the lab and is added to foods as a preservative. Nisin has been used to combat food-borne pathogens since the late 1960s.

Researchers have long known the sequence of the nisin gene, and they can assemble the chain of amino acids (called a peptide) that are encoded by this gene. But the peptide undergoes several modifications in the cell after it is made, changes that give it its final form and function. Researchers have tried for more than 25 years to understand how these changes occur.

"Peptides are a little bit like spaghetti; they're too flexible to do their jobs," said University of Illinois chemistry professor Wilfred van der Donk, who led the research with biochemistry professor Satish K. Nair. "So what nature does is it starts putting knobs in, or starts making the peptide cyclical."

Special enzymes do this work. For nisin, an enzyme called a dehydratase removes water to help give the antibiotic its final, three-dimensional shape. This is the first step in converting the spaghetti-like peptide into a five-ringed structure, van der Donk said.

The rings are essential to nisin's antibiotic function: Two of them disrupt the construction of bacterial cell walls, while the other three punch holes in bacterial membranes. This dual action is especially effective, making it much more difficult for microbes to evolve resistance to the antibiotic.

Previous studies showed that the dehydratase was involved in making these modifications, but researchers have been unable to determine how it did so. This lack of insight has prevented the discovery, production and study of dozens of similar compounds that also could be useful in

fighting food-borne diseases or dangerous microbial infections, van der Donk said.

Through a painstaking process of elimination, Manuel Ortega, a graduate student in van der Donk's lab, established that the amino acid glutamate was essential to nisin's transformation.

"They discovered that the dehydratase did two things," Nair said. "One is that it added glutamate (to the nisin peptide), and the second thing it did was it eliminated glutamate. But how does one enzyme have two different activities?"

To help answer this question, Yue Hao, a graduate student in Nair's lab, used X-ray crystallography to visualize how the dehydratase bound to the nisin peptide. She found that the enzyme interacted with the peptide in two ways: It grasped one part of the peptide and held it fast, while a different part of the dehydratase helped install the ring structures.

"There's a part of the nisin precursor peptide that is held steady, and there's a part that is flexible. And the flexible part is actually where the chemistry is carried out," Nair said.

Ortega also made another a surprising discovery: transfer-RNA, a molecule best known for its role in protein production, supplies the glutamate that allows the dehydratase to help shape the nisin into its final, active form.

"In this study, we solve a lot of questions that people have had about how dehydration works on a chemical level," van der Donk said. "And it turns out that in nature a fairly large number of natural products – many of them with therapeutic potential – are made in a similar fashion. This really is like turning on a light where it was dark before, and now we and other labs can do all kinds of things that we couldn't do previously."

More information: "Structure and mechanism of the tRNA-dependent lantibiotic dehydratase NisB," *Nature*, 2014. DOI: 10.1038/nature13888

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