Salmonella stays deadly with a 'beta' version of cell behavior

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Salmonella cells have hijacked the protein-building process to maintain their ability to cause illness, new research suggests.

Scientists say that these bacteria have modified what has long been considered typical cell behavior by using a beta form of an amino acid – as opposed to an alpha form – during the act of making proteins.

Beta versions of amino acids occur in nature under rare and specific circumstances, but have never been observed as part of protein synthesis. Before this finding, in fact, researchers had determined that virtually all proteins were constructed with the alpha forms of amino acids.

This work has shown that when researchers delete any one of three genes from the process that makes use of the beta form of the amino acid, or if they insert the alpha form in the beta version's place, *Salmonella* cells are no longer able to cause disease. The amino acid in question is lysine, one of 22 genetically encoded amino acids that are strung together in cells to make proteins.

"When these genes were knocked out, the cells became sensitive to antibiotics. And if we put beta lysine into the medium where cells were growing, they became resistant to antibiotics," said Michael Ibba, professor of microbiology at Ohio State University and a senior author of the study. "So we could see the beta amino acid being taken up and used. The cells really do need the beta amino acid to be resistant to antibiotics, and for other aspects of their virulence."

This finding suggests that the process using this specific beta amino acid could be an attractive antibiotic target for this common pathogen, the researchers say.

The Centers for Disease Control and Prevention estimates that about 1.4 million people in the United States are infected with *Salmonella* each year, though only 40,000 cases are reported. Most people infected with *Salmonella* develop diarrhea, fever and abdominal cramps. Though recovery can occur within a week without treatment, some severe cases require antibiotic treatment and hospitalization.

The study is published in the Aug. 14 online edition of the journal *Nature Chemical Biology*.

This work began when University of Toronto scientists exploring the origins of *Salmonella*'s virulence identified three genes that were clear players in the process. These three genes – called YjeK, PoxA and EF-P – were unusual in this context.

Genes that confer virulence in bacteria typically have a specific job, such as producing toxins or transporters. But these three virulence genes all looked like they should have a role in the protein synthesis machinery – which is Ibba's expertise.

Under normal circumstances in cells, an enzyme will select amino acids in the cell and place them on a molecule called transfer RNA, or tRNA, which leads to translation of the genetic code into proteins.

In *Salmonella* cells, these steps are similar, but with a few surprising twists, Ibba said. He and colleagues confirmed that the YjeK gene makes beta lysine, and showed that the PoxA gene takes that beta lysine and attaches it to EF-P – a protein that partially mimics the shape and function of tRNA.

"It's a really unexpected pathway," said Ibba, also an investigator in Ohio State's Center for RNA Biology. "It is a mimic of what normally makes protein in a cell. Where a cell would normally be expected to use an alpha amino acid, *Salmonella* puts on a beta amino acid. And it ends up making molecules that lead to the cells being virulent."

The research team first reconstructed this unusual protein synthesis process in test tube experiments, and then followed with studies in cell cultures. Even before they took on studying the mechanism, however, they knew that the effects of these virulence genes were powerful: In earlier animal studies, deleting any one of the three genes and then infecting mice with these altered *Salmonella* cellshad no effect on the animals. When the genes were left intact and cells were injected into mice, the resulting *Salmonella* infection killed the animals.

In addition, when the researchers tricked *Salmonella* cells into using alpha lysine for this pathway instead of beta lysine, the cells lost their ability to cause illness.

"This tells us the cell is not going to be able to easily replace the beta amino acid," Ibba said. "It is essential for virulence in *Salmonella*."

And that, he said, is why that amino acid might be such an effective drug target, especially as humans don't seem to make beta amino acids at all. "You have to make an antibiotic look like something natural, only different. If you have something that's already different like a beta amino acid, you've potentially got a much better drug target because it involves chemistry that's comparatively rare in the cell. It's harder for the cell to try to alter its own chemistry to develop resistance," Ibba said.

From here, the researchers are observing cell behavior later in the protein-building process to figure out how this hijacked system actually gives Salmonella its virulence.

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