Scientists identify proteins that help bacteria put up a fight

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Scientists have identified the role of two proteins that contribute to disease-causing bacteria cells' versatility in resisting certain classes of antibiotics.

The finding is a step toward development of drug therapies that could target bacterial resistance at its cellular source. Before researchers can design such drugs, they must understand all of the activities at play in the conflict between bacteria and the agents that kill them.

This finding by Ohio State University microbiologists extends the understanding of how bacteria cells resist antibiotics through the activities of two genetically distinct forms of what are called MprFs, or multiple peptide resistance factors. The proteins they studied are MprF1 and MprF2.

These proteins were found to be key to the mechanism allowing bacteria cells to change the electrical charge of their membrane, which is how the cells develop their resistance to certain antimicrobial agents and, more generally, how they adapt their membrane to new environmental conditions, such as those provided by their host organism.

"Both of these proteins are potentially very good drug targets because in theory, if you can target them and inhibit their action, you can make bacteria strains more susceptible to existing antibiotics," said Michael Ibba, associate professor of microbiology at Ohio State and a coauthor of the study.

The findings are described online in this week's issue of *Proceedings of the National Academy of Sciences*.

Scientists have already observed that the cell membranes of many disease-causing bacteria develop resistance by changing their electrical charge from negative to positive. Many antibiotics work because they carry a positive charge that attracts them to negatively charged bacteria cells. The opposite charges allow antibiotics to penetrate and kill bacteria. But by changing their naturally occurring negative charge to positive, some bacteria cells establish a protective "coat" that repels the antibiotic.

A common example of antibiotic resistance is Methicillin-resistant Staphylococcus aureus (MRSA), the strain of bacteria responsible for thousands of difficult-to-treat infections reported in humans each year.

"There is a dispute that remains unresolved as to whether or not this pathway we're investigating is involved in MRSA. It's very unclear. By understanding the mechanism, we might be able to find out if this is involved in MRSA or not," Ibba said.

Ibba and Hervé Roy, a postdoctoral researcher at Ohio State and lead author of the study, concentrated on exploring the activities of these specific MprF proteins, which are just two of dozens of forms of a class of genes associated with the development of resistance in about 200 bacteria species. They investigated the activity of two forms of MprF from the pathogen Clostridium perfringens, one of the most common sources of food poisoning in the United States.

MprF proteins affect the membrane's charge by using an adapter molecule, called transfer RNA (tRNA), to transfer amino acids to the lipids that make up the cell membrane. This action leads to modification of the membrane and the change in its charge.

Ibba and Roy found that both MprF1 and MprF2 perform this same function, but they use different amino acids that lead to the modification. The amino acid lysine has already been identified as a player in this modification, and is used by MprF2. Ibba and Roy found that MprF1, however, uses the amino acid alanine instead. This amino acid also contributes to cell membrane modification and seems to have additional functions that remain unknown.

“This is a new function that we discovered, that MprF1 uses alanine, which then allows the cell to fine tune the properties of the membrane,” Roy said. “Earlier studies found these effects on the membrane, but no one knew what protein caused it.”

What makes these proteins even more potent in the resistance effort is that they can use the adapter molecule in a variety of forms to achieve membrane modification. When the researchers manipulated the tRNA’s structure and properties to match differences in the molecule that would occur in different species of bacteria, the proteins could still recognize the molecule and put it to use to perform the amino acid transfer that changes the cell membrane.

“This means that there is no species barrier for the spread of this virulence factor among other bacteria because this protein can recognize tRNA in any species, no matter what it looks like,” Roy said.

Ibba and Roy describe their findings as only the beginning of investigating the role of the MprF family of proteins in bacteria. They believe other amino acids could also be used that would modify bacteria cell membranes, and are investigating additional pathways within the cells that lead to remodeled membranes.

“We know the change to the membrane is key to resistance,” Ibba said. “We now know there is not just one way that can happen. We have just

found a second way an organism can do this, and it is able to make the change to the membrane in two different ways. From our findings there are almost certainly even more ways that the membrane can be modified, and that's what we're looking for next."

Source: Ohio State University