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Researchers seek to make cavity-causing bacteria self-destruct

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Larger goal to eliminate key enzyme's contribution to all strep and staph disease

Bacteria that eat sugar and release cavity-causing acid onto teeth may soon be made dramatically more vulnerable to their own acid. Researchers have identified key genes and proteins that, if interfered with, can take away the ability of a key bacterial species to thrive as its acidic waste builds up in the mouth.

The ability of Streptococcus mutans (S. mutans) to survive in acid is one reason that the species is the main driver of tooth decay worldwide. Past research has shown that this ability has several components including a bacterial enzyme called fatty acid biosynthase M (FabM), which when shut down, makes S. mutans almost precisely 10,000 times more vulnerable to acid damage.

In addition, early work suggests that FabM or one of its relatives may also help all Streptococci (strep) and Staphylococci (staph) infections to resist the human body's defenses, which include immune cells that subject bacteria to acid. Between them, "strep" and "staph" bacteria are responsible for meningitis, pneumonia, sepsis, methicillin-resistant staph aureus, the "flesh-eating" infection (fasciitis), as well as infections on heart valves and around stents.

While FabM represents a major target for the design of new drugs, the focus of the next round of work is to identify and rank every one of the 2,000 known S. mutans genes that contributes to its "fitness" (ability to

survive, out-compete other strains and cause disease). A research team at the University of Rochester Medical Center today announced that it has received a $3.6 million fitness profiling grant from the National Institute of Dental and Craniofacial Research (NIDCR), part of the National Institutes of Health (NIH). Grant-funded projects will seek to create a catalogue of proteins that, along with FabM, can serve as targets for a multi-pronged attack on bacteria that tend to evolve around single-thrust treatments.

“Our first goal is to force the major bacterium behind tooth decay to destroy itself with its own acid as soon as it eats sugar,” said Robert G. Quivey, Ph.D., professor of Microbiology & Immunology at the University of Rochester Medical Center and principal investigator for the grant. “After that, this line of work could help lead to new anti-bacterial combination therapies for many infections that have become resistant to antibiotics.”

Study Details

In 2002, Charles O. Rock, Ph.D., a faculty member within the Department of Infectious Diseases at St. Jude Children's Research Hospital, published his research describing the existence of the FabM enzyme. Rock, a consultant on Quivey’s grant application, also established the role that the FabM gene plays in the construction of compounds called fatty acids in the membranes of strep bacteria, a barrier they present to surrounding world. Applying the FabM line of work to oral disease for the first time, Quivey and colleagues about two years later published research that FabM enzymes were behind dramatic changes seen, in response to increasing acidity, in the fatty acids that compose the S. mutans membrane.

S. mutans produces lactic acid as a waste product of fermentation, the process by which some ancient lifeforms convert sugar into energy for

life without using oxygen. After a great many generations of exposure to its own acid waste, the membranes for this species have become “acid durable.” Quivey’s team has shown that FabM contributes to this durability by making carbon chains, the main functional feature of S. mutans membrane fatty acids, grow longer. In fact, as many as 60 percent of the fatty acids in a bacterial outer membrane undergo this change as acidity increases, Quivey said.

Researchers have already shown that such structural changes protect membranes, presumably by making it more difficult for acids to donate hydrogen ions to them, but they do not yet know why. Forcing hydrogen ions on other compounds gives acid its bad reputation. Remaining questions that the team will be seeking to solve over the next five years include how do longer fatty acids in membranes protect against acid specifically, and how do bacteria sense changes in acidity.

To help answer these questions, Quivey’s team has genetically engineered the first and only mutant form of S. mutans with the FabM gene removed.

This FabM “knockdown” mutant is a living model that shows the exact impact of the enzyme in live bacteria. Without FabM, the mutant fills its outer membrane with other, smaller fatty acids that are much less acid resistant than those normally created via FabM, but that still provide some protection from acid. Thus, a goal is to design a treatment that would prevent S. mutans from forming both straight chain and “smaller chain” fatty acids.

As Quivey and others design next-generation antibacterial drugs, they are looking not just for a single way to stop the action of a single disease-causing enzyme, but how to shut down its three or four back-up systems. The process of cutting off genetic escape routes for bacteria applies to every trait central to the ability of the bacteria to survive and cause

disease. Beyond acid durability, the team will also look at the genes and proteins that enable S. mutans to stick to teeth enamel like no other, which it does by producing a sugary polymer (plaque). Tooth decay is the result of plaque combined with acid.

Quivey's partners in the grant application were Elizabeth Grayhack, Ph.D., research associate professor of Biochemistry and Biophysics, Robert Marquis, Ph.D., professor of Microbiology and Immunology, and Eric Phizicky, Ph.D., professor of Biochemistry and Biophysics. The grant application succeeded with the NIH, Quivey said, because the team and proposal combined many years of experience in genomic projects (Grayhack and Phizicky) with extensive microbial experience (Marquis and Quivey).

As part of the grant, Grayhack and Phizicky will create a library of mutant strains for the 2,000 known S. mutans genes, with each strain having just one of the 2,000 genes shut off. They will then subject the library to acid, for example, and see which strains thrive. Knowing which gene is missing from each strain, researchers will then be able to draw conclusions about each single gene's contribution to not only to acid durability, but also to many aspects of the strep bacteria's ability to survive and cause disease.

"Down the road, the finished library will enable researchers to determine every bacterial protein involved in oral disease, to learn their exact structure and to tailor drugs that interfere with them," said Marquis. "Identifying and turning off say the top four ways in which bacteria might try to resist treatment is the team's strategy."

Source: University of Rochester Medical Center