New design may produce heartier, more effective salmonella-based vaccines

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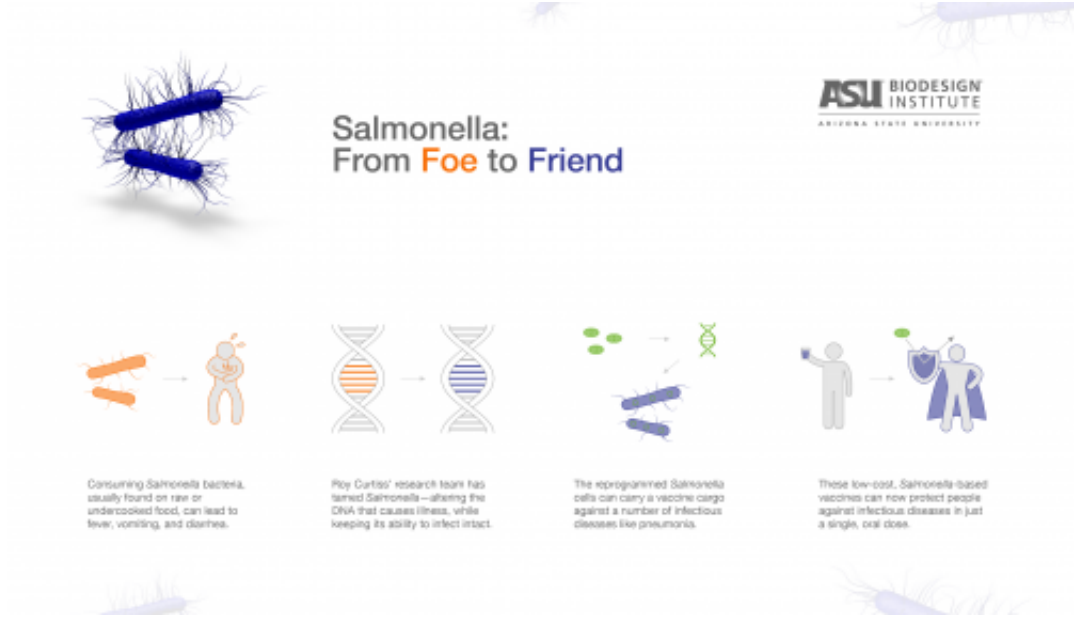

ASU researchers can tame salmonella to act as a vaccine Trojan horse and trigger an immune response against a number of infectious diseases. Credit: Jason Drees, Biodesign Institute

The bacterial pathogen *Salmonella* has a notorious capacity for infection. Last year alone, according to the Center for Disease Control, various species of *Salmonella* caused multistate disease outbreaks linked with contaminated peanut butter, mangoes, ground beef, cantaloupe, poultry, tuna fish, small turtles and dry dog food.

The troublesome invader, however, can be turned to human advantage. Through genetic manipulation, the species *S. Typhi* can be rendered harmless and used in vaccines in order to prevent, rather than cause illness.

In new research, reported in the *Journal of Bacteriology*, lead author Katie Brenneman and her colleagues describe efforts to improve the effectiveness of a Recombinant Attenuated *Salmonella* Vaccine (RASV) by modifying its ability to survive the hostile environment of the stomach.

"Even though wild-type strains of *Salmonella* are quite capable of surviving the acidic environment of the stomach, it is surprisingly difficult to deliver a live *Salmonella* vaccine orally," Brenneman says. "Many vaccines have mutations that leave them especially vulnerable to low pH, which means a large proportion of the vaccine cells are killed before they reach the intestine and thus are unable to do their job of delivering vaccine targets to the immune system. We're trying to compensate for that increased acid sensitivity by increasing expression of the normal acid resistance systems."

The group demonstrated experimental strategies to restore acid resistance in several *Salmonella* vaccine strains, thereby improving their ability to survive low pH conditions in the stomach. The improved survival rate allows more of the bacterial cells to continue their infection sequence, colonizing intestinal tissues and generating a strong immune response.

Further, the acid resistant vaccine strains may behave more like unmodified *Salmonella*, which are cued by low pH conditions to prepare for the later stages of the infection process by up-regulating a key suite of genes involved in host interactions. These factors, the authors suggest, may significantly improve the effectiveness of *Salmonella* vaccines.

Co-authors of the study include Crystal Willingham, Wei Kong, Roy Curtiss III, and Kenneth L. Roland.

At the Biodesign Institute's Center for Infectious Diseases and Vaccinology at ASU, researchers have been harnessing *Salmonella*'s impressive ability to infiltrate human tissues and stimulate immune responses, producing *Salmonella*-based vaccines targeting a range of illnesses.

The Center is under the direction of Roy Curtiss III, whose team has been genetically modifying the pathogen in efforts to produce a new breed of safe, efficient and cost- effective vaccine.

Salmonella vaccines offer great potential in meeting growing needs for effective protection against existing and emergent threats. One such vaccine—designed by the Curtiss group and currently in Phase I FDA trials—targets infant pneumonia, a disease that continues to kill some 2 million people per year, many of them in the developing world. Other RASV's are in various stages of development.

Such vaccines are attractive for a number of reasons. They can typically be produced much more cheaply than conventional vaccines, they may be delivered orally rather than through injection and can confer long-term immunity without the requirement of a subsequent booster dose. Further, *Salmonella* powerfully stimulates both cellular and humoral immunity, producing a robust, systemic response in the vaccine recipient.

The basic idea behind RASVs is to genetically retool the *Salmonella* bacterium in such a way that it retains its strong, immunogenic properties without causing illness. It is then outfitted with antigens for the particular disease the vaccine is designed to protect against. This Trojan-horse method introduces the disease antigens hidden in the

Salmonella carrier, which then stimulate the immune responses.

But as the authors of the current study explain, the promising technique—potentially applicable for vaccines against virtually any pathogen—is not without its challenges. One of the most significant hurdles concerns the ability of *Salmonella* to survive the harsh environment of the stomach, where highly acidic (low pH) conditions prevail.

Naturally occurring, unmodified *Salmonella* have evolved sophisticated strategies of acid tolerance and acid resistance that allow them to survive the stomach environment. By contrast, modified *Salmonella* strains cultured in the laboratory are weakened or attenuated to improve their safety. The process has the negative effect of greatly reducing *Salmonella*'s acid tolerance.

A number of features allow normal *Salmonella* to survive low pH conditions. Two of the most important have been studied in some detail. The first is known as the acid tolerance response (ATR) and the second, the arginine decarboxylase acid resistance system. The latter of these mechanisms is not expressed in *Salmonella* cultured for vaccine use and the remaining ATR system is often insufficient to protect bacterial cells from stomach acid.

One approach to the problem has been to protect the vaccine strain from low pH conditions by shrouding it in an enteric capsule. Alternately, vaccines have been administered in conjunction with an antacid to lower stomach pH, (typically with sodium bicarbonate).

These strategies improve the survival of vaccine strains but have the disadvantage of depriving *Salmonella* of encountering the low pH environment, which acts to signal the bacteria that they have entered the host environment. These signals stimulate the upregulation of genes to

help *Salmonella* survive the next phase of infection in the intestine, where it is threatened by short-chained fatty acids, antimicrobial peptides and osmotic stress. Further, induction of normal acid tolerance response improves *Salmonella*'s ability to invade epithelial cells in the intestine.

In the current study, researchers attempted to restore acid tolerance in modified *Salmonella* at pH levels of 3 and 2.5 in order to overcome the loss of tolerance imposed by three common gene mutations used for RASVs. To accomplish this, a hybrid version of the arginine decarboxylase acid resistance system was created. This system was not only capable of inducing acid resistance in cultured *Salmonella* cells, but could be tightly controlled by means of a special promoter, triggered by the presence of the sugar rhamnose.

Use of the rhamnose promoter to induce acid resistance allows cultured cells to be prepared to withstand the low pH rigors of the stomach. Following the initial stages of infection, the bacterial cells expend their storehouse of rhamnose and their acid resistance is then rapidly downregulated.

Experiments confirmed that the rhamnose-regulated system could indeed rescue *Salmonella* from exposure to low pH conditions and that it provided bacterial cells with an equivalent degree of protection from acidic environments to the native arginine decarboxylase system.

The three acid unadapted mutants used in the study all showed significant improvement in survival at pH conditions of 3 and 2.5. The results open the door to the efficient and cost-effective creation of highly acid resistant vaccine strains that can exhibit fine-grained control under a rhamnose promoter and can be produced on demand. Allowing vaccine strain exposure to low gastric pH should further improve performance, by triggering virulence genes necessary for subsequent

survival and colonization in the intestine.

"In future studies, we will need to validate that this system, or other similar systems under construction, will improve the immunogenicity of an RASV," says co-author Ken Roland. "This work is ongoing, but I can tell you that we have preliminary data supporting the idea that our rhamnose-regulated arginine decarboxylase system can significantly enhance the immunogenicity and protective immunity of an RASV."

More information: jb.asm.org/content/195/13/3062.abstract

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