Study shows CRISPR effectiveness against colitis pathogen

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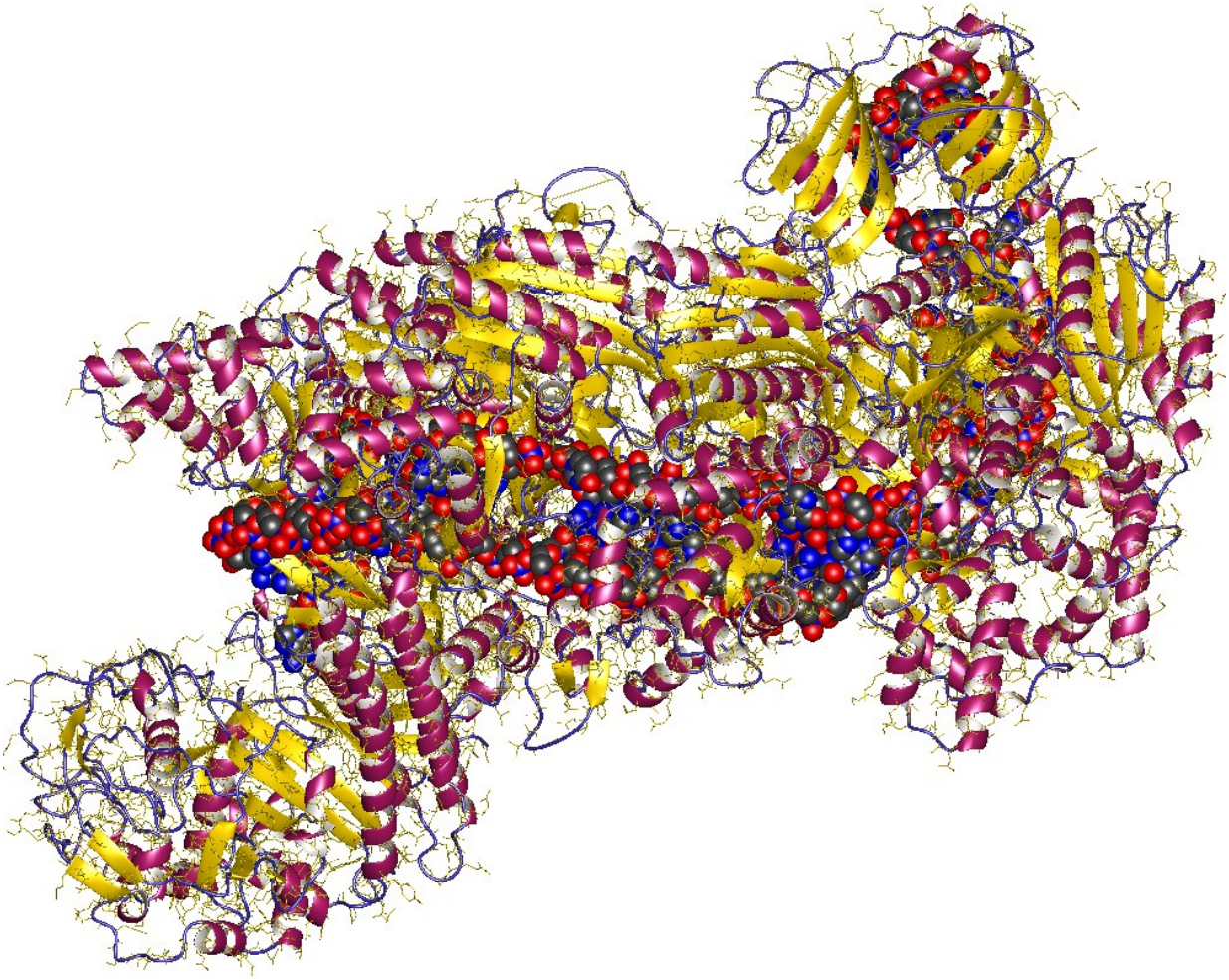

CRISPR (= Clustered Regularly Interspaced Short Palindromic Repeats) + DNA fragment, E.Coli. Credit: Mulepati, S., Bailey, S.; Astrojan/Wikipedia/ CC BY 3.0

Research at North Carolina State University shows that the CRISPR-Cas system can be used to effectively target and eliminate specific gut bacteria, in this case Clostridioides difficile, the pathogen that causes colitis—a chronic, degenerative disease of the colon.

In a proof-of-concept study published in the journal *mBio*, researchers were able to show pathogen reductions in experiments conducted both on the lab bench and in mice.

Microbiologists from two different NC State colleges teamed with NC State startup company Locus Biosciences to test the effectiveness of using a virus called a bacteriophage to carry a programmable CRISPR to specifically target and eliminate C. difficile bacteria, a search-and-destroy mission that holds promise for human gut health.

"We wanted to engineer phages with self-targeting CRISPR payloads and deliver them to the gut of an organism of choice—in this case a mouse—in order to have a beneficial impact on host health and to prevent disease," said Rodolphe Barrangou, the Todd R. Klaenhammer Distinguished Professor of Food, Bioprocessing and Nutrition Sciences at NC State and co-corresponding author of a paper describing the research.

Co-corresponding author Casey M. Theriot, an assistant professor of infectious disease at NC State, said that use—and overuse—of antibiotics increases susceptibility to C. difficile infection, as antibiotics wipe out both good and bad bacteria in the gut. Relapses occur in some 30% of human patients treated with a standard antibiotic to eliminate C. difficile.

"We need to target the precise pathogen without disturbing the rest of the microbiome, and that's what this approach does," she said.

CRISPR technologies have been used to precisely remove or cut and replace specific genetic code sequences in bacteria. The CRISPR method used in this study involved Cas3 proteins that acted like an arcade game Pac-Man, Barrangou said, chomping C. difficile bacteria and causing extensive DNA damage.

In the lab, the CRISPR-Cas systems effectively killed C. difficile bacteria. After that, the researchers tested this approach in mice infected with C. difficile. Two days after the CRISPR treatment, the mice showed reduced C. difficile levels, but those levels grew back two days later.

"C. difficile is really difficult to work with, hence its name," Theriot said.

"This was a positive first step in a long process," Barrangou said. "The results of using phages to deliver CRISPR payloads open up new avenues for other infectious diseases and beyond."

Next steps include retooling the phage to prevent C. difficile from returning after the initial effective killing. The researchers said that future work will also involve developing a library of different phages for various C. difficile strains.

More information: Kurt Selle et al, In Vivo Targeting of Clostridioides difficile Using Phage-Delivered CRISPR-Cas3 Antimicrobials, *mBio* (2020). DOI: 10.1128/mBio.00019-20

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