

Listeria protein provides a CRISPR 'kill switch'

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A single protein derived from a common strain of bacteria found in the soil will offer scientists a more precise way to edit RNA.

The protein, called AcrVIA1, can halt the CRISPR-Cas13 editing process, according to new research from Cornell, Rockefeller University and the Memorial Sloan Kettering Cancer Center published in the journal *Science* July 3.

"We're expanding our scientific toolbox to effectively use a CRISPR without causing side effects," said co-author Martin Wiedmann, Ph.D. '97, the Gellert Family Professor in Food Safety and director of Cornell's Food Safety Laboratory and Milk Quality Improvement Program. "Thanks to this bacterium, we're getting a chance to turn off and on our ability to make changes to RNA."

CRISPR, or the clustered regularly interspaced short palindromic repeats, is a laboratory mechanism that can act like microscopic scissors and precisely edit the genes contained in DNA. Among the half-dozen types of CRISPRs in use today, CRISPR-Cas13 can edit RNA, which until

now had lacked a brake in the editing process.

Since SARS-CoV-2, the coronavirus that causes the COVID-19 disease, is an RNA virus, this new editing accessory may be useful to coronavirus researchers, the scientists said.

Lead author Alex Meeske, postdoctoral researcher in the lab of senior author Luciano Marraffini, professor at Rockefeller University, had suspected that a protein (bacteriophage) housed in *Listeria* could be useful for RNA editing.

At the start of this study, Meeske reached out to Wiedmann, a [food safety](#) expert, to obtain genetic bacterial samples from his food pathogen collection. Doctoral candidate in the Wiedmann laboratory Jingqiu Liao narrowed down the prospects from about 1,500 bacterial candidates to 62 strains.

The Wiedmann lab transferred those samples to Rockefeller, where intern Alice Cassel sequenced the 62 strains and isolated 20 candidate proteins.

One strain stood out: *Listeria seeligeri*, a harmless bacteria found everywhere in the soil. Unlike its fierce cousin—the foodborne pathogen *L. monocytogenes*—it does not cause human disease.

The Rockefeller scientists found that the protein AcrVIA1—derived from *L. seeligeri*—instantly stopped the CRISPR editing process. "AcrVIA1 can be very useful in controlling application of Cas13. Anything that the Cas13 edits, this anti-CRISPR [protein](#) can shut off," Meeske said. "It's a 'kill switch' you can use during the CRISPR editing process, and it has become an additional tool we have at our disposal."

Wiedmann explained that scientists can now work on RNA problems with more exacting means. "This tool gives us more precision," he said.

More information: Alexander J. Meeske et al. A

phage-encoded anti-CRISPR enables complete evasion of type VI-A CRISPR-Cas immunity, *Science* (2020). DOI: [10.1126/science.abb6151](https://doi.org/10.1126/science.abb6151)

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