Glowing molecule can reveal live tuberculosis bacteria

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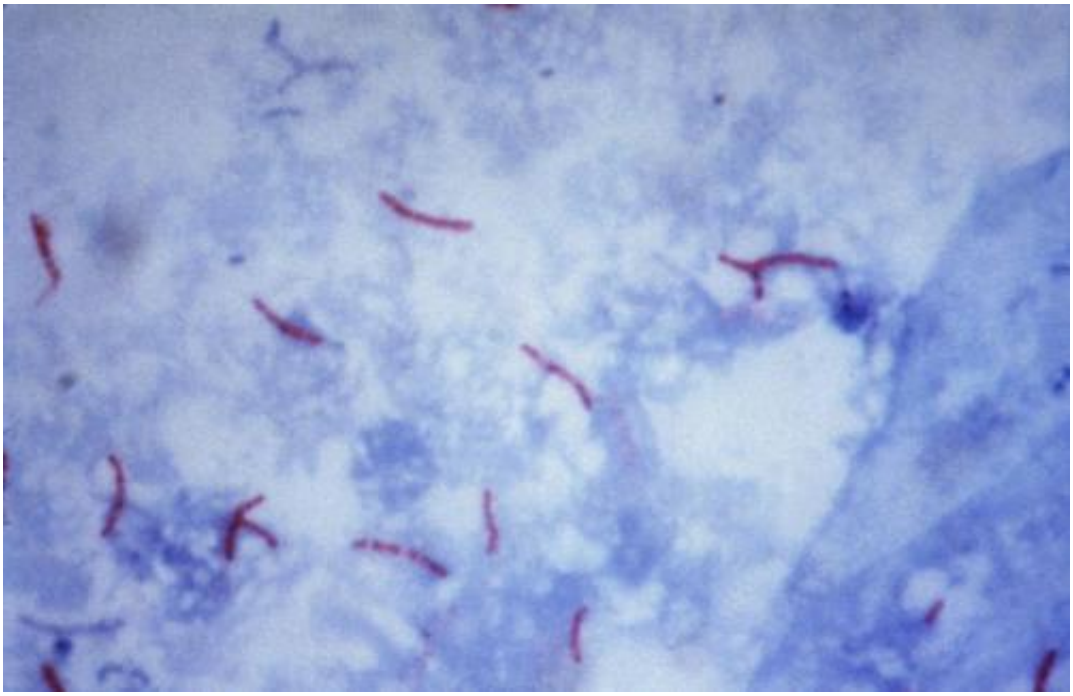

This photomicrograph reveals Mycobacterium tuberculosis bacteria using acid-fast Ziehl-Neelsen stain; Magnified 1000 X. The acid-fast stains depend on the ability of mycobacteria to retain dye when treated with mineral acid or an acid-alcohol solution such as the Ziehl-Neelsen, or the Kinyoun stains that are carbolfuchsin methods specific for M. tuberculosis. Credit: public domain

A molecule made in the lab may change the way tuberculosis is diagnosed in the field.

Chemically tweaking a sugar molecule known as trehalose lets it slip inside the bacteria that causes tuberculosis (TB) and glow. The method offers a quick, simple way to detect the pernicious bug, and may help counter TB, a deadly lung infection that's particularly common in developing countries.

Howard Hughes Medical Institute (HHMI) Investigator Carolyn Bertozzi and colleagues report the work February 28, 2018, in the journal *Science Translational Medicine*.

Despite its devastating toll on health, the bacteria behind TB, *Mycobacterium tuberculosis*, can be hard to spot. Current tests rely on chemical stains that have been around for decades and can be finicky. Estimates put the sensitivity of these stains anywhere from 32 percent to 94 percent.

Better detection methods are sorely needed to combat TB, which killed more than 1.7 million people worldwide in 2016, says Bertozzi, of Stanford University. "If you can't even get an accurate diagnosis, how do you treat people?"

As a chemist, Bertozzi, along with her colleagues, studies the molecules that make up bacterial cell walls. Early discoveries by her lab revealed that some bacteria use sugar molecules called trehalose as building blocks. Bertozzi found the cell walls of *M. tuberculosis* particularly compelling. "There's some really interesting biology there." She began working with a team of scientists who held personal stakes in tuberculosis research. After a chance encounter at a meeting at Janelia Research Campus, Bertozzi decided to collaborate with study coauthor Professor Bavesh Kana of the University of Witwatersrand in Johannesburg, South Africa.

The researchers realized that trehalose molecules - those cell wall

building blocks - might offer a way to flag living *M. tuberculosis* cells. But first, the team needed to find a chemical beacon that would make the flag visible. One chemical, called DMN, seemed to fit the bill. DMN can glow under certain wavelengths of light - but only when it is out of water. Because the *M. tuberculosis* cell wall contains a membrane that's a "thick layer of grease," Bertozzi says, it's the perfect place for DMN to light up.

That insight - that DMN was "off" until a cell tucks it into its membrane - was key, Bertozzi says. "It's such a simple thing, but simple things like that make all the difference between something that can be deployed or not."

After linking trehalose to DMN in the lab, the researchers tested their hybrid molecule, called DMN-Tre, on an *M. tuberculosis* relative. Just as they had hoped, the bacteria grabbed the molecule and, within minutes, incorporated it into their cell membranes, where it began to glow under a fluorescent microscope.

In tests on sputum samples from 16 people with TB, DMN-Tre picked up *M. tuberculosis* cells in all of the samples. The new technique performed similarly to the standard - but more complex and time-consuming - labeling method based on the Auramine O stain, a dye that sticks to acids in bacterial cell walls.

Other tests showed that DMN-Tre is selective to Actinobacteria, the bacterial phylum that includes *M. tuberculosis*. Human cells and other types of bacteria, both of which are plentiful in sputum samples, don't incorporate the molecule, the researchers found.

Unlike existing TB detection methods, DMN-Tre can also distinguish cells that are metabolically active from those that are not. Because the molecule relies on bacteria to actively incorporate it into the membrane,

only healthy cells are labeled, whereas cells that are compromised by drug treatment do not label as well. That property may allow clinicians to monitor how well treatments are working in people, and perhaps even test whether certain mixtures of drugs would work against specific strains of *M. tuberculosis*.

More work remains before the molecule is ready for use in the field, Bertozzi says. But she's optimistic that the new method could prove useful in the global fight against TB.

More information: M. Kamariza el al., "Rapid detection of Mycobacterium tuberculosis in sputum with a solvatochromic trehalose probe," *Science Translational Medicine* (2018). stm.sciencemag.org/lookup/doi/ … scitranslmed.aam6310

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