Legionella bacteria's escape route revealed

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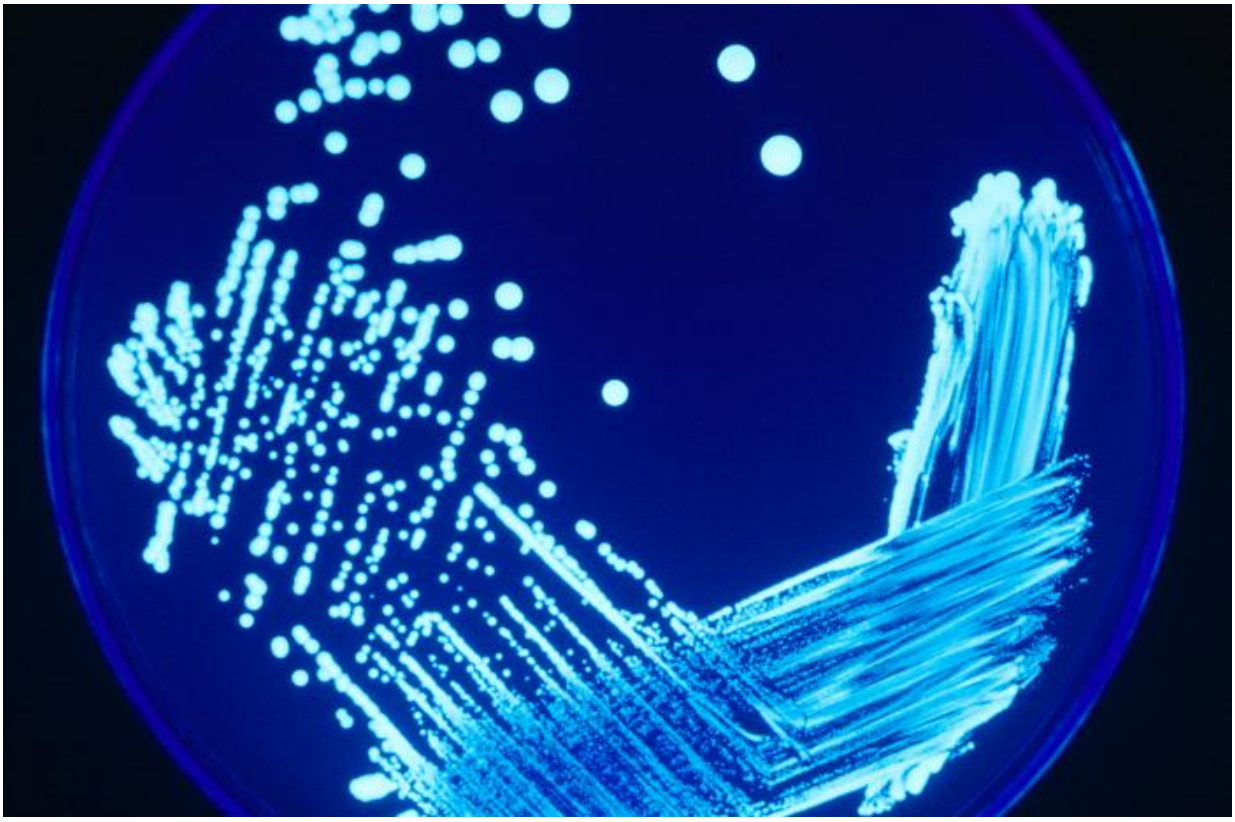

Legionella sp. colonies growing on an agar plate and illuminated using ultraviolet light to increase contrast. Obtained from the CDC Public Health Image Library. Credit: CDC/James Gathany (PHIL #: 7925)

The precise mechanism used by Legionella bacteria to escape the body's defences has been unpicked in intricate detail and is described for the first time in the journal *eLife*.

The study reveals a potential new therapeutic approach to tackle infection by Legionella pneumophila, which is a common cause of community and hospital-acquired pneumonia and causes death in almost a third of cases.

One of the ways the body rids itself of infection is to gobble up bacteria or viruses within its cells - a process called autophagy. But particularly dangerous bacteria, such as Legionella, have evolved ways to evade this process, allowing them to survive in host cells. Legionella does this by producing a molecule called RavZ to disrupt the autophagy machinery, but until now it was not known exactly how RavZ achieves this effect.

"We set out to understand the molecular mechanism by which Legionella evades host autophagy, specifically by establishing how RavZ breaks apart a key molecule in the autophagy process called LC3-PE," explains senior author Dr. Yaowen Wu, Group Leader at the Chemical Genomics Centre of the Max Planck Society in Germany.

LC3-PE is a crucial molecule for one of the main events during autophagy - the creation of a membrane-bound 'sac' that engulfs bacteria or other debris, so that the cell can get rid of it. Dr. Wu and his colleagues show that the RavZ molecule uses a 'tweezer' and 'scissor' process to first extract LC3-PE from the cell membrane, and then break it into its two components, preventing autophagy.

Analysing interactions between molecules such as RavZ and LC3-PE is extremely challenging because it is hard to isolate LC3-PE in its natural form and impossible to change the structure of LC3-PE using a traditional biochemical approach. To tackle this, the team produced 'semisynthetic' versions of the LC3 molecule which allowed them to retain important natural features while altering specific components, so that they could study the interaction with RavZ in intricate detail.

Using these state-of-the-art chemical tools, they first looked at how the RavZ molecule finds and latches onto the LC3-PE molecule. Knowing that other molecules that bind to LC3 bear a special motif, they looked at the RavZ molecule to see if this motif was also present. They found that RavZ bears three of these motifs, but uses only one of them to specifically home in on the LC3 portion of its target molecule.

Having pinpointed how RavZ recognises LC3, they looked at whether they could work out how it binds to the LC3-PE molecule. By studying the physical structure of RavZ, they identified the binding site that it uses to latch onto the LC3 portion, as well as evidence that it changes its shape to enclose the LC3 molecule within. Once securely bound, RavZ pulls the entire LC3-PE molecule out of the host cell membrane before cleaving it into two pieces. This means it can no longer be useful to the host cell and frees up RavZ to seek out and destroy the next LC3-PE molecule, ensuring autophagy cannot occur.

Having established this mechanism, the team found they could block it by using a peptide that prevents RavZ from recognising and binding to LC3, highlighting a promising avenue for developing drugs against Legionella.

"Legionella bacteria have evolved a very smart and efficient mechanism during evolution to avoid being eaten by our cells," says Dr. Wu. "It will be interesting to see whether this 'tweezer' extraction model is used by other pathogens, such as Shigella and Yersinia bacteria, which both produce substances that disrupt the trafficking of important molecules within host cells. We hope that understanding these mechanisms will be beneficial for the development of new drugs against infection by Legionella."

More information: Aimin Yang et al, Elucidation of the anti-autophagy mechanism of theeffector RavZ using semisynthetic LC3

proteins, *eLife* (2017). DOI: 10.7554/eLife.23905

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