Amoeba builds barriers for protection against bacteria

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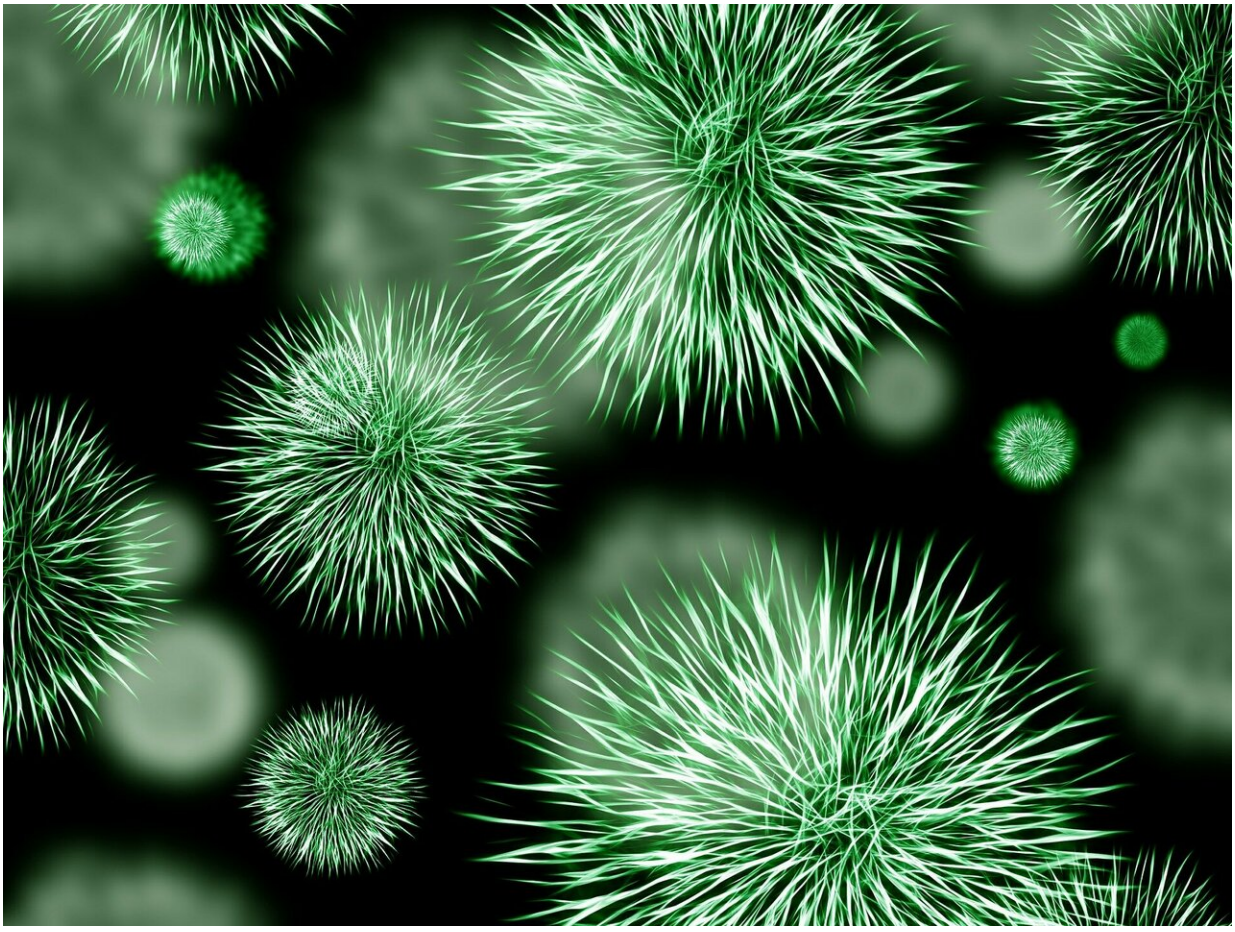

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In some respects, animals and amoebae are not that different. For

instance, both are at risk of potentially deadly attacks by bacteria and have evolved ways to prevent them. Researchers at Baylor College of Medicine report in the journal *Science Advances* that *Dictyostelium discoideum*, the soil-dwelling single-celled amoeba that feeds on bacteria, builds a barrier around its colonies that counteracts bacterial attempts to penetrate them, facilitates amoebal feeding and protects them from oxidative stress.

"We were surprised to discover that, when exposed to some types of Gram-negative bacteria such as *Klebsiella pneumoniae*, but not Gram-positive bacteria, *D. discoideum* secretes into its surroundings large amounts of CadA, a protein until now known only as a cell adhesion molecule that contributes to the amoeba's development," said corresponding author Dr. Adam Kuspa, professor of biochemistry and molecular biology and senior vice president and dean of research at Baylor. "We analyzed CadA and determined that it is a lectin, a molecule that binds to carbohydrates, and mixing CadA with *K. pneumoniae* resulted in clumps of bacteria."

Kuspa and his colleagues then investigated the effect of the lack of CadA on the amoeba's ability to form colonies or plaques on a film of bacteria, typical laboratory conditions to study amoebae. They deleted the CadA gene and found that only 20 percent of the amoebae survived and formed plaques when set to grow on a film of *K. pneumoniae*. But the same CadA-deficient amoebae grew the same as amoebae with CadA when set to grow on Gram-positive bacteria.

"It was totally unexpected that secreted CadA was important for amoebal colony formation on some type of bacteria, but not other," said first author Dr. Timothy Farinholt, who was a graduate student in the Kuspa lab when he was working on this project. "When we added exogenous CadA to CadA-deficient amoeba, the amoeba formed threefold more colonies than when CadA was not available."

Altogether, the experiments suggested that CadA is important for the amoeba to go through the initial stages leading to plaque formation and that once the plaque reaches certain size, CadA is no longer needed to keep the amoebae alive on a bacterial film.

But, how was CadA helping *D. discoideum* survive on a sea of life-threatening *K. pneumoniae*?

A series of microscopic examinations showed a sharp border between the amoebal colony and the surrounding bacteria, suggesting that CadA is forming a barrier that prevents live bacteria from entering the amoebal plaque. As CadA binds to *K. pneumoniae* and agglutinates or clumps the bacteria, the amoebae are attracted toward these bacterial clumps and feed on them at the edges to the plaque. Additional experiments showed that when CadA is present, the amoebae actively feeding at the plaque edges have lower levels of oxidative stress than when CadA is absent.

"Amoeba living in soil are constantly exposed to vast numbers of heterogeneous groups of bacteria and CadA and other lectins we and others have so far identified enable amoebae to recognize specific species and adapt to survive," Kuspa said.

More information: "Social amoebae establish a protective interface with their bacterial associates by lectin agglutination," *Science Advances* (2019). advances.sciencemag.org/content/5/7/eaav4367

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