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Research shows the success of a bacterial community depends on its shape

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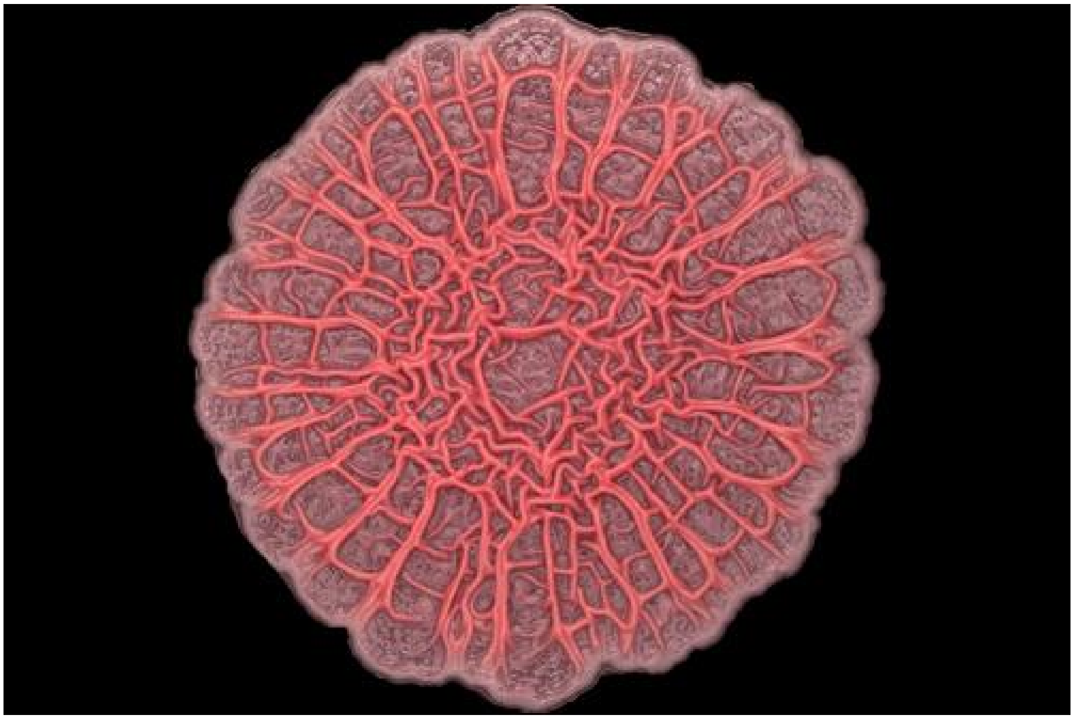

A mutant strain of P. aeruginosa forms a hyper-wrinkled bacterial colony with prominent spokes. Credit: Yu-cheng Lin/Columbia university

For some microbes, the motto for growth is not so much "every cell for itself," but rather, "all for one and one for all."

MIT researchers have found that cells in a bacterial colony grow in a way that benefits the community as a whole. That is, while an individual cell may divide in the presence of plentiful resources to benefit itself, when a cell is a member of a larger colony, it may choose instead to grow in a more cooperative fashion, increasing an entire colony's chance of survival.

"Once cells make that decision to live as a communal set of organisms, what other things do they have to start doing to make living as good as possible?" asks Chris Kempes, who did much of the work as a graduate student in MIT's Department of Earth, Atmospheric and Planetary Sciences. "Once you enter into that intimate cooperation with neighboring cells to benefit the group, it starts to hint at becoming complex, multicellular life."

Kempes and his colleagues explored the dynamics of communal growth in the bacterial species Pseudomonas aeruginosa, a common strain found in soil and water that can cause infection in humans—particularly in cystic fibrosis, in which thick bacterial biofilms spread through the lungs, making it difficult to breathe.

The team paired laboratory experiments with theoretical modeling to show that as a colony takes shape, it not only grows outward, but also upward, forming a complex pattern of ridges, or wrinkles. This wrinkling, the researchers hypothesized, acts to increase the surface area with which a colony can take in oxygen and grow.

Through simulations, the group could predict the pattern of wrinkles based on certain environmental and genetic factors, such as the amount of oxygen available. In particular, the team observed that as wrinkles form, at a certain point, the cells involved continue to grow in height, but not in width. Wrinkles grow upward once they've reached a certain width—a width, it turns out, that is optimal for the success of the colony as a whole.

"This starts to hint at some really interesting cooperative dynamics going on," says Kempes, who is now a postdoc at NASA's Ames Research Center and a principal investigator at the SETI Institute. "This really paints a picture of how this community geometry responds to a variety of factors and finds what's best for the growth of the entire community."

Kempes and co-authors Lars Dietrich, Chinweike Okegbe, and Zwoisaint Mears-Clarke of Columbia University and Mick Follows of MIT have published their results this week in the *Proceedings of the National Academy of Sciences*.

A wrinkle through time

In experiments, Dietrich, Okegbe, and Mears-Clarke grew bacterial cells on solidified agar, finding that initially, cells clumped together to form a smooth disk that continued to grow outward across the growth medium. After a while, the disk also started to grow upward, forming wrinkles in a radial pattern as each wrinkle expanded in both height and width. But after a certain point, wrinkles stopped growing in width, though they continued growing taller. Curiously, every wrinkle in the colony stopped growing after reaching the same width.

Thinking that oxygen may play a role in regulating colony growth, Kempes developed a mathematical model to simulate internal availability of oxygen in the colony. The model accurately predicted the distribution of oxygen within a community, compared with the group's experimental results.

Kempes next varied the width of wrinkles in the model to see how the growth rate of the entire colony responded, finding that with a given amount of oxygen, there exists an optimal width to which wrinkles will grow in order to maximize a colony's survival; any wider or narrower, and the entire community grows less quickly.

Back in the lab, the team then looked at a colony's growth pattern under different conditions of oxygen availability. Oxygen is about 21 percent of Earth's atmosphere. The group grew samples of bacteria in a chamber with 15, 21, and 40 percent oxygen. In each scenario, wrinkles adapted accordingly, growing narrower with less oxygen, and wider with more.

"It turns out the morphology of the colony can be clearly related to something that might do with the fitness of the colony," Follows says. "It's very beautiful to see that in a simple way."

Dietrich adds, "In principle this could apply for most bacteria and even organisms from other domains of life, including microbial eukaryotes."

Growing into genes

The researchers also found that there was a genetic component that may regulate a colony's shape. In their experiments, they used a mutant strain of P. aeruginosa that lacks phenazines— chemicals that mimic the effect of oxygen, effectively helping a cell to breathe. As they wanted to observe how a colony's growth responds to oxygen, the researchers chose to work mostly with the mutant strain to avoid confusing their results.

But out of curiosity, the team performed similar growth experiments with the bacteria's wild strain, which produces phenazines and therefore has access to both oxygen and phenazines as substrates. As the wild colony formed, wrinkles grew wider compared with the mutant strain, suggesting that more oxygen-like resources encourage such growth. A second mathematical model simulating the wild strain, developed by Kempes, showed similar results.

Joao Xavier, a computational biologist at Memorial Sloan-Kettering Cancer Center, says understanding the spatial patterning of bacteria has important implications in the study of certain diseases.

"There is a growing literature on how bacterial populations can be used as model systems to understand other populations, such as cancer [cells]," says Xavier, who was not involved in the research. "This paper makes an important contribution because it shows that simple metabolic processes can explain spatial structure that looks quite complex."

Kempes says the model may be useful in determining how a colony grows in the presence of certain chemicals or drugs, such as those that target cystic fibrosis.

"You could imagine searching for a chemical that disrupts a cell's ability to figure out the optimal geometry, so they would die," says Kempes, adding that while it may be difficult to limit a colony's oxygen intake without also starving the lung, the group's model and experiments "open up a playground for starting to think about testing different drugs."

More information: Morphological optimization for access to dual oxidants in biofilms, www.pnas.org/cgi/doi/10.1073/pnas.1315521110

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