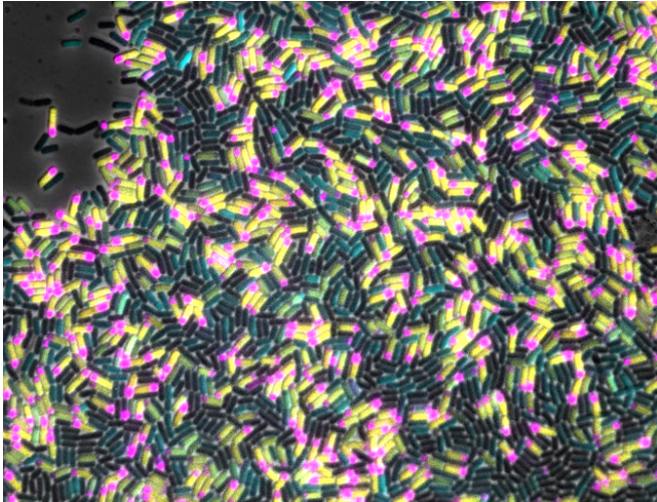


Study decodes genetic circuitry for bacterial spore formation

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A mixed population of starving *Bacillus subtilis* cells includes both nonsporulating cells (dark blue) and cells that have begun spore formation by dividing asymmetrically into large (yellow) and small (pink) chambers. Credit: M. Fujita/UH

A team led by Rice University bioengineering researchers has decoded the mechanism that some bacteria use to make life-or-death decisions during extremely tough times.

Deciphering how bacteria respond to stress could yield new clues for combating food spoilage and for controlling food-borne pathogens. The new study was published in *Molecular Systems Biology* and sheds light on a long-standing debate about one of the field's fundamental questions: What causes stressed-out bacteria to make the drastic move to cease normal functions and form spores?

"What people in our field have long wondered is, How do spore-forming bacteria like *Bacillus* make this decision?" said study co-author Oleg Igoshin, associate professor of bioengineering at Rice and a senior investigator at Rice's Center for

Theoretical Biological Physics (CTBP). "Is there a specific biochemical trigger that activates one of the network proteins or is sporulation more of a general physiological response?"

To form a hard-shelled spore, which can survive for years without food, the organism must pour its energy into sporulation. Becoming a spore too soon can lead to death by competition—from neighbors that keep multiplying—but delaying the decision can lead to death by starvation before the spore is complete.

"It's a high-stakes decision, which suggests that the decision mechanism has come about through intense evolutionary pressure," Igoshin said. "It's also possible that organisms have adopted this same mechanism to make other critical decisions."

B. subtilis is a common soil bacteria and a well-known survivor. It isn't harmful to humans and is even used as a probiotic in some traditional foods. It is so good at forming spores that it's the model organism of choice for biologists who study sporulation.

Almost a decade ago, Igoshin, a computational biologist, began studying the regulatory genes that *B. subtilis* uses to make sporulation decisions. He and members of his lab interpret the work of experimental collaborators and develop computer simulations to decipher the workings of the regulatory network, such as the switches, feedback loops and signal amplifiers, that *B. subtilis* uses to make its decision.

In 2012 Igoshin and graduate student Jatin Narula showed how the [regulatory network](#) employs a series of nested "feed-forward" loops to filter signal noise, and in 2015 they revealed the network's timing mechanism, a circuit that uses the organism's clock-like DNA replication cycle.

In the new study, which builds upon the 2015 work,

Narula, Igoshin and collaborators used their computer model to show how a general physiological cue—the slowdown of cellular growth—can trigger *B. subtilis*' sporulation decisions. Provided by Rice University
Igoshin said the sporulation network is very sensitive to the concentration of a key protein that the cell produces at an essentially constant rate. During starvation, when the cell's growth rate slows, the concentration of this protein builds up, and the bacteria are more likely to form spores. The theoretical work at Rice was experimentally tested in the lab of co-author Gürol Süel of the University of California at San Diego.

Experiments performed by two graduate students in Süel's lab, Anna Kuchina and Fang Zhang, confirmed the main model prediction: Only cells that slow down their growth beyond a threshold value proceed to sporulation. The experimental data indicated that the amount of sporulation network proteins—but not the activity of the proteins—was modulated by cell growth, a finding that contradicts the theory that there is a specific biochemical trigger for sporulation.

Igoshin said the finding has important implications for food safety and general microbiology.

"Sporulation by some of the close relatives of *B. subtilis* is a big hassle for the food-preservation industry because many of those spores can survive boiling temperatures," Igoshin said. "To kill those spores, you need to apply both heat and high pressure. So people have been looking for other methods to inhibit sporulation. If sporulation was triggered by a specific molecule, then perhaps a drug could be found to block that molecule, but our research suggests that sporulation is a general physiological response and that food safety engineers will need to look for other methods of control.

"Moreover, there is a good chance that this mechanism controls key decisions in other bacterial species," he said. "It ties to very basic bacterial physiology, and as a result, I think it may be universal."

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