

Thinking ahead: Bacteria anticipate coming changes in their environment

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A new study by Princeton University researchers shows for the first time that bacteria don't just react to changes in their surroundings -- they anticipate and prepare for them. The findings, reported in the June 6 issue of *Science*, challenge the prevailing notion that only organisms with complex nervous systems have this ability.

"What we have found is the first evidence that bacteria can use sensed cues from their environment to infer future events," said Saeed Tavazoie, an associate professor of molecular biology, who conducted the study along with graduate student Ilias Tagkopoulos and postdoctoral researcher Yir-Chung Liu.

The research team, which included biologists and engineers, used lab experiments to demonstrate this phenomenon in common bacteria. They also turned to computer simulations to explain how a microbe species' internal network of genes and proteins could evolve over time to produce such complex behavior.

"The two lines of investigation came together nicely to show how simple biochemical networks can perform sophisticated computational tasks," said Tavazoie.

In addition to shedding light on deep questions in biology, the findings could have many practical implications. They could help scientists understand how bacteria mutate to develop resistance to antibiotics. They also may help in developing specialized bacteria to perform useful

tasks such as cleaning up environmental contamination.

In one part of the study, the researchers studied the behavior of *E. coli*, the ubiquitous bacterium that travels back and forth between the environment and the gut of warm-blooded vertebrates. They wanted to explain a long-standing question about the bug: How do its genes respond to the temperature and oxygen changes that occur when the bacterium enters the gut?

The conventional answer is that it reacts to the change -- after sensing it -- by switching from aerobic (oxygen) to anaerobic (oxygen-less) respiration. If this were true, however, the organism would be at a disadvantage during the time it needed to make the switch. "This kind of reflexive response would not be optimal," Tavazoie said.

The researchers proposed a better strategy for the bug. During *E. coli*'s life cycle, oxygen level is not the only thing that changes -- it also experiences a sharp rise in temperature when it enters an animal's mouth. Could this sudden warmth cue the bacterium to prepare itself for the subsequent lack of oxygen?

To test this idea, the researchers exposed a population of *E. coli* to different temperatures and oxygen changes, and measured the gene responses in each case. The results were striking: An increase in temperature had nearly the same effect on the bacterium's genes as a decrease in oxygen level. Indeed, upon transition to a higher temperature, many of the genes essential for aerobic respiration were practically turned off.

To prove that this is not just genetic coincidence, the researchers then grew the bacteria in a biologically flipped environment where oxygen levels rose following an increase in temperature. Remarkably, within a few hundred generations the bugs partially adapted to this new regime,

and no longer turned off the genes for aerobic respiration when the temperature rose.

"This reprogramming clearly indicates that shutting down aerobic respiration following a temperature increase is not essential to *E. coli*'s survival," said Tavazoie. "On the contrary, it appears that the bacterium has "learned" this response by associating specific temperatures with specific oxygen levels over the course of its evolution."

Lacking a brain or even a primitive nervous system, how is a single-celled bacterium able to pull off this feat? While higher animals can learn new behavior within a single lifetime, bacterial learning takes place over many generations and on an evolutionary time scale, Tavazoie explained. To gain a deeper understanding of this phenomenon, his team developed a virtual microbial ecosystem, called "Evolution in Variable Environment." Each microbe in this novel computational framework is represented as a network of interacting genes and proteins. An evolving population of these virtual bugs competes for limited resources within a changing environment, mimicking the behavior of bacteria in the real world.

To implement this framework, the researchers had to deal with the sheer scale and complexity of simulating any realistic biological system. They had to keep track of hundreds of genes, proteins and other biological factors in the microbial population, and observe them as they varied over millions of time points. "Simulations at this scale and complexity would have been impossible in the past," said Tagkopoulos. Even with the vast number crunching power the supercomputers provided by the University's computational science and engineering support group, their experiments took nearly 18 months to run, said Tagkopoulos.

In this virtual world, microbes are more likely to survive if they conserve energy by mostly turning off the biological processes that allow them to

eat. The challenge they face then is to anticipate the arrival of food and turn up their metabolism just in time. To help them along, the researchers gave the bugs cues before feeding them, but the cues had to appear in just the right pattern to indicate that food was on its way.

"To predict mealtimes accurately, the microbes would have to solve logic problems," said Tagkopoulos, a fifth-year graduate student in electrical engineering and the principal architect of the Evolution in Variable Environment framework.

And sure enough, after a few thousand generations, an ecologically fit strain of microbe emerged which did exactly that. This happened for every pattern of cues that the researchers tried. The feeding response of these gastronomically savvy bugs peaked just when food was offered, said Tagkopoulos.

When the researchers examined a number of fit virtual bugs, they could at first make little sense out of them. "Their biochemical networks were filled with seemingly unnecessary components," said Tagkopoulos. "That is not how an engineer would design logic-solving networks." Pared down to their essential elements, however, the networks revealed a simple and elegant structure. The researchers could now trace the different sequences of gene and protein interactions organisms used in order to respond to cues and anticipate mealtimes. "It gave us insights into how simple organisms such as bacteria can process information from the environment to anticipate future events," said Tagkopoulos.

The researchers said that their findings open up many exciting avenues of research. They are planning to use similar methods to study how bacteria exchange genes with one another (horizontal gene transfer), how tissues and organs develop (morphogenesis), how viral infections spread and other core problems in biology.

"What is really exciting about our discovery is that it brings together and establishes deep connections between the traditionally separate fields of microbial ecology, network evolution and behavior," said Tavazoie.

Source: Princeton University

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