Researchers discover how microbes survive in freezing conditions

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Most microbial researchers grow their cells in petri-dishes to study how they respond to stress and damaging conditions. But, with the support of funding from NASA, researchers in LSU's Department of Biological Sciences tried something almost unheard of: studying microbial survival in ice to understand how microorganisms could survive in ancient permafrost, or perhaps even buried in ice on Mars.

Brent Christner, associate professor of biological sciences, and colleagues at LSU including postdoctoral researcher Markus Dieser and Mary Lou Applewhite Professor John Battista, recently had results on DNA repair in ice-entrapped microbes accepted in the journal of *Applied and Environmental Microbiology*. To understand how microbes survive in frozen conditions, Christner and colleagues focused on analysis of DNA, the hereditary molecule that encodes the genetic instructions used in the development and function of all organisms.

"Microbes are made up of macromolecules that, even if frozen, are subject to decay," Christner said. "We know of a range of spontaneous reactions that result in damage to DNA."

The worst kind of damage is known as a double-stranded break, where the microbe's DNA is cleaved into two separate pieces that need to be put back together to make the chromosome functional.

"This kind of damage is inevitable if cells exist frozen in permafrost for thousands of years and cannot make repairs," Christner said. "Imagine that a microbe is in ice for extended periods of time and its DNA is progressively getting cut into pieces. There will eventually be a point when the microbe's DNA becomes so damaged that it's no longer a viable informational storage molecule. What is left is a corpse."

The situation would seem dire for the longevity of microbes in ice. But curiously, researchers have been able to revive microbes buried in ice and permafrost for hundreds of thousands to millions of years. In fact, Christner managed to revive several different types of bacteria from near the bottom of the Guliya ice cap on the Qinghan-Tibetan plateau in Western China – ice that is 750,000 years old, from long before the age of humans.

But how is it possible for microbes to counter expectations and survive for such long periods when frozen? The survival of microorganisms in ancient glacial ice and permafrost has typically been ascribed to their ability to persist in a dormant, metabolically inert state. But even this explanation does not account for the background levels of ionizing radiation that cause damage to these microbes' DNA, frozen at the bottom of a glacier or not.

"In order to survive that long, different studies for instance point towards dormancy, or 'slow motion metabolism,' but regardless of the physiological state, without active DNA repair an organism will accumulate DNA damage to an extent that will lead to cell death," Dieser said.

Results from Christner and colleagues' recent paper point to another explanation: mechanisms that repair DNA can operate even under freezing conditions. In laboratory experiments, Christner and colleagues took frozen suspensions of bacteria native to Siberian permafrost and exposed them to a dose of DNA-damaging ionizing radiation equivalent to what the microbes would have experienced during ~225,000 years buried in permafrost. The researchers then let the microbes incubate at low temperature (5oF) for a period of two years, periodically checking the integrity of the microbes' DNA.

As expected, ionizing radiation damaged the circular microbial chromosome, transforming it into a slurry of smaller pieces. What surprised the researchers was that, over the course of two years in the freezer, the pieces of DNA began to come back together in their proper order.

"This isn't a random process," Christner said. "This tells us that the cells are repairing their DNA. This is important because we don't typically think of these as being conditions under which complex biological processes are going on."

Christner said that these findings make it reasonable to speculate that if life ever evolved on Mars and microbes are still frozen somewhere in the subsurface, those microbes might still be viable if given the right conditions.

"It just keeps looking better for conditions of habitability on Mars," Christner said. "This is relevant in an astrobiological sense because if these DNA repair mechanisms operate in Earth's cryosphere, extraterrestrial microbes might be using this survival mechanism to persist on other icy worlds in the solar system. We are very excited about these results."

More information: aem.asm.org/content/early/2013 … 845-13.full.pdf+html

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