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New research discovers surprising activity among organisms thriving in extremely deep, hot subseafloor

January 25 2022

Sediment samples from the deep, hot subseafloor biosphere were collected during the IODP 370 expedition onboard the Japanese scientific drilling ship. Credit: JAMSTEC

Since the discovery of the deep subseafloor biosphere in the mid-1990s, scientists have studied the conditions under which organisms thrive in this isolated and generally food-deprived environment and wondered which conditions set a limit to the existence of life. In 2016, a group of international scientists set out to sea on board the Japanese scientific drillship, Chikyu, to study the temperature limit of the deep subseafloor biosphere. Sediment samples were collected from a drill hole that cut through the geological subduction zone of the Nankai Trough off Japan.

At this site, temperature increases steeply with depth to reach 120 °C, a temperature suggested close to the limit for life, at 1200 meters beneath the seafloor. To their surprise, the scientists found a very small, but very active microbial community thriving under these deep and hot conditions.

The scientists determined the number of cells in the sediment and measured their metabolic rates by highly sensitive radiotracer measurements of methane production and sulfate reduction. They discovered that the metabolic rates per cell were extraordinarily high for the deep biosphere. New findings, published on Jan. 25 in the journal *Nature Communications*, about the samples collected in 2016 are shedding light on the survival strategies of organisms living in this harsh environment.

"We propose that the organisms are forced to maintain a high metabolic turnover, which approaches the activity of microbes living in surface sediments and in laboratory cultures, to provide the energy required to repair thermal cell damage," said Felix Beulig from the University of Bayreuth, who is the lead author of the study. "The energy required to repair thermal damage to cellular components increases steeply with temperature, and most of this energy is likely necessary to counteract the continuous alteration of amino acids and loss of protein function," said the study leader, Tina Treude, UCLA professor of marine geomicrobiology.

It is far from trivial to detect microbial metabolic activity in sediments with less than 500 cells per cubic centimeter sediment, which is seven orders of magnitude lower than in the average surface sediment. "We worked under extremely controlled, sterile conditions and performed a large number of control experiments simultaneously with the sample incubations," said Florian Schubert from the German Research Centre for Geosciences, who conducted these analyses as part of his Ph.D. studies. "We even incubated sediment sterilized with high gamma radiation, as well as drill fluid from the drill hole, to detect any potential non-biological reactions or contamination-induced microbial activity," said Jens Kallmeyer, who is the mentor of Florian Schubert.

Because the metabolic rate determinations were conducted under laboratory conditions, some uncertainty remained whether the microbes would show the same metabolic activity in their natural environment. The scientists therefore compared the measured metabolic sulfate reduction rates to the calculated depletion time of dissolved sulfate in the deep sediment. "Given that we are comparing two very different methodological approaches that act on time scales of days versus millions of years, the agreement between the experimental rate determination and the calculated depletion time is gratifyingly good," said Arthur Spivack from the University of Rhode Island.

The high per-cell activity of sulfate reducers and methanogens in the deepest and hottest sediment is apparently fueled by hydrogen and acetate from the sediment porewater. "Acetate, which is a small organic molecule that is also present in vinegar, is of particular interest as a potential food source," said Verena Heuer from MARUM in Germany, who was the co-chief scientist of the expedition. "Acetate reaches concentrations of more than 10 mmol per liter in the sediment porewater, which is exceptionally high for marine sediments."

For Bo Barker Jørgensen from Aarhus University, who is one of the pioneers of deep biosphere research, the detection of high cell-specific rates in the deep biosphere is a fascinating discovery. "We always found that microbes in the deep biosphere are an extremely sluggish community that slowly nibbles on the last remains of million-year-old, buried organic matter. But the deep biosphere is full of surprises. To find life thriving with high metabolic rates at these high temperatures in the deep seabed nourishes our imagination of how life could evolve or survive in similar environments on planetary bodies beyond Earth."

Fumio Inagaki and Yuki Morono from JAMSTEC in Japan were the other two co-chief scientists of the expedition and responsible for the detection of cells in the sediment. When asked what they thought about the fact that the expedition did not detect the upper temperature limit of the deep biosphere, both said, "We have to go back and drill deeper. The ultimate limits of the biosphere inside the Earth remain unknown. As shown by this project, the boundary resides somewhere in the oceanic crust beneath the sediments. It will be explored in the future through scientific ocean drilling."

More information: Tina Treude, Rapid metabolism fosters microbial survival in the deep, hot subseafloor biosphere, *Nature Communications* (2022). DOI: 10.1038/s41467-021-27802-7. www.nature.com/articles/s41467-021-27802-7

Provided by University of California, Los Angeles