Bacterial evolution in ancient sub-seafloor sediments

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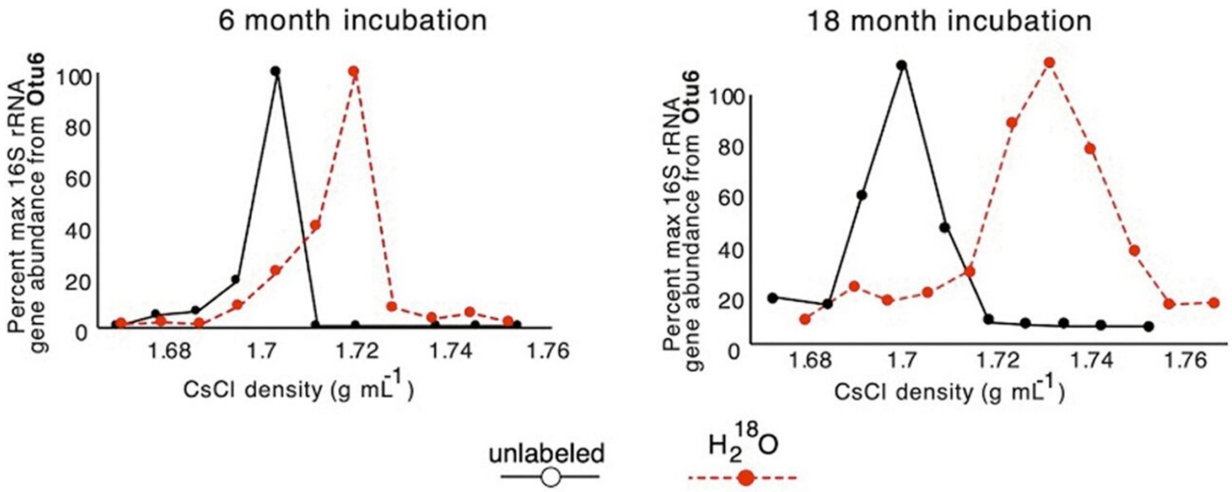
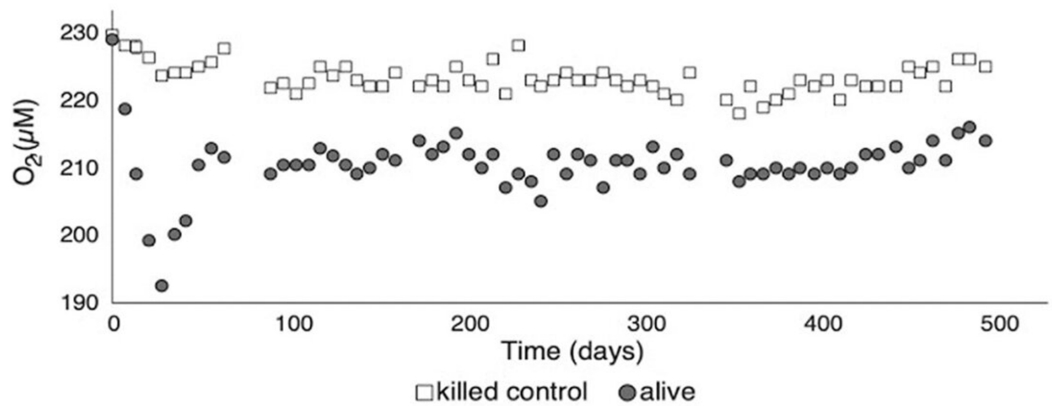

6 month incubation
18 month incubation
Percent max 16S rRNA gene abundance from Otu6
CsCl density (g mL^{-1})
unlabeled
$H_2^{18}O$


O_2 (μM)
Time (days)
killed control
alive

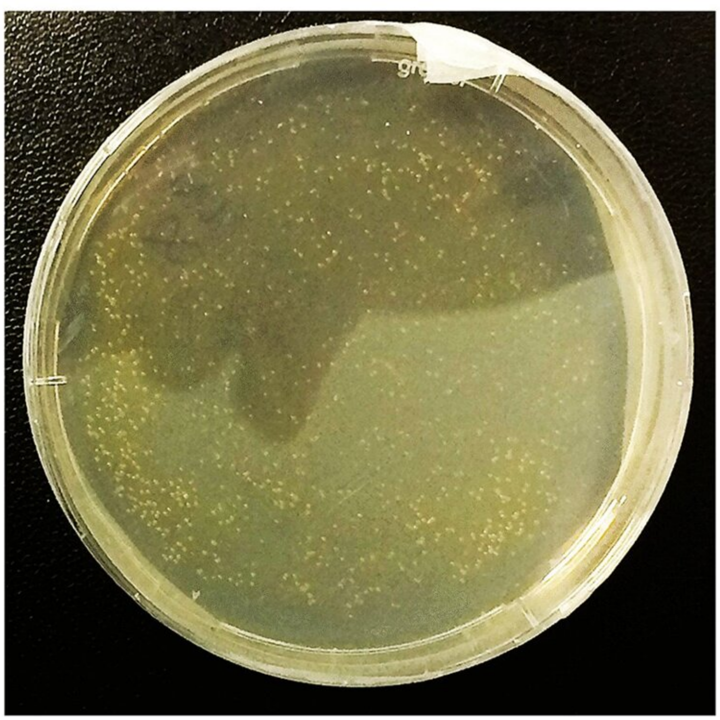


Figure 1 (Top) ^{18}O labeling of 16S rRNA genes from Thalassospira OTU_6 after 6 and 18 months of incubation with ^{18}O-labeled water from the 3-mbsf sediment (17). OTU_6 was one of >30 OTUs that were identified as ^{18}O labeled and actively growing in the incubations (17). (Middle) Oxygen concentration over time in the 18-month slurry from the 3-mbsf incubation (filled circles) and slurries containing labeled water and autoclaved sediment (killed control). The O_2 consumption rate was 0.04 μmol O_2 liter−1 day−1. (Bottom) Cultivation of colony-forming bacteria on solid medium after the 18-month incubation of sediment slurries in sterile ^{18}O-labeled artificial seawater. No bacterial colonies formed on petri dishes that were inoculated with the killed-control slurries. A total of 21 colonies were picked for genome sequencing, all of which were affiliated with the genus Thalassospira. Credit: DOI: 10.1128/mBio.01150-21

Micro-organisms persisting deep below the seafloor for millions of years continue to evolve despite living at the energy limit to life.

On the surface of the Earth where nutrients are in ample supply, bacteria proliferate and evolve at an accelerated rate compared to plants and animals. Cells divide rapidly, and random mutations accumulate at a correspondingly high rate. Since many of these genetic changes alter the properties of the cells that carry them, their effects soon become fixed features of the genome. Bacteria also make use of various modes of genetic exchange, which enables recombination between diverse genomes. This process serves to mitigate the effects of deleterious mutations, while allowing favorable mutations that confer beneficial traits to be propagated. In addition, environmental factors such as the presence of antibiotics exert selective pressures that further contribute to bacterial evolution.

The type of genetic exchange and mode of evolution outlined above is common for bacteria in terrestrial habitats like soil and in the oceans. But in the deep sea, bacteria can also be found at depths of more than 10 m below the seafloor where they persist under extreme energy limitation, close to the energy limit to life, in sediments that more than one million years old. In these settings nearly all the nutrients are consumed at the seafloor surface leaving extremely low food supply for the bacteria that persist deep below the seafloor. Can cells in such an extreme and ancient setting continue to divide and evolve?

"Some scientists have posed the hypothesis that the rates of cellular division are too slow and evolution cannot take place under such conditions," says Professor William Orsi, geomicrobiologist in the Paleontology & Geobiology Section of the Department of Earth and Environmental Sciences at Ludwig-Maximilians-Universitaet (LMU) in Munich, and a member of the GeoBio Center. The rationale for this assessment is based on the fact that the extreme energy limitation that bacterial populations face in ancient sediments several meters below the seafloor results in very low rates of metabolism, and should therefore bacteria will divide very slowly—or possibly not at all. Under such conditions, the slow rate of growth and the speed at which new traits can spread through populations may simply be too slow to allow evolution to happen.

The theory seems plausible. But a new study shows that in fact evolution does indeed take place under these rather peculiar environmental conditions, as an LMU team led by Orsi, in collaboration with researchers at the University of Arizona and colleagues at the LMU have now shown. The study was conceived more than five years ago, when William Orsi and Gert Wörheide(who holds the Chair of Paleontology and Geobiology at LMU) conceived an idea to test the hypothesis that evolution is actually possible deep below the surface of the Earth. After Orsi had isolated bacteria belonging to the genus Thalassospira from samples of sub-seafloor sediments that were several millions of years old, they went on to analyze the genomes of the bacteria. They discovered that the genomes were characterized by the presence of a relatively high proportion of 'pseudogenes." Pseudogenes are the descendants of formerly functional genes that have acquired so many mutations over the course of time that they are effectively inactivated.

This finding implies that these bacteria have a tendency to accumulate deleterious mutations, which can ultimately lead to loss of the original functions of these genes. Moreover, in collaboration with colleagues at the University of Montreal, the LMU team discovered that the subseafloor Thalassospira bacteria genomes exhibited ten times lower levels of genetic recombination compared to surface world bacteria. The lack of recombination from neighboring bacteria resulted in newly acquired errors becoming fixed in the genomes of the subseafloor bacteria and can be passed on when the cells divided. Physical isolation from the surface world therefore resulted in genetic isolation. In the long term, these genetically isolated subseafloor organisms become established as idiosyncratic subtypes of their respective species that were buried in the sediments millions of years ago.

"A similar phenomenon has been observed in endosymbiotic bacteria, which live within the cells of other species," says Orsi. Unlike free-living species, endosymbionts are genetically isolated, and little or no recombination takes place, so random mutations can pile up, giving rise to what is known as 'genetic drift." Purifying selection cannot take place, and genes are gradually inactivated, altered beyond recognition and ultimately lost. "The fact that we have found clear indications for comparable evolutionary mechanisms in bacteria recovered from sub-seafloor sediments suggests that such evolutionary processes occur in free-living bacteria far more often than we have hitherto assumed," Orsi concludes.

More information: William D. Orsi et al, Genome Evolution in Bacteria Isolated from Million-Year-Old Subseafloor Sediment, *mBio* (2021). DOI: 10.1128/mBio.01150-21

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