https://doi.org/10.1038/s41564-025-02048-x

Defining ultra-slow-growing extremophilic microorganisms as aeonophiles

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In environments like the deep subsurface, microorganisms with long doubling times can remain metabolically active for millions of years — we propose referring to this class of extremophile as aeonophiles.

Time, for microorganisms, is usually measured in minutes or hours. The fastest-growing bacteria can double every ten minutes or less, whereas species whose minimum doubling time is around 24 hours are considered extremely slow growers. However, this range of growth rates represents a tiny fraction of those that exist on Earth[1] (Fig. 1). Here, we suggest that microbial strains that grow much more slowly, meaning doubling times of weeks to tens of years or longer, constitute a distinct class of extremophiles. Deep subsurface environments, in particular, require ultra-slow growth owing to extremely low energy delivery, with resulting cellular lifespans of potentially up to millions of years. Although this lifestyle is best characterized in Earth's subsurface, it may exist in other places as well[2]. The ability to grow extremely slowly may or may not be an inherited trait, but it is certainly a dominant mode of life for a large number of microorganisms and is therefore an important but often overlooked focus of microbiology research. Naming ultra-slow-metabolizing organisms will facilitate their study and hopefully spark new discoveries by making them easier to refer to and to perform text searches for across diverse research domains. We suggest referring to these slow-growing microorganisms as aeonophiles (aeono, meaning a very long period of time, and phile, meaning love).

Earth's subsurface, the sediments and rocks that underlie all oceans and continents, contains an enormous biosphere, with an estimated 10^{30} cells[3]. Remarkably, individual cells in the subsurface persist in a living, metabolically active state for more than 100 million years[4] while dividing on timescales of decades or longer, or not at all. Instead, they seem to primarily turn over their biomass without dividing, suggesting a fundamentally different mode of microbial life than the cell-division-focused growth that is familiar from exponentially growing cells in the lab.

The first line of evidence for a near or total absence of cell division in the subsurface comes from comparing the so-called minimum maintenance power for microbial life to the power available from chemical reactions that fuel cellular metabolism. Maintenance power is the energy delivery rate necessary to keep a cell in a living but non-dividing state. In a maintenance state, cells just repair broken DNA, proteins, lipids and carbohydrates, but do not undergo mitosis to make a new daughter cell. Geochemical models of marine sediments show that the microbial consumption of substrates in these environments is exceptionally slow, measuring in at $<1.9 \times 10^{-19}$ watts per cell[5]. This is just 1% of the lowest maintenance power ever measured in the natural environment (measured for *Chlorobium* sp. under extreme low-light limitation) and 0.001% of the lowest maintenance power for heterotrophic laboratory cultures grown under energy-limiting conditions. The lowest maintenance power ever recorded for *Escherichia coli* was a whopping 10,000 times faster, approximately 2×10^{-14} watts per cell in an energy-starved chemostat. Lab-first approaches are clearly not yet suited to probing the limits or physiologies of aeonophiles.

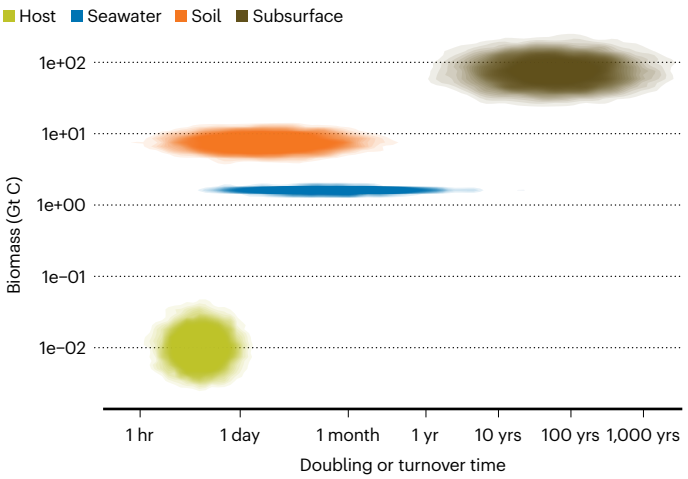

Fig. 1 | Global estimates of microbial biomass in biomes where organisms have different turnover times. Global estimates of the amount of microbial biomass (measured in gigatonnes of carbon) in animal and plant hosts, seawater, soil or subsurface environments plotted against a selection of published doubling or turnover times for the microorganisms in these environments. Data sources and calculations are listed at https://github.com/adsteen/aeonophiles.

Second, cells in marine sediments show almost no accumulation of genetic mutations over thousands of years, implying that they have undergone little to no cell divisions during that time period[6]. One extraordinary result suggests a near absence of cell division in deep continental aquifers: nearly genomically identical organisms were found in deep aquifers on three continents that have been separated since the breakup of Pangea more than a hundred million years ago[7]. This extreme slow growth over geological timescales sets aeonophiles apart from seasonally dormant organisms, as discussed in more detail below.

Third, direct evidence that these communities are not replicating on more typical microbiological timescales comes from the rate of consumption of isotopically labelled substrates into new biomass in deep marine subsurface communities. Such work suggests that each cell takes up 0.001 to 0.1 femtograms of carbon per day[8]. With 19–31 femtograms of carbon per cell in marine sediments, this results in half-a-year to eighty-four years for each cell to turn over its biomass

without replicating, agreeing with the predictions made by reaction transport models and thermodynamics[1]. These studies provide direct support for extremely slow growth of aeonophiles, rather than just relying on inferences from the availability of energy in the environment or accumulated genetic changes.

Nevertheless, there is ample evidence that these slow-metabolizing cells are alive in the subsurface. All living organisms primarily synthesize amino acids with the L chiral form, maintaining a higher ratio of L to D forms in living cells. In dead cells, the concentration of D- and L-amino acids will eventually become equal over timescales of tens of thousands of years due to abiotic racemization reactions. However, subsurface organic matter contains a greater ratio of L to D amino acids than would be expected based on abiotic reactions, implying ongoing synthesis of new L amino acids[1]. mRNA transcripts, which decay on a timescale of minutes, and would be absent in long-dead cells, are detectable in many subsurface environments[9], showing ongoing production of these short-lived molecules. Enzymes decay more slowly than mRNA, but their lifetimes are measured in days or (perhaps) years, so the presence of active enzymes in the subsurface is further evidence of ongoing metabolic activity. Finally, the cells themselves are intact, maintaining their ribosomes and taking up substrate in sediments up to 101.5 million years old[4]. Based on this evidence, aeonophiles appear to be the dominant organisms in Earth's subsurface.

It is likely that aeonophiles differ in important and fundamental ways from fast-growing microorganisms. They are distinct from dormant cells, as the latter are organisms that normally grow fast but persist in a metabolically inactive state during relatively short-term nutrient limitations or environmental stresses. Because subsurface conditions provide extremely low energy, aeonophiles must remain in a slow-growing condition for thousands or perhaps millions of years. They are also distinct from persister cells, which grow slowly within a population that includes genetically identical fast growers. We consider aeonophiles, on the other hand, to be species in which all cells are obligated to grow slowly for many years or longer. Grouping aeonophiles, which belong to a wide range of phyla, into a functional class of extremophiles will facilitate study of the physiology and ecology of slow life, in the same way grouping specialists that are adapted to extreme heat (thermophiles), cold (psychrophiles), acid (acidophiles), high pH (alkaliphiles), pressure (barophiles and piezophiles) and salt (halophiles) has been useful. Rather than extreme chemical or physical conditions, however, aeonophiles are specialists at using extreme timescales.

If the evolutionary success of a class of organisms can be measured by their total biomass, aeonophiles are tremendously successful. Current estimates indicate that the marine subsurface contains 2.9×10^{29} living cells, plus another $2–6 \times 10^{29}$ in the continental subsurface[3]. This is four orders of magnitude more than the total number of cells in the collected host-associated microbiomes (for example, animal guts and so on) of the world[3]. In addition, the organisms that seem to be aeonophiles tend to belong to specific taxonomic groups such as Atribacterota, Bathyarchaeota and Promethearchaeota (formerly Asgardarchaeota) that are not commonly found in environments that promote fast growth, suggesting adaptation of the whole clade to aeonophilic conditions[10].

By definition, extremeophily confers a fitness advantage under extreme environmental conditions. Usually, this advantage is understood to mean an increased growth rate. However, growth rate is an inappropriate proxy for fitness when the fitness is conferred by extremely slow growth. Evolutionary success in aeonophiles is instead determined by who dies the slowest, rather than who grows the fastest.

There is no evidence that faster-growing 'typical' organisms such as *E. coli* persist in aeonophilic conditions, as they are not found in the deep subsurface, suggesting that these traits are only present in certain organisms. In agreement with this, certain aeonophilic adaptations have been observed in subsurface communities. Subsurface microorganisms produce extracellular enzymes adapted to degrading the types of organic matter found in deeper sediments[11]. Metabolic and transcriptomic evidence supports the prevalence of DNA repair and expression of trehalose, a compound that can increase lifespan by preventing aggregation of partially degraded proteins[9]. The few cultures that exist of aeonophilic taxa are extremely slow growing by the standards of other pure cultures, with doubling times of weeks to months under ideal conditions (for example, see ref. 12). Slow growth under ideal conditions suggests physiological trade-offs that facilitate surviving long time periods with no growth.

For such traits to be adaptive, they need to have been established through natural selection, which requires producing progeny. Natural selection for these organisms is possible if they replicate in shallow sediments or other environments where resources are more abundant[13]. Then, the individuals that have traits enabling survival in a non-growth state during burial will be the first to replicate if they return to the surface via geological events such as mud volcanoes or submarine mud slides[14], which would also provide mechanisms for dispersal of organisms with the adaptive traits. An exact evolutionary mechanism has yet to be worked out, and we hope that being able to refer to the trait as aeonophily will facilitate its development.

We suggest that aeonophiles are defined as organisms that persist in a maintenance state (metabolically active but not undergoing appreciable cell division) for years or longer. Such organisms can have minimum doubling times of weeks to months or longer under ideal conditions, such as ANME-1, Bathyarchaeia, *Atribacter* sp. and *Promethearcheum* sp. Some aeonophiles, such as these listed taxa, can be coaxed to grow on human timescales with specialized laboratory techniques but are too slow for facile experimental manipulation. In nature, aeonophiles persist in a low- or non-growth state with cell biomass turnover times of much longer than the lifespan of a human researcher, possibly allowing them to survive for up to millions of years or longer. It is possible that some of these environmental aeonophiles are incapable of ever growing quickly enough for their cell division to be observed in a laboratory. Aeonophiles would be evolutionarily adapted to wait in a metabolically active state for years, thousands of years or even longer until conditions improve enough to allow cell division, resulting in cellular lifespans that span geological timescales and events.

Here, we have focused on aeonophiles in Earth's marine and terrestrial subsurface as these environments are stable over extremely long timescales. Virtually all ecosystems contain both dormant microorganisms and a long tail of rare but ecologically relevant taxa. The presence of relatively slow-growing microorganisms in high-energy environments (for example, *Mycobacterium tuberculosis* in the human lung) suggests that ultra-slow-growing aeonophiles might also be present in low abundance in high-energy ecosystems on Earth's surface.

Even if aeonophiles are limited to subsurface environments, they represent a large diversity of life on Earth and contribute to important biogeochemical cycles such as those that maintain free oxygen in the atmosphere and affect the redox state of the planet[15]. Many are independent from surface inputs and comprise deep branches on the tree of life that co-occur with the origin of the eukaryotes[12]. By dint of their extremely slow lifestyles, they are likely to have traits that are distinct from those of laboratory model organisms that could enable fundamental biological

discoveries, perhaps with ultra-stable biomolecules useful for biomedical or industrial applications. Therefore, whether these traits are found to be evolutionarily adaptive or not, the study of aeonophiles is likely to yield benefits for humanity. By naming aeonophiles, we hope to encourage and facilitate study of these important microorganisms and their contributions to the coevolution of life and Earth.

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Published online: 20 June 2025

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Acknowledgements

The authors thank D. Giovannelli, D. E. Larowe, K. A. Kormas and J. Arft for helping develop the term aeonophile. Funding was provided by NSF grants EAR-2121670 and OCE-2151015 (to K.G.L.) and NSF-OCE 2145434 (to A.D.S.).

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Microbiology* thanks John Spear and Alfred Spormann for their contribution to the peer review of this work.