





## Sparking a candle in the dark

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## ABSTRACT

Cyanobacteria have always lived on solar power... yet we found them living in the Earth's crust, 600 meters below the surface. Our results suggest that they have repurposed their metabolic machinery to burn hydrogen for energy.



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More than two billion years ago, a group of microorganisms called <u>cyanobacteria</u> invented oxygenic photosynthesis, the process that allows light, carbon dioxide, and water into chemical energy and oxygen. Cyanobacteria are the plants of the microbial world – in fact, plants can perform photosynthesis because they tamed free-living cyanobacteria early in their evolutive history. Ever since they appeared, cyanobacteria have become the most successful group of microorganisms on our planet, having colonized almost every habitat, from the ocean to the aridest deserts. They'll survive anywhere – even in space – as long as they get the light they need. But what if there is no light at all?

Enter the deep subsurface. When we think about life, we usually focus on the ecosystems that we find in our daily lives: the soil, the forests, the oceans. But beneath them, there lies a vast underground that is far from being inhabited: where we see just a bunch of solid rocks, microorganisms see a lot of tiny cracks waiting to be colonized. In fact, by some accounts, the deep terrestrial biosphere harbors a significant, if not the largest, part of the total microbial biomass on Earth. This biomass is however mostly unexplored, since getting science-worthy samples from the deep subsurface is expensive and difficult.

We performed a 613-meter deep drilling in the <u>Iberian Pyrite Belt</u> (southwestern Spain), a region that is considered by NASA as a Mars analogue due



to its unique geological characteristics. We collected samples from different depths and searched for life using a combination of techniques, including fluorescence microscopy, DNA sequencing, and an antibody-based life detector chip. Strikingly, cyanobacteria appeared to be the dominant organisms at several of the samples. These cyanobacteria were absent from our drilling and laboratory controls, confirming that they were not contaminants, and their presence was confirmed by fluorescence microscopy using specific probes against cyanobacterial ribosomes. Ribosomes are the factories used by cells to synthesize proteins, and they quickly degrade upon cellular death. Thus, the positive signals found in our samples strongly suggested that those cyanobacteria were alive and well.

So what could they be doing hundreds of meters away from the nearest source of light? We were able to isolate and sequence microbial DNA from two of the cyanobacteria-rich samples, located at 420 and 607 meters deep respectively. The genome of an organism encodes all the proteins that it can synthesize: that is to say, the set of "abilities" of that organism. So we looked at the cyanobacterial genomes in our samples and searched for functions that could be useful in the dark, anoxic conditions of the deep subsurface. We found hydrogenases enzymes used to draw energy from hydrogen - in both samples. Interestingly, we also found out that, in our samples, cyanobacterial abundance was negatively correlated with hydrogen concentrations. Could cvanobacteria be burning subsurface hydrogen to survive?

Cyanobacteria are indeed well acquainted to hydrogen, as it is a byproduct of some of their metabolic pathways, such as nitrogen fixation. On the other hand, cyanobacteria are able to switch from photosynthesis (solar power) to respiration (burning fuel) – after all, they can't photosynthesize at night. In order to perform respiration, you need to draw electrons from a donor (the fuel) to an acceptor (the oxygen), releasing energy in the process. But there is little oxygen in the deep subsurface. So even if the cyanobacteria down there can use hydrogen as fuel, they still need something to burn it with. The solution might come from a safety valve present in some cyanobacteria - including the ones in our samples – which, paradoxically, is primarily used to protect them against an excess of light. Too much light would overcharge their membranes with electrons, so they redirect those electrons outside the cell - to metals or other extracellular compounds. Deep subsurface cyanobacteria would actually face a similar problem – having no oxygen, their membranes would slowly become charged with hydrogen electrons – so they can benefit from the same solution.

Just as McGyver saved the day by improvising surprisingly complex devices out of ordinary materials, evolution often repurposes old traits in order to solve new problems. Our findings not only extend the already large known ecological range of cyanobacteria. It also reminds us, yet again, that once biology has appeared, it will adapt to survive even in the most adverse conditions. Places that look barren to us – be it on this or other planets – may, in fact, be thriving with life. Who can tell what we might find in the future?