

Methanosphaerula subterranea EG compromises cell defense systems in exchange for stable energy metabolism pathways under high CO₂ conditions

Microbial adaptation to high CO₂, especially through evolution at the genomic level, can provide insights into the metabolism and survival strategies that may have been employed by the primitive life forms on early, CO₂-rich Earth. From the natural high CO₂ subsurface environment in Hartousov, Eger rift area, Czech Republic, we isolated an active methanogen strain, *Methanosphaerula subterranea* EG. Both *M. subterranea* EG and its sister species, *M. palustris* E1-9c, share a similar lacustrine origin but have since adapted to contrasting environments (ambient vs. high-CO₂) and therefore provide an ideal system for exploring genomic adaptation to high-CO₂ conditions. Pan-genomics analysis of *Methanosphaerula* and their closest relative, the uncultivated genus UBA288, reveals a significant genome reduction on the cell defense systems against viruses. Specifically, strain EG contains only one set of type IV restriction-modification system that relies on a standalone m5C specific restriction enzyme, a single set of type I-E CRISPR system, and limited toxin-antitoxin system, even though virus communities are as influential in high CO₂, as in low-CO₂ environments. By comparison, most of the reference genome and metagenome-assembled genomes (MAGs) possess more than one set of related systems. In contrast, energy metabolism pathways are much more conserved, as the pathway completeness involving energy harvesting and carbon fixation across the analyzed genomes and MAGs show far fewer differences. The persistence of the high CO₂-adapted strain EG in maintaining its methanogenesis and acetyl CoA pathway intact, while compromising its other functional systems, supports the hypothesis that these pathways represent the most ancient biological processes.

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