Reconstructed microbial metabolic networks in the deep terrestrial subsurface

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While microbial biomass is present in deep subsurface strata, there is little known about the metabolic activity in those environments. Opalinus Clay rock is a low porosity rock whose mineralogy and physical characteristics have been extensively studied. The lack of pore space inhibits microbial activity, even though organic carbon sources (i.e., acetate, fossilized carbon) and electron acceptors (i.e., sulfate) are available. Another likely source of energy and electrons in this system is H_2, known to be produced by the weathering of ferric mineral species, which are present in this rock.

When porosity alone is increased (by drilling a borehole into which porewater flows), microbial activity is detectable through the accumulation of sulfide. Based on a metagenomic analysis, the corresponding microbial community comprises two main organisms representing 85% of the biomass: a *Pseudomonas* sp. that breaks down and ferments organic macromolecules and a Peptococcaceae that oxidizes these compounds to CO_2 and reduces sulfate.

When H_2 is added, the microbial activity increases along with the complexity of the community. Metaproteomic analysis highlighted two autotrophic organisms oxidizing H_2: a sulfate-reducing Desulfobulbaceae representing 42% of community, and a Rhodospirillaceae reducing Fe(III). They actively release acetate and other organic molecules, feeding heterotrophic sulfate-reducing bacteria, which oxidize these compounds to CO_2. A *Hyphomonas* sp. is the only organism able to utilize organic macromolecules, oxidizing them to CO_2 by transferring the electrons to Fe(III). No fermenting organism could be detected .

This work provides unique insights into deep subsurface microbiology and significantly expands our knowledge of microbial metabolism on these environments.