Large sulfur oxidizing bacteria of the Thiovulaceae (Campylobacterota) thriving in the sulfidic groundwater of Movile Cave, in Romania

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Abstract

Life in Movile Cave (Romania) relies entirely on carbon fixation by bacteria oxidizing sulfide, methane and ammonia, using oxygen, nitrate, sulfate, and ferric iron as electron acceptors. There, our attention was drawn by a white veil-like structure at the water surface. Microscopic analysis of the veil revealed a dense population of microorganisms of various shapes and sizes, some of which being much larger than the other spirilla, rods and cocci that could be observed. We studied these larger microorganisms with optical and electron microscopy techniques, sequenced their genome and analyzed the main physiological abilities. Their shape is spherical to ovoid, 12-16 µm in diameter, and their cytoplasm is rich in intracellular sulfur globules. They are present in densities of up to 5.5×10^3 cells/ml and they are very motile. These cells were identified as *Thiovulum* sp. (Campylobacterota), forming a separated cluster from marine *Thiovulum* sp., consisting mostly of cave bacteria. The *Thiovulum* microhabitat is located at the water surface in the lower, partially submerged, level of Movile Cave. Here, H_2S is brought in by diffusion and

convection, and some water flow is only present deep below the water surface. Frequent attachment is observed between cells, which is consistent with other reports of *Thiovulum* sp. being clustered in dense, veil like, aggregates attached to polysaccharide matrices. Nevertheless, the Movile Cave strain occurs in the water and is not attached to any rocky surface. The Movile Cave *Thiovulum* genome is small at 1.72 Mbp, contains 1804 coding sequences, 3 rRNA operons, and has a GC content of 28%. The genome suggested that the Movile Cave Thiovulum strain can switch between aerobic and anaerobic sulfide oxidation using O_2 and NO_3^- as electron acceptors, respectively. In the latter case, NO_3^- is likely reduced by *Thiovulum* to NH_3 via dissimilatory nitrate reduction, thereby contributing to the complete nitrogen cycle in this environment. Additionally, coupling the genomic analysis, with new electron microscopy images, we suggest that in absence of motor-like structures along the membrane, the short peritrichous filamentous structures, typical to *Thiovulum*, are pili, likely of type IV, for which genes were found in all 6 available *Thiovulum* genomes. These pili may play a role in veil formation, connecting adjacent cells and support the exceptionally fast swimming behavior of these bacteria.

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