

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

Traian Brad^{‡,§}, Mina Bizic^l, Danny Ionescu^l, Lucian Barbu-Tudoran^{¶,#}, Joost Aerts[□], Radu Popa[«], Luca Zoccarato[»], Jessica Ody[^], Jean-François Flot[∧], Scott Tighe[?], Daniel Vellone[?], Serban Sarbu^{‡,‡,‡}

‡ Emil Racoviță Institute of Speleology, Cluj-Napoca, Romania

§ Institutul Român de Știință și Tehnologie, Cluj-Napoca, Romania

l Leibniz Institute for Freshwater Ecology and Inland Fisheries, IGB, Berlin, Germany

¶ Electron Microscopy Center "Prof. C. Craciun", Faculty of Biology and Geology, "Babes-Bolyai" University, Cluj-Napoca, Romania

Electron Microscopy Integrated Laboratory, National Institute for R&D of Isotopic and Molecular Technologies, Cluj-Napoca, Romania

□ Department of Molecular Cell Physiology, Faculty of Earth and Life Sciences, Free University Amsterdam, Amsterdam, Netherlands

« River Road Research, Buffalo, United States of America

» Institute of Computational Biology, Department of Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

^ Royal Museum for Central Africa, Brussels, Belgium

∧ Evolutionary Biology and Ecology, Université libre de Bruxelles (ULB), Brussels, Belgium

∧ Interuniversity Institute of Bioinformatics in Brussels – (IB), Brussels, Belgium

? Vermont Integrative Genomics Lab, University of Vermont Cancer Center, Health Science Research Facility, Vermont, United States of America

‡ Emil Racoviță Institute of Speleology, Bucharest, Romania

‡ Department of Biological Sciences, California State University, Chico, United States of America

Corresponding author: Traian Brad (traian99@gmail.com), Mina Bizic (mina.bizic@igb-berlin.de), Danny Ionescu (danny.ionescu@igb-berlin.de)

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 O₂ and NO₃⁻ as electron acceptors, respectively. In the latter case, NO₃⁻ is likely reduced by *Thiovulum* to NH₃ 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.

Presenting author

Traian Brad

Funding program

Project number PN-III-P4-IDPCCF-2016-0016, within PNCDI III

Grant title

Food chains in the dark: diversity and evolutionary processes in caves (DARKFOOD)

Conflicts of interest