

The microbial diversity of the Su Bentu cave, Italy and the influence of human exploration. S. Leuko¹, K. Koskinen², I. D'Angeli³, L. Sanna⁴, J. De Waele³, P. Marcia⁵, C. Moissl-Eichinger², and P. Rettberg¹, ¹⁾ German Aerospace Center (DLR e.V.), Institute of Aerospace Medicine, Radiation Biology Department, Linder Höhe, 51147 Cologne, Germany; ²⁾Medical University Graz, Section of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, 8036 Graz, Austria; ³⁾ Italian Institute of Speleology, University of Bologna, Via Zamboni 67 - 40127 Bologna, Italy; ⁴⁾ Institute for Biometeorology, National Research Council of Italy, Traversa La Crucca 3, I-07100 Sassari, Italy; ⁵⁾Dipartimento di Scienze della Natura e del Territorio, Università di Sassari, Via Muroni 25, I-07100, Sassari, Italy. Email presenting author: stefan.leuko@dlr.de

Introduction: The microbial diversity in the Su Bentu Cave (Sardinia, Italy) was investigated by means of Illumina MiSeq analysis. The hypogean environment is of great interest for astrobiological research as cave conditions may resemble those in extra-terrestrial regions. Furthermore, they hold high potential to identify novel, extremely adapted organisms to severely oligotrophic habitats. However, the influence of human is not neglectable and in-depth investigations are needed to determine the impact of exploration on an otherwise mostly pristine ecosystem. The cave investigated in this study develops for several kilometres into the mountain, two hundred metres below the topographic surface and is characterized by a strong air circulation. Its structure is composed of huge passages carved in limestone where an ephemeral underground stream creates some lakes, close to which seven samples of visible calcite rafts, manganese deposits and moonmilk (a hydrated calcium carbonate speleothem), were sampled during an expedition in 2014. Other samples were retrieved from a frequently used campsite and from some dry cave passages leading deeper into the cave.

Methods: The community composition and diversity of Archaea and Bacteria in cave samples were studied using amplicon sequencing method: a variable region of 16S rRNA gene, present in Bacteria and Archaea, was amplified with universal PCR primers 515F and 876R [1]. The produced fragments were subjected to Illumina MiSeq sequencing process followed by Mothur analysis [2]. Taxonomic assignment was performed by querying the sequence reads against a silva SSU 123 reference database [3] and various diversity indices and richness estimates were calculated. Abundance of archaeal and bacterial ammonia oxidizing (*amoA*) genes was determined by qPCR.

Results & Conclusion: Analysis of the core microbiome of this environment revealed that the phylum *Proteobacteria* is dominant (47.27%) with the phylum *Nitrospira* next most abundant (29.76%). Within the *Proteobacteria*, the classes of α , β , and γ were the most abundant sequences recovered, with β – *Proteobacteria* being the dominant one (38.99%). *Nitrospira* are capable of autotrophic C fixation [4] and are involved in the two-step autotrophic nitrification, suggesting the pres-

ence of the CO₂ fixation coupled ammonia oxidation process, which likely is also the source of primary production in other cave systems. Furthermore, a broad diversity of *Actinobacteria* and *Acidobacteria* has been recovered from this subterranean setting.

In-depth investigations into the phylogenetic relationship of archaeal and bacterial *amoA* genes revealed a significantly higher abundance of archaeal *amoA* genes compared to bacterial *amoA* genes. Recovered archaeal sequences cluster closely to other *amoA* gene sequences previously recovered from soil, sediment or freshwater samples. The analysis of bacterial *amoA* gene phylogeny is currently ongoing.

The human impact of previous explorations was only detectable at two of the seven sampling points, which were located near the cave entrance and at a frequently used campsite within the cave system. *Propionibacterium* was among the most abundant genera recovered from this site, with *Propionibacterium acnes* being a major inhabitant of the adult human skin. Furthermore, a high abundance of *Staphylococcus* and *Streptococcus* was recovered, which are both well-established human associated genera with representatives such as *Staphylococcus epidermidis* and *Streptococcus mutans*.

Our study shows that there is a complex microbial community hosted in this ecosystem with indications that ammonia-oxidation is one of the major sources of energy. The impact of human exploration in this cave is only localized near the entrance and at the campsite, which indicates that these organisms are not transported into this cave by the movement of air masses.

References:

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