

**The archaeal diversity in a cave system: An analogue environment for life on other planets?** S. Leuko<sup>1</sup>, J. De Waele<sup>2</sup>, L. Bessone<sup>3</sup>, and P. Rettberg<sup>1</sup>, <sup>1</sup>German Aerospace Center (DLR e.V.), Institute of Aerospace Medicine, Radiation Biology Department, Research Group 'Astrobiology', Linder Hoehe, D - 51147 Cologne (Köln), Germany <sup>2</sup>Department of Biological, Geological and Environmental Sciences, Bologna University, Via Zamboni 67, 40126 Bologna, Italy, <sup>3</sup>European Astronaut Centre, Linder Hoehe, D - 51147 Cologne (Köln), Germany. Email presenting author: [stefan.leuko@dlr.de](mailto:stefan.leuko@dlr.de)

**Introduction:** The quest of exploring and looking for life in new places is a human desire since centuries. Nowadays, we are not only looking on planet Earth any more, but our endeavors focus on nearby planets in our solar system. At this point in time, we are not able to send manned missions to other planets, but to be ready and prepared for the day, training today is pivotal. Developed by the European Space Agency (ESA) since 2008, these CAVES missions (Cooperative Adventure for Valuing and Exercising human behavior and performance Skills), prepare astronauts to work safely and effectively and solve problems as a multicultural team while exploring uncharted underground natural areas (i.e. caves) using space procedures. The hypogean environment is also of great interest for astrobiological research as cave conditions may resemble those in extra-terrestrial environments. Besides the main focus of exploration and skill training, future astronauts are also trained in taking microbiological samples during the exploration for further analysis in the lab.

**Results:** During the 2013 mission, astronauts collected soil samples and employed flocked swaps to sample areas with little or no visible soil. Microscopic analysis back in the lab revealed a diverse spectrum of different cell shapes and sizes. Samples were further analyzed employing molecular tools such as RFLP analysis, 16s rRNA clone libraries and sequence analysis. These analyses revealed that recovered sequences closely cluster either to the archaeal marine group I, the South African Gold mine group, the soil Crenarchaeota group or the Thermoplasmatales, respectively. Differences in the diversity could be seen depending on the soil type and on the sampling methods employed. The archaeal community of this cave contained two dominant groups which comprises 18 clones in SC01 and 18 clones in SC04. Clones in SC01 are closely related to the marine group I archaea and with the recently sequenced *Nitrosopumilus maritimus* [1] this group belongs to the most abundant microorganisms in the global oceans [2].

Since Crenarchaeota have been implicated in the nitrogen cycle, the environment was investigated for the presence of the ammonia monooxygenase subunit A (*amoA*) gene. The sequences of *amoA* related genes from Thaumarchaeota were clustered into 4 OTU'S. AC01, comprised of 9 recovered clones, as well as AC02, comprised of 6 recovered clones,

showed similarities of 78% to the *amoA* gene of *Nitrososphaera gargensis* Ga9.2a. The OTU's AC03 and AC04 showed similarities of 83% to *Nitrosopumilus maritimus* isolate SF\_AOA\_A10.

#### Conclusions:

The hypogean environments are difficult to sample and only provide limited possibilities for scientists to obtain samples. Expeditions such as the ESA CAVES training course offer a rare opportunity to gain access to samples from deep within a cave. The extraterrestrial subsurface has been suggested as potentially harboring life due to the protection from harsh surface conditions and recent evidence from Mars and other bodies in the Solar System suggests the presence of volcanic caves [3]. In the investigated cave we found a diverse community of archaea able to utilize ammonia as energy source. By analyzing low energy systems like a cave, we may be able to find clues for what could be necessary to survive on a different planet.

#### References:

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- [2] Agogué H. et al. (2008) *Nature*, 456, 788.
- [3] Northup D. E. et al. (2011) *Astrobiology*, 11, 601.