Europlanet TA Scientific Report

PROJECT LEADER

Project number: 20-EPN-083
Name: Federico Biagioli
Home Institution: University of Tuscia (Italy)
TA Facility visited: Centre for Microbial Life Detection at the Medical University of Graz (Austria).

Project Title: Beyond Antarctica: a survey on detection of life in endolithic fossils supporting future space exploration missions

Scientific Report Summary.
(plain text, no figures, maximum 250 words, to be included in database and published)

Endolithic growth is the ultimate microbial adaptation and the predominant life-form in the far extreme ice-free areas of Antarctic deserts, considered among of the best analogues of the Martian environment on Earth. Although recent molecular studies have started to shed light on the biodiversity, distribution and composition of crypto-endolithic communities in visibly colonized rock samples, fossilized or apparently not colonized endolithic communities remain largely unexplored. Amplicon-sequencing analysis of fungal and prokaryotic domains on both not colonized and fossil samples were here performed to gain information about preservation of extinct life traces within rock and evidences of possible life detection also in eventual extra-terrestrial samples. Genomic DNA was extracted and sequenced from 24 fossil and 6 apparently not colonized rocky samples, resulting in a high number of reads and mapped ASVs for fungal (657,626 quality-filtered reads and 161 ASVs), bacterial 16S rDNA (1,296,594 reads mapped into 839 ASVs) and 16S archaeal specific (2,813,402 validated clustered in 3,514 ASVs) datasets. The high number of reads and ASVs achieved allows us to suppose that rocks not only represent a perfect refuge from harsh external environmental conditions, but also an important preservation ark for biosignatures of past life forms.

Full Scientific Report on the outcome of your TNA visit

Endolithic growth is one of the most spectacular microbial adaptations to extreme environmental constraints and the predominant life-form in the ice-free areas of Antarctic deserts. In Antarctica, they were first described from the McMurdo Dry Valleys (Ross Desert), considered one of the best analogues of the Martian environment on Earth [1]. There, where the extremely low temperature, aridity, oligotrophy and UV irradiation are the main challenges for life, rock represents the main substratum and last refuge for microbial colonization. Antarctic endolithic communities represent the limits of surface life on Earth [2], and are considered a suitable analogue for extra-terrestrial life detection [3] as terrestrial model for the last stages of life on early Mars [4].

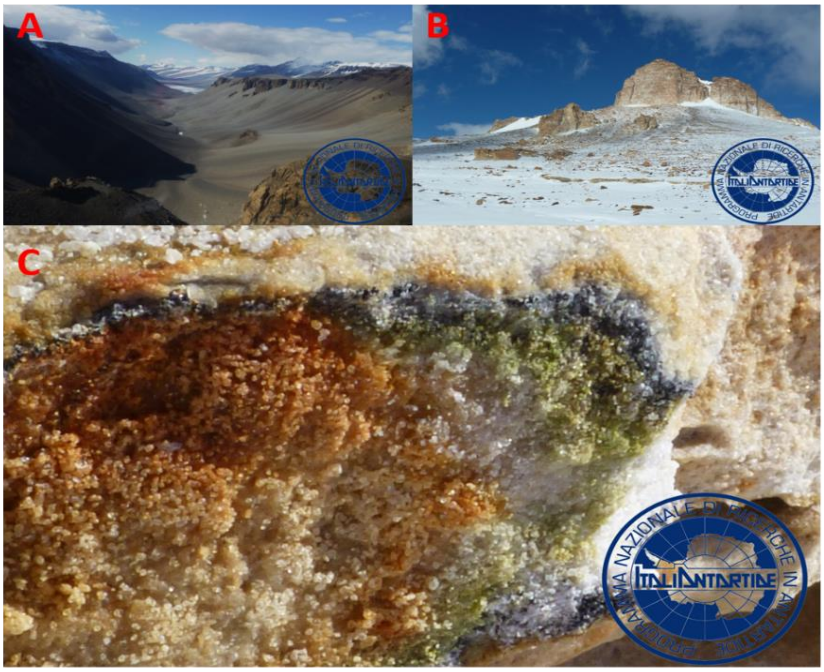

Figure 1. (A-B) In the ice-free areas of Antarctic deserts (A: Labyrinth Valleys detail; B: Linnaeus Terrace locality), endolithic growth (C: detailed view of a lichen dominated crypto-endolithic community) represent the most spectacular microbial adaptations to extremes and the predominant life-form.

Although recent molecular studies have started to shed light on the biodiversity, distribution, and composition of crypto-endolithic communities in visibly colonized rock samples [5], also defining drivers and effects of environmental pressure on these endolithic settlers [6], several localities harbouring fossilized or apparently not colonized communities remain largely unexplored.

With this in mind, we propose to delineate a suitable protocol for DNA extraction, amplification and amplicon-sequencing analysis of fungal compartment and prokaryotic domains on both not colonized and fossil samples. Data here obtained will deepen our knowledge on Antarctic crypto-endolithic communities overall, opening new scenarios on the possibility for searching life beyond Earth. Indeed, the Antarctic microbial endolithic communities represent an invaluable tool for answering some main biological questions: i) how life adapts and evolves under the extremes; ii) which are the responses to environmental pressure and external perturbations; iii) which are the limits of adaptability for life in terms of life/extinction; iv) which are the limits of habitability on Mars-like environments on Earth, and, hence, to speculate the possibility of life in the solar system. If such lifeforms were present on the Red Planet, during loss of atmosphere, water, and concomitant cooling of Mars, these organisms may have withdrawn into porous rocks as the last habitable niche in a deteriorating environment. Under these conditions, trace fossils may have been formed and the preservation of such near-surface fossils is a distinct possibility. The search for such structures is a legitimate goal to solve the question about the habitability and for future exploration of Mars. To accomplish the study, we have selected the Centre for Microbial Life Detection at the Medical University of Graz, as the

designated structure to develop our project as the facility's team has great experience with microbial detection and quantification in samples from extreme environments.

Working phase

Sample selection and preparation

During the preliminary preparation phase, conducted at the Antarctic Geomycology Laboratory of the University of Tuscia (Italy), a total of 30 rock samples were selected and crushed under sterile conditions and successively stored in 5ml sterile tubes for transport to the Centre for Microbial Life Detection at the Medical University of Graz (Austria). The selected rock samples were, in detail:

1) Not Colonized rocks

- 3 rock samples from Linnaeus Terrace
- 3 rock samples from Mt New Zealand

2) Fossilized rocks

- 10 rock samples from Timber Peak
- 10 rock samples from Mt Fleming
- 4 rock samples from Horseshoe Mt

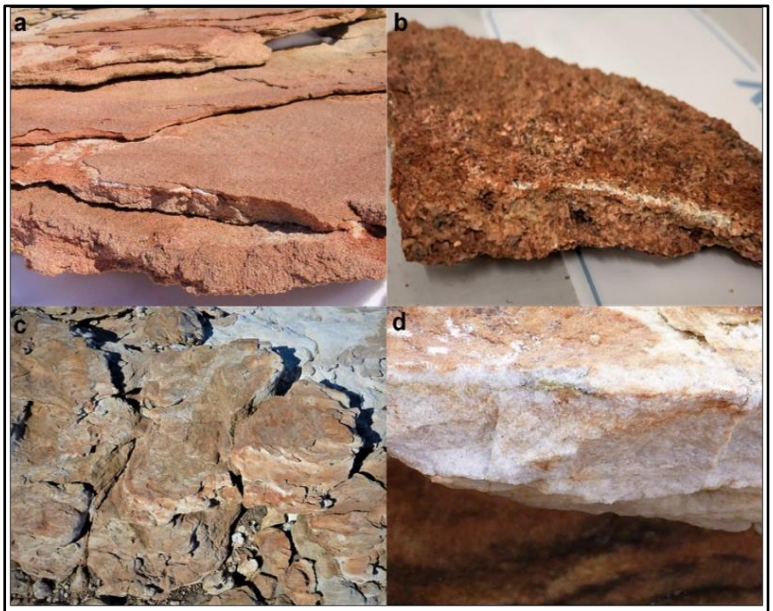

Figure 2. A-B) Fossil setting of past crypto-endolithic colonization at Timber Peak (Northern Victoria Land) and C) fossil sandstones collected at Mt. Fleming (McMurdo Dry Valleys, Southern Victoria Land); D) apparently non-colonized Beacon sandstone collected at Linnaeus Terrace (McMurdo Dry Valleys, Southern Victoria Land).

All samples have been collected along the Victoria Land (Continental West Antarctica) during the XXXIV Italian Antarctic Expedition, in the framework of ongoing projects funded by the Italian National Program for Antarctic Researches (PNRA). In particular samples from some of the most significant locations for the presence of cryptoendolithic colonization will be considered as, for instance: i) Linnaeus Terrace, (77°35'S 161°0.49'E, 1650 m asl) in the McMurdo Dry Valleys is the region from which Antarctic cryptoendolithic communities have been described for the first time [7]. This area is designed as an ASPA (Antarctic Specially Protected Areas) to protect these outstanding environments and the access is possible with special permission only; ii) Mt New Zealand, (74°10'S 162°30'E, 3100 m asl) where cryptoendolithic colonization have been still reported at very high altitude (3200 m asl) [5]. Additional fossils will be selected among Timber Peak (74°10'S 162°25'E, 2800 m asl), Mt Fleming (77°32'S 160°17'E, 1645 m asl, Dry Valleys) and Horseshoe Mt (77°33'S 160°1'E 2000 m asl, Dry Valleys) where fossilized communities have been observed [2].

Genomic DNA extraction and Amplicon-sequencing library preparation

During the 5 working days, at the Centre for Microbial Life Detection at the Medical University of Graz as planned, eDNA was extracted from all samples and the amplicon-sequencing analysis library was prepared, targeting the 3 main crypto-endolithic communities' components: Fungi, Bacteria and Archaea.
Metagenomic DNA was extracted from 0.3 g of sample using Qiagen DNeasy PowerSoil Kit (Carlsbad, CA, USA) according to manufacturer's protocol. Extracted DNA concentration was quantified via Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). The Internal Transcribed Sequence 1 ribosomal region (ITS1) and hypervariable region V4 of 16S ribosomal gene were targeted to assess the fungal and prokaryotic community composition, respectively. The ITS1 region was amplified using barcoded primers ITS1F/ITS2R, suitable for shorter read length [9], while for the variable V4 region of 16S, barcoded F515/R806 primer set was used according to Caporaso et al. (2012) [10]. To target the archaea-specific prokaryotic component, a nested PCR approach was performed, using the primer pair 344F and 1041R at the first and 519F and 806R for the second PCR run. For protocols and technical specifications see [11]. Finally, Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq platform.

Bioinformatic processing data and Results

The focus of the 3 additional working days, held remotely, was on the bioinformatic analysis of all raw sequence datasets. Demultiplexed ITS, Bacterial and Archaeal raw sequence datasets were processed using AMPtk [12] v.1.5.1 software. Briefly, barcodes/indexes and primer sequences were removed from raw data. Reads were then subjected to quality trimming to a maximum of 250 bp and discarding reads less than 100 bp in length, and sequencing artefacts were dropped by using USEARCH v.9.1.13 with default parameters [13]. Sequence quality filtering was performed with the expected error parameter of 0.9 [13; 14] and the cleaned reads were merged and then clustered at 99% similarity using VSEARCH [15] v.2.15.1, with DADA2 [16] algorithm that uses a statistical error model to correct sequencing errors to infer the Amplicon Sequence Variants (ASVs). Global singletons and rare taxa (<5 reads) were skipped as likely false positives due to sequencing errors, following Lindahl et al. (2013) [17]. Finally, taxonomic identification was performed with a sequence identity of 97% as Global Alignment threshold, using hybrid database SINTAX/UTAX [13].
The ITS1 dataset generated 1,253,902 raw sequence reads, resulting in 657,626 gene quality-filtered reads, ranging from 173 up to 170,104 per sample. After singletons and rare taxa (<5 reads) removal (55 out of 216 ASVs total), a total of 161 high-quality ASVs were obtained. A total of 1,735,770 raw reads were generated from Bacterial 16S rDNA dataset and accounted for a total of 1,296,594 which were grouped into 839 ASVs (out of a total of 1339 ASVs) after quality filtering, with sequencing depths between samples ranging from 118 to 139,741 reads. The 16S archaeal specific dataset produced a total of 8,395,172 primarily, reduced in 2,813,402 validated post processing reads, ranging from 269 up to 206,709 reads per sample and clustered in 3,514 mapped ASVs (out of a total of 4,562 ASVs).

Conclusion and Future Prospects

We were able to successfully extract, amplify and sequence metagenomic DNA, both from rocks with no visible colonization and from rocks with traces of past colonization, for all investigated microbial components. Furthermore, the high number of reads and mapped ASVs obtained, considering the peculiar features and nature of analyzed samples, allows us to suppose that rocks not only represent a perfect refuge from harsh external environmental conditions, but also an important preservation ark for biosignatures of past life forms. Further biodiversity analyses will allow us to deeply understand the taxonomic composition and distribution of different microbial components across two types of rock analyzed. In addition, we will be able to gain more insight about the strategies and adaptive skills of these microorganisms, even to explore the limits of life in one of the best analogues of the Martian environment on Earth.

References

1: Cockell CS, Bush T, Bryce C, Direito S, Fox-Powell M, Harrison JP, et al. Habitability: a review. Astrobiology. 2016; 16:89-117.

2: Friedmann EI, Druk AY, McKay CP. Limits of life and microbial extinction in the Antarctic desert. *Antarctic Journal of the United States*. 1994; *29*:176-180.

3: Friedmann EI. The Antarctic cold desert and the search for traces of life on Mars. *Advances for Space Research,* 1986; 6(12):265-268.

4: McKay CP, Friedmann EI, Wharton RA, Davis WL. History of water on Mars: A biological perspective. *Advances in Space Research, vol. 12*. 1992; 4:231-238.

5: Coleine C, Stajich JE, Zucconi L, Onofri S, Pombubpa N, Egidi E, et al. Antarctic cryptoendolithic fungal communities are highly adapted and dominated by Lecanoromycetes and Dothideomycetes. *Frontiers Microbiol.* 2018; 9:1392.

6: Selbmann L, Onofri S, Coleine C, Buzzini P, Canini F, Zucconi L. Effect of environmental parameters on biodiversity of the fungal component in the lithic Antarctic communities. *Extremophiles*. 2017; 21(6):1069–1080.

7: Friedmann EI. Endolithic microorganisms in the Antarctic cold desert. *Science.* 1982; 215:1045–1053.

8: Coleine C, Stajich, JE, Pombubpa N, Zucconi L, Onofri S, Canini F, Selbmann L. Altitude and fungal diversity influence the structure of Antarctic cryptoendolithic Bacteria communities. *Environmental Microbiology Reports*. 2019; 11(5):718-726.

9: Smith, D.P., Peay, K.G. (2014) Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLoS ONE 9: 90234

10: Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6: 1621-1624.

11: Kumpitsch, C., Moissl-Eichinger, C., Pock, J. et al. Preliminary insights into the impact of primary radiochemotherapy on the salivary microbiome in head and neck squamous cell carcinoma. Sci Rep 10, 16582 (2020). https://doi.org/10.1038/s41598-020-73515-0

12: Palmer, J.M., Jusino, M.A., Banik, M.T., Lindner, D.L. (2018) Non-biological synthetic spike in controls and the AMPtk software pipeline improve mycobiome data. PeerJ 6: e4925.

13: Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. J. Bioinform. 26: 2460-2461.

14: Edgar, R.C., Flyvbjerg, H. (2015) Error filtering, pair assembly and error correction for next generation sequencing reads. J. Bioinform. 31: 3476-3482.

15: Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F. (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4: e2584.

16: Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. Nature Met. 13(7): 581-583.

17: Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., and Kauserud, H. (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol 199: 288-299

- Give details of any publications arising/planned (include conference abstracts etc)

- Host confirmation

Please can hosts fill in/check this table confirming the breakdown of time for this TA project:

Dates for travel to accommodation for TA visit (if physical visit by applicant)	Start Date of TA project at facility	Number of lab/field days spent on TA Visit pre-analytical preparation	Number of days in lab/field site for TA Visit	Number of days spent in lab for TA Visit data analysis (remote data analysis)	End Date of TA project at facility	Dates for travel home (if physical visit by applicant)
Departed: 14-11-2021 Arrived: 15-11-2021	15-11-2011	[0]	[5]	[3] 21.04.2022 22.04.2022 26.04.2022	26-04-2022	Departed: 19-11-2021 Arrived: 20-11-2021

The host is required to approve the report agreeing it is an accurate account of the research performed.

Host Name	**Christine Moissl-Eichinger**
Host Signature	
Date	**26.04.2022**

- Project Leader confirmation

Do you give permission for the full version of this TA Scientific Report (in addition to the 250 word summary) to be published by Europlanet 2024 RI on its website and/or public reports? YES

Project Leader Name	**Federico Biagioli**
Project Leader Signature	
Date	**28.04.2022**