Europlanet TA Scientific Report

PROJECT LEADER

Project number: 20-EPN2-035
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Project Title: Characterization of the composition and metabolic profiles of microbial communities in Antarctic dry soils as a model for Mars explorations.

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

McMurdo Dry Valleys (MDVs) of Antarctica, the coldest and driest desert on Earth, characterized by strong UV irradiation and strong winds, are considered one of the closest terrestrial analogue of the Martian environment. Only microbial life-forms inhabiting the rocks and soils can survive in these harsh conditions. Endolithic communities have been thought to be the predominant life forms and have been widely characterized, while the drivers of microbial colonization in MDVs dry permafrost are still barely known. It is still not clear if life forms found in soils through molecular approaches are metabolically active or present as dormant wind-transported spores, propagules or death cells. Additionally, the possible contribution of the endolithic communities to soils diversity has never been investigate in MDVs. As the endolithic growth has been hypothesized as one of the adaptive strategies of putative Martian microbial life-forms, the hypothesis of rock fragments dispersal should be investigated, to give new perspectives on life-traces search on Mars.
The microbial diversity and metabolic activity have been characterized in soils collected in MDVs at increasing distance from a colonized sandstone outcrop and in the corresponding rocks samples. The microbial composition and functionality have been investigated through shotgun metagenomics, while the metabolomics profiles have been defined through NMR metabolomics. This description may give unprecedented information on whether the cells found are active or not and on how they metabolically adapt and thrive in this environment. The results may be useful in the frame of search for chemical biosignatures within future Mars explorative missions.

Full Scientific Report on the outcome of your TNA visit

We encourage you to add figures to your report, which should be approx. 1 page of text plus figures.

Studying the adaptations of life forms in pristine environments as the Antarctic deserts is fundamental to understand how life evolved on our planet and on extra-terrestrial planets with similar characteristics. In this regard, the McMurdo Dry Valleys (MDVs) show many characteristics such as low temperatures, extreme dryness, strong irradiation regimes, and local high salinity, making them one of the closest analogues of the Martian environments. In particular, dry permafrost, which is unique to MDVs on Earth, is ubiquitous in the Northern hemisphere of Mars, where many exploratory missions are being carried out. However our knowledge of the diversity and the factors determining life establishment in such peculiar conditions is still limited. In this regard, the project aimed to answer to many open questions in this field. First of all, one of the objective was to determine if life forms present in these soils are metabolic active in their native environment and to disentangle. Additionally, it aimed to disentangle the effect of rocks communities, already well described, on the diversity of the surrounding soils, given the exfoliation phenomena induced by subsurface microbial growth on rocks. Finally, it is the first attempt to characterized the metabolomics profiles in these analogues environments, that could provide unprecedented insights into the life detection on Mars.

To answer these questions, samples collected at Battleship Promontory (76°54'04''S 160°54'41''E), during the austral summer 2019/2020 have been analysed. In particular, samples available corresponded to soils collected as close as possible to a sandstone outcrop (0 meters; labelled as S1) showing an evident endolithic colonization and at increasing distances of 50 (labelled as S2) and 100 meters (labelled as S3). Samples of the corresponding rock outcrop have been collected as well (R1-R3). All samples were collected in triplicate. During the TNA visit the DNA has been extracted from the samples. The extraction were performed from 500 mg of soil or grounded rocks using the DNeasy PowerSoil Kit (QIAGEN). Given the extremely low biomass of the samples, multiple extractions have been performed form the same sample (in some cases up to 12 extractions have been performed from one sample) and pooled to obtain enough DNA for the sequencing. The required amount was obtained for 10 out of 12 samples available. The samples were sent to an external company for libraries construction and Illumina paired-end sequencing. For many of the samples, an high number of reads was obtained (reported in table 1) with a Q30 score of 89.25%. During the two days remote access the sequences obtained have been analysed using the ATLAS pipeline. This include quality control, assembly, genomic binning, and annotation.

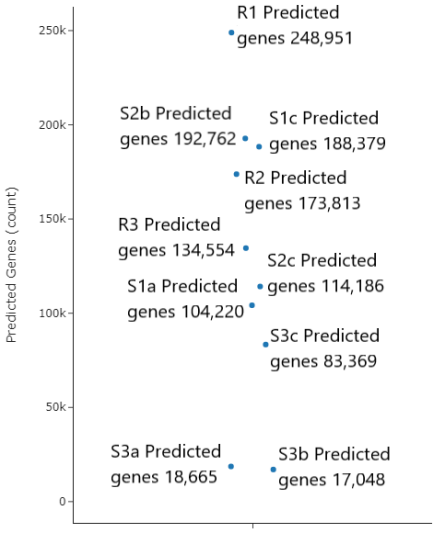

Figure 1. Number of genes predicted for each sample after annotation

Table 1. Reads obtained for the Illumina sequencing of each sample

Sample name	Total number of reads
R1	65,628,872
R2	51,386,496
R3	41,092,320
S1a	31,073,530
S1c	65,630,168
S2b	64,821,972
S2c	44,129,170
S3a	12,368,034
S3b	8,402,882
S3c	37,802,468

The analyses of diversity and metabolic competences of the communities in relation to different physicochemical parameters and the comparisons of soils and rocks communities are still ongoing.

For the metabolomics analyses 100 mg of soil or grinded rock samples were extracted with Precellys beads in H_2O/MeOH as described in Zhou et al. (Metabolites. 2021;12(1):17). The obtained supernatant was lyophilized, and dissolved in NMR buffer for measurements as described in Zhou et al. (Cell Reports Methods. 2021;1(2):100016). The metabolomics profiles have been obtained for all the samples available. In the figure 2 the spectra obtained for rocks samples are reported as an example. Metabolites with isolated signals on the spectra obtained were assigned. Also in this case, the analyses of the metabolites differentially expressed in the different sample types and in relation to the environmental variables are being performed. However

a strong clustering of the metabolomics profiles obtained for rocks and soil samples has been highlighted (fig.3).

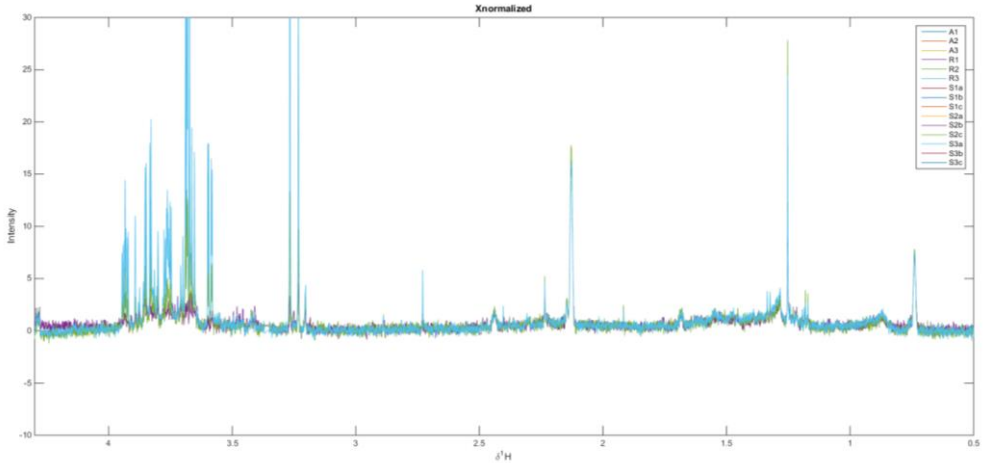

Figure 2. NMR spectra for the rocks samples

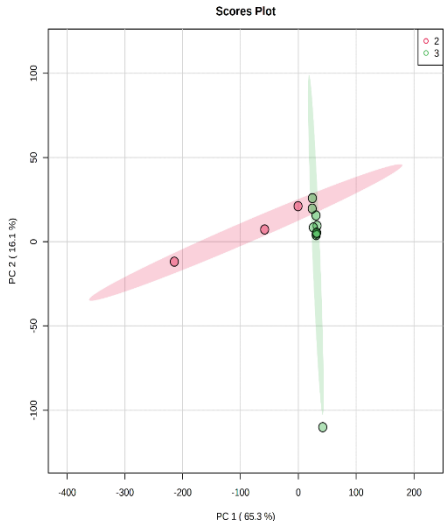

Figure 3. PCA cluster of the rocks (red) and soil samples (red)

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

Two papers on ISI/WOS journals are expected to arise from the data obtained from the activities carried out during the realization of the project. The first one will report the metagenomic dataset, the comparisons of the soils and rocks communities, and the correlation of their composition and metabolic diversity with different soil physicochemical parameters. The second one will include the NMR metabolomics characterization of the soil and rocks samples, also in this case performing multiple comparisons among the samples and with the parameters available.
The data could be of great interest in different field of research, therefore they will be object of presentations in conferences of different context, dealing with Antarctic environments (such as the SCAR conferences), with astrobiology research (such as the EANA or the BEACON meetings), and with microbial ecology in general.

- 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	End Date of TA project at facility	Dates for travel home (if physical visit by applicant)
Departed: 03-10-21 Arrived: 03-10-21	04-10-21	[0]	5	[2] Remote analyses of data obtained	08-10-21	Departed: 09-10-21 Arrived: 09-10-21

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	**19.05.2022**

- Project Leader confirmation

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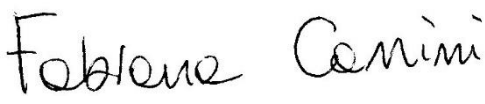
Project Leader Name	**Fabiana Canini**
Project Leader Signature	
Date	18/05/2022