Europlanet TA Scientific Report

PROJECT LEADER

Project number: 20-EPN-061
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TA Facility visited: Makgadikgadi Salt Pan (Botswana)

Project Title: Life in extreme environments: Distribution and importance of far-red light driven photosynthesis to primary production in Martian-like environments

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

The aim of this project was to confirm the richness and abundance of chlorophyll *f*-containing cyanobacteria, and their ability to use low-energy light to perform oxygenic photosynthesis in Martian-like environments. This study was a follow-up to a 2019 sampling trip to the sabkhas of the Western Sahara (Morocco), for which we could show that chlorophyll *f*-cyanobacteria are highly abundant. Here we expanded this research by collecting samples from the hypersaline environments of the Sua and Ntwetwe Pans in Makgadikgadi (Botswana). Microbial mat and rock samples containing endolithic and hypolithic phototrophs were collected. Light microscopy on site confirmed the abundance of cyanobacteria of various morphologies in most collected samples. The microbial mat samples were especially rich in cyanobacteria, forming a 1-2 mm thick layer at various depths depending on the absorption properties of the top layer. Preliminary analyses with high-performance liquid chromatography (HPLC) in combination with hyperspectral confocal fluorescence microscopy confirmed the presence of red-shifted chlorophylls in some of these samples but to less extent as observed in the sabkhas. Genomic DNA has been extracted and will be used for sequencing and phylogenetic analyses based on 16S rRNA and specific far-red light genes. This will allow to fully evaluate the microbial diversity and their ability to perform chlorophyll *f*-driven oxygenic photosynthesis. In addition, the enrichment and isolation process of new chlorophyll *f*-containing cyanobacteria has been started by transferring the samples to growth media of various salinity and keeping them under selective far-red light illumination.

Full Scientific Report on the outcome of your TNA visit

In order to assess the importance of near-infrared photosynthesis in Martian-like environments, samples were collected from various sites in the Makgadikgadi region (Botswana). These sites included the hypersaline environments of the Sua and Ntwetwe Pans (Figure 1). In total, 77 samples were taken from 9 different sites. As results from a previous Europlanet field trip by Nürnberg and Canniffe in 2019 to the sabkhas in the Western Sahara (Morocco) indicated that far-red cyanobacteria are highly abundant in microbial mats in these environments and can be phototrophs living within and under rocks, similar samples were collected during this research trip. All samples showed clear green layers (Figure 2A,B) and the examination of those by light microscopy indicated the presence of cyanobacteria of various morphologies, ranging from unicellular to filamentous forms with specialised cells for nitrogen fixation (heterocysts).

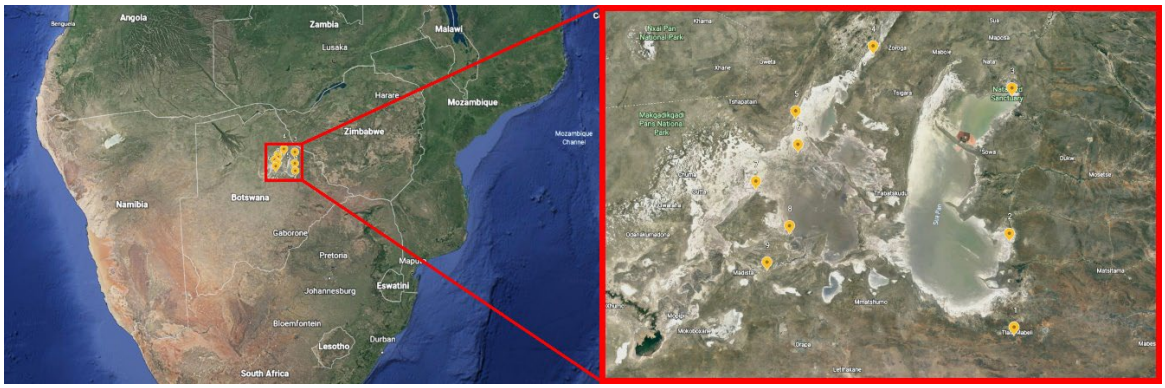

Figure 1. Sampling sites in the Makgadikgadi region, northern Botswana. Zoomed-in view shows locations of these sites in Sua and Ntwetwe Pans.

After collection, the samples were either stored in DNA/RNA Shield solution (Zymo Research) to protect the DNA from degradation and allow future metagenomics studies at the FU Berlin or kept in storage containers in darkness or under far-red illumination for pigment analysis, microscopy and the isolation of chlorophyll *f*-cyanobacteria.

We furthermore tested a small-scale pigment extraction method using a low-cost 3D-printed centrifuge (3D-Fuge; Byagathvalli *et al*., 2019) in combination with a portable spectrometer (Ocean Insight) and a self-assembled sample holder with LEDs for illumination. The fluorescence emission spectra of the pigments were recorded and used as an indicator for the presence of red-shifted chlorophyll *f*. Although we could not detect any chlorophyll *f* in the selected samples, which might be due to spectral overlap with other pigments, we successfully established the method for the future development of a portable HPLC system for the direct analysis of pigment composition in the field.

After the return to the applicant's laboratories the samples were further analysed by (i) extracting pigments and performing HPLC analyses (in Liverpool, UK) and (ii) performing hyperspectral confocal fluorescence microscopy (in Berlin, Germany). Preliminary results confirm the presence of the red shifted-chlorophyll *f* in some samples (Figure 2C) and the morphological diversity of the cyanobacteria, containing both filamentous and unicellular but aggregating forms (Figure 2D). Further analyses shall clarify their phototrophic diversity. Genomic DNA for sequencing and phylogenetics analyses will be extracted using the *Quick*-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research). The analysis will be performed based on 16S rRNA and far-red light specific genes as previously reported (Antonaru *et al.*, 2020). In parallel, we have started to isolate the chlorophyll *f*-containing strains by transferring small pieces of the samples into diverse cyanobacterial growth media (with eukaryotic inhibitors) of different salinity under selective far-red light illumination.

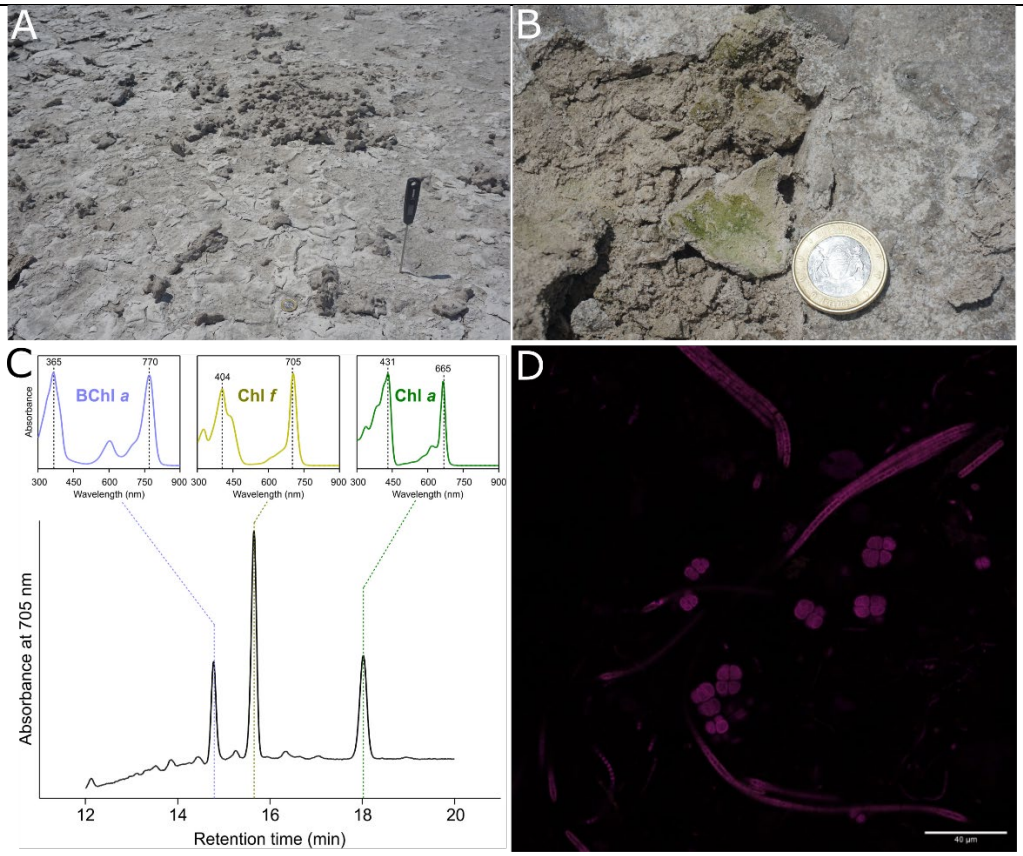

Figure 1. Characterisation of chlorophyll *f*-containing samples from the North site of Ntwetwe Pan. (A) Sampling location. (B) Cyanobacterial layer underneath salt/sand crust. (C) Pigment composition as per HPLC analysis. The samples contain bacteriochlorophyll *a* (BChl *a*) and chlorophyll (Chl) *a* and *f*. (D) Fluorescence image showing cyanobacteria of various morphologies. Excitation at 488 nm; Emission from 650-700 nm. Scale bar, 40 µm.

References:
Antonaru *et al*. (2020). ISME J, 14: 2275-87.
Byagathvalli *et al*. (2019). PLoS Biol, 17: e3000251.

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

The results of this study are planned to be published in a top-tier microbial ecology journal, such as Environmental Microbiology or ISME J, authored by Canniffe, Nürnberg and Wu, a PhD student working with the PI. The results will also be presented by Nürnberg at the International Symposium of Photosynthetic Prokaryotes from 21-25 August 2022 in Liverpool. All sequence data will be made publicly available through databases such as NCBI. New isolates of cyanobacteria will be deposited with culture collections.

- 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: 09-02-22 Arrived: 10-02-22	10-02-22	1	7	1	19-02-22	Departed: 19-02-22 Arrived: 20-02-22

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

Host Name	
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Date	02/05/2022

- Project Leader confirmation

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Project Leader Name	**Dennis Nürnberg**
Project Leader Signature	
Date	**2 May 2022**