NASA Astrobiology ECCA Report

Microbial Biofilms in Serpentinizing Ecosystems: Exploring Life at the Extremes

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Overview and objectives

My NASA Astrobiology Early Career Collaboration Award (ECCA) supported a research stay in Prof. Gael Erauso's laboratory at the Mediterranean Institute of Oceanography (MIO; Aix-Marseille University) in Marseille, France. The purpose of the visit was to advance my dissertation research on microbial biofilms in serpentinizing ecosystems by studying Extracellular Polymeric Substances (EPS) in microbial strains isolated from the Prony Hydrothermal Field in New Caledonia.

The main objectives of the ECCA project were met. I screened alkaliphilic and acidophilic isolates for EPS and biofilm forming potential and characterized major EPS associated components. The strains included *Exiguobacterium pronyensis* BJCHIT, *Clostridium sp.* strain PROH2, *Alkalicella caledoniensis*, *Alkaliphilus serpentinius*, *Aciditoga* spp., and *Marinitoga* spp., representing organisms adapted to alkaline conditions up to approximately pH 11 and acidic conditions below pH 5.

Research completed and what I learned

During the visit, I learned anaerobic culturing approaches (**Figure 1**), a major expertise of the host laboratory, and tested strains for biofilm formation using crystal violet and Congo red assays (**Figure 2**). I refined a crude EPS extraction workflow for pure cultures, normalized EPS yield by culture density, and quantified matrix associated components using phenol-sulfuric acid assays for polysaccharides, Bradford assays for proteins, and Qubit fluorometry for extracellular DNA. Lipid characterization by FAME/GC-MS was initiated in collaboration with Vincent Grossi from the MIO team.

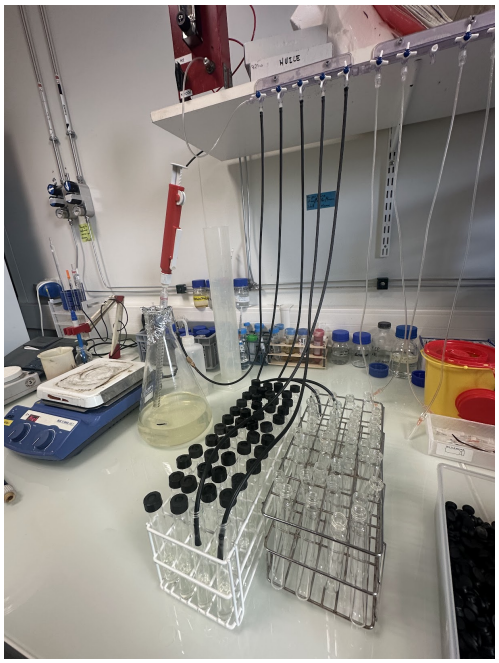

Figure 1. Anaerobic culture setup at MIO used to grow extremophile isolates.

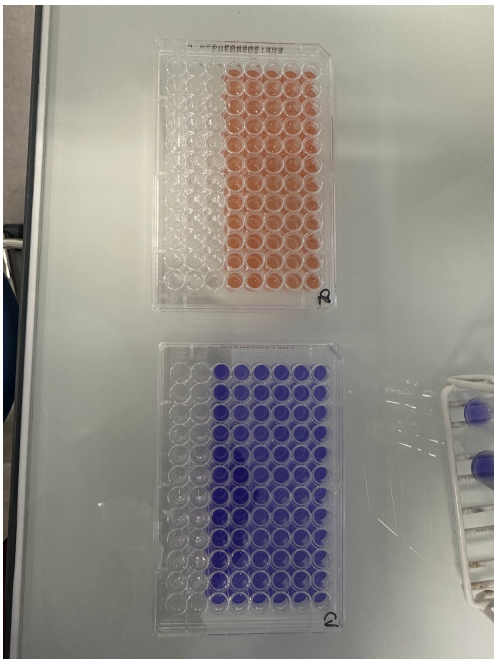

Figure 2. Representative 96-well biofilm, EPS screening assays using Congo red and crystal violet.

The overall results showed that EPS related traits were strongly strain specific. BJCHIT and PROH2 emerged as the strongest candidates for follow up work, but their matrix profiles differed. PROH2 produced more crude EPS when glucose was added to the growth medium. In contrast, sucrose, especially at 2%, increased the polysaccharide signal in both PROH2 and BJCHIT. BJCHIT also showed higher levels of extracellular DNA and protein material, suggesting that its biofilm matrix may have a different composition than PROH2.

Confocal microscopy with DAPI, Direct Yellow 96, and Nile Red supported these biochemical differences. BJCHIT appeared dominated by dense cellular aggregates, consistent with a biofilm associated lifestyle, whereas PROH2 showed a more heterogeneous pattern with both aggregates and dispersed cells. Overall, this visit taught me that carbon source, physiology, and growth mode can strongly influence the amount and composition of EPS produced by extremophilic microorganisms.

Collaborations and future plans

This work was carried out in Prof. Erauso's laboratory with continued guidance from my advisor, Dr. Matthew Schrenk at Michigan State University. I interacted with the interdisciplinary MIO team, including Vincent Grossi, Manon Bartoli, Anne Postec, Marianne Quemeneur, Linda Guentas, and other laboratory members. These interactions strengthened the MSU-MIO collaboration and helped me learn new approaches for culturing extremophiles that I can bring back to MSU. The results produced during this internship will be integrated into a manuscript comparing biofilm genetic potential and EPS associated phenotypes across serpentinizing systems.

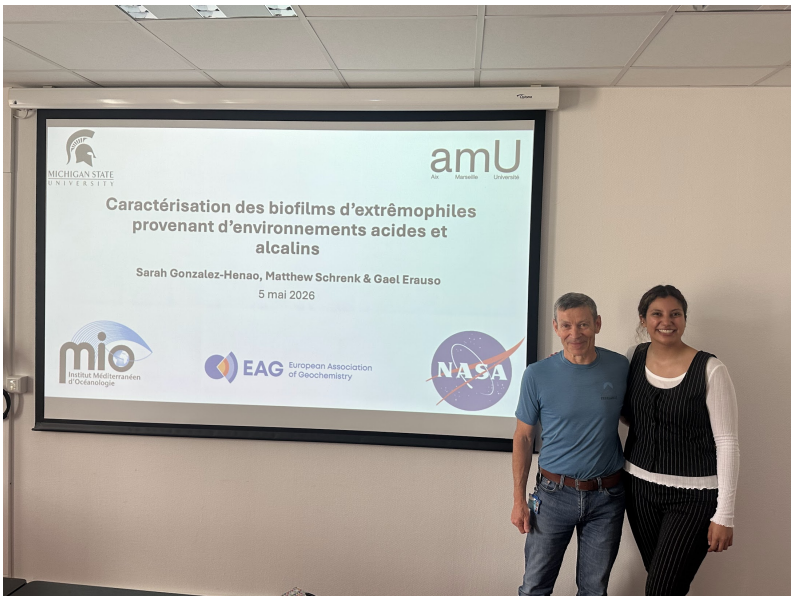

Figure 3. Final research presentation at MIO with Prof. Gael Erauso.

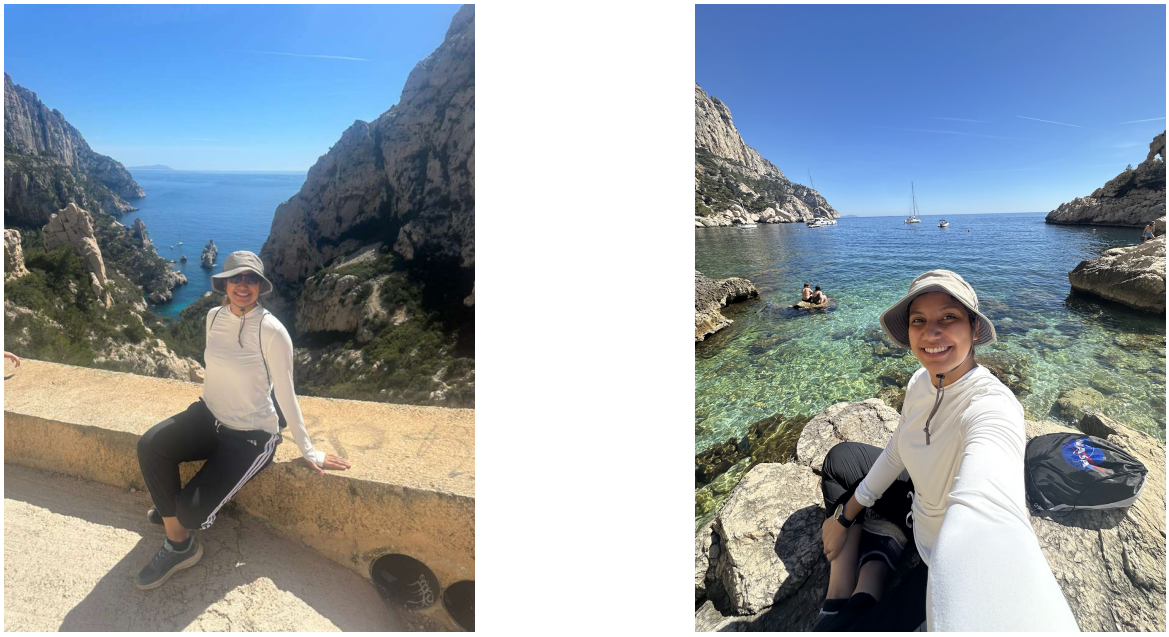

Figure 4. Trip images from the Calanques National Park during the ECCA research stay.