The microbiology of anaerobic analog environments – The MASE project.

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Introduction: Astrobiology seeks to understand the limits of life and to determine the physiology of organisms in order to be able to better assess the potential habitability of other worlds and improve our ability to assay them for the presence of life. To successfully achieve this we require microorganisms from environments on Earth that in physical and/or chemical conditions approximate to extraterrestrial environments [1]. The most challenging of these environments with respect to the sample collection and follow on isolation and cultivation of microorganisms are anaerobic environments. Here we describe an approach to this challenge and results from the European-funded MASE (Mars Analogues for Space Exploration) project which seeks to understand anaerobic analog environments.

Methods: We selected a number of anaerobic environments based on characteristics that make them analogous to past and present locations on Mars (Icelandic lakes, sulfidic springs, deep hypersaline environments, acidic iron-rich environments and permafrost). We implemented a culturing approach to enrich organisms from these environments under anaerobic conditions using a defined medium that would allow for all organisms to be grown under identical culturing conditions in future physiological comparisons. We then isolated anaerobic microorganisms, carried out a study of their basic physiology and deposited these organisms in the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) culture collection to make them available to astrobiologists and microbiologists.

Results: In total 1131 enrichments were obtained and 118 yielded growth (10.4%). Among these, 131 enrichments were set up to enrich for autotrophs (no organic carbon supplement). Thirty-four of these displayed growth. Thirty-one isolates (30 bacteria and one archaea) were obtained from high cell density enrichments. We isolated a range of organisms that used carbon sources or redox couples of relevance to Mars, including iron oxidisers and reducers and organisms capable of metabolising carbon sources expected on Mars including amino acids and PAHs. Among these, acidophiles, psychrotolerant/philic and halophilic organisms were also isolated reflecting the link between not just carbon and energy use, but the tolerance to extreme physical and chemical stresses relevant for Martian environments. We obtained six isolates from Lake Grænavatn in Iceland and one isolate, Yersinia sp. (MASE-SM-9) for which no organic carbon supplement was used. These may either be true autotrophs or using low levels of residual organic carbon in the medium. The microorganisms that we isolated are now deposited in the culture collection, DSMZ. They have been used to undertake fossilization studies to better understand how biosignatures may be preserved from anaerobic microorganisms [2].

Discussion: A range of microorganisms were isolated from these analog environments and alongside these organisms environmental DNA provided a complete picture of the microbiome [3].

In addition to the samples collected for the isolation, other samples were collected. These samples are crucial for carrying out the characterisation of the total microbial community and determining the geochemical and environmental context of the collected samples. These samples included samples for FISH (Fluorescent In-Situ Hybridisation) to quantify organisms and study their morphology and spatial distribution using DNA probes, DNA extraction samples to undertaken environmental rRNA gene and metagenomics analysis to determine the presence of functional genes and the phylogeny of the total anaerobic population. Samples were collected to determine the geochemical context of the solutions and sediments from which organisms were enriched. They were DOC analysis samples for determining dissolved organic carbon, Cations and anions samples to determine inorganic geochemistry, and total nitrogen and total carbon samples. Other samples were also collected for projects within MASE, which were samples to be analysed by the Spanish Centre for Astrobiology using the SOLID (Signs of Life Detector) instrument to investigate the use of antibody detection methodologies for detecting organisms and their biomarkers.

References: [1] Preston, L.J. and Dartnell, L.R. (2014) *Intern Journ Astrobiol* **13**:81-98; [2] Gaboyer F. *et al.* (2017) in review [3] Perras, A. *et al* (2017) Abscicon abstract