

Mars Analogues for Space Exploration – the MASE project. P. Schwendner^{1,2}, C. Cockell¹ and the MASE team:
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Introduction: Is life unique to our planet? Based on recent data Mars is representing the most prominent example to contemplate the occurrence of microbial life other than Earth. Despite the fact that the geologic and climatic conditions prevalent on Mars bear a challenge for the survival and evolution of Earth-like organisms, past and possible present Mars may have hosted conditions that could be habitable. Assessing the habitability of Mars and detecting life, if it was ever there, depends on knowledge of whether the combined environmental stresses experienced on Mars are compatible with life and whether a record of that life could ever be detected. However, our current ability to make these assessments is hampered by a lack of knowledge of how the combined effect of different environmental stresses influence the survival and growth of anaerobic organisms. Many combinations of stress, such as high radiation conditions combined with high salt and low temperature, relevant for early Mars, have not been investigated.

In particular, osmotic stress is one of the major limitations for cell growth. Microorganisms have evolved a number of adaptations, both structural and metabolic, to counteract osmotic stress. These strategies are well-understood for organisms in NaCl-rich brines such as the accumulation of certain organic solutes (known as either compatible solutes or osmolytes). Less well studied are responses to ionic environments such as sulfate-rich brines which are prevalent on Earth but can also be found on Mars.

Therefore, the MASE (Mars Analogues for Space Exploration) project [1] was proposed to gain knowledge on Mars habitability and on adaptation of anaerobic life forms to extremes by analysing Mars analogues on Earth while also allowing to optimise mission operations and life detection by applying and testing the instruments on-field.

Methods: We selected a number of anaerobic environments based on characteristics that make them analogous to past and present locations on Mars (Fig. 1).

We implemented a cultivation approach to enrich microorganisms from these environments under anaerobic conditions using a defined basal medium that would facilitate future physiological comparisons. We then not only isolated anaerobic microorganisms, carried out a study of their basic physiology and deposited these organisms in the DSMZ (Deutsche Sammlung

Site	Anoxia	Low temp	Aridity	High salinity	Acidity	Low nutrient availability
Rio Tinto sediments and subsurface (Spain)	X				X	X
Cold sulfidic springs of the Sippenauer Moor (Germany)	X	X				X
Basaltic environments at Lake Graenavatn (Iceland)	X	X	X		X	X
Boulby salt mine (UK)	X		X	X		X
Permafrost (Canada, Russia)	X	X				X



Fig. 1 Summary of sampling sites

von Mikroorganismen und Zellkulturen GmbH) culture collection but also applied molecular techniques such as high-throughput sequencing [2] and metabolomics [3].

We chose *Yersinia intermedia* MASE-LG1 a strain isolated from an Icelandic lake as test organism. It is known for its abilities to adapt to a wide variety of habitats of rapidly changing environmental conditions. In order to identify which roles the different salts play in the global metabolic response, *Y. intermedia* was exposed sustained salt stress induced by either MgSO₄ or NaCl. After metabolite extraction, metabolic profiles from three replicate cultures of *Y. intermedia* MASE-LG-1 grown under three different conditions (e.g. control salt stressed in MgSO₄, and salt stressed in NaCl) were obtained.

Results: A range of microorganisms were isolated from these analog environments and alongside these organisms environmental DNA provided a complete picture of the microbiome [2].

In total 1,131 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. The microorganisms that we isolated are now deposited in the culture collection, DSMZ [4]. They have been used to i) undertake fossilization studies to better understand how biosignatures may be preserved from anaerobic microorganisms [5] and ii) investigate their response to various stresses such as radiation, desiccation, oxidizing compounds, osmotic stress and combinations thereof [6]. In more detail, we investigated the global metabolic response of the anaerobic bacterium *Yersinia intermedia* MASE-LG-1 to osmotic salt stress induced by either magnesium sulfate (MgSO₄) or

NaCl at the same water activity (0.975). Using a non-targeted mass spectrometry approach, the intensity of hundreds of metabolites was measured. The compatible solutes L-asparagine and sucrose were found to be increased in both MgSO₄ and NaCl compared to the control sample, suggesting a similar osmotic response to different ionic environments. We were able to demonstrate that *Yersinia intermedia* MASE-LG-1 accumulated a range of other compatible solutes. However, we also found the global metabolic responses, especially with regard to amino acid metabolism and carbohydrate metabolism, to be salt-specific, thus, suggesting ion-specific regulation of specific metabolic pathways.

References:

[1] www.mase-eu.org [2] Perras A. *et al* (2017) in review. [3] Schwendner P. *et al* (2017) in review. [4] Cockell C. S. *et al* (2017) *Intern Journal Astrobiol* <https://doi.org/10.1017/S1473550417000246>. [5] Gabyoyer F. *et al* (2017) *Scientific Reports* 7,8775. [6] Beblo-Vranesevic K. *et al* (2017) *PLoS One*. 12:e0185178.

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