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Metabolic response of *Yersinia MASE-LGI* to osmotic stress and ionizing radiation

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The MASE (Mars Analogues for space exploration) project intends to gain deeper insights into the habitability of Mars by searching for anaerobic extremophiles in Mars analogue environments on Earth like the cold sulfidic springs in Germany, the deep-subsurface salt mine in UK, the iron-rich Rio Tinto and the cold acidic lake Graenavatn in Iceland. From the latter, the MASE team isolated a *Yersinia* sp. strain.

The surface of Mars is known to host deposits of magnesium and iron sulfates, suggesting that liquid water on that planet might contain high concentrations of sulfates. Halites have also been identified. Therefore, of significance to astrobiology and understanding the habitability of Mars is to understand the microbial response to sulfate and chloride salt exposure in combination with the ubiquitous ionizing radiation in the near-surface of Mars.

We used metabolomics to investigate the global metabolic response of *Yersinia MASE-LG-I* strain to sustained salt stress induced by either MgSO₄ or NaCl. In addition to the effects of salt, the effect of ionizing radiation was analysed both individually and in combination with salt stress. The metabolites were extracted from cells grown in salt supplemented MASE-medium or from cells that were exposed to ionizing radiation. Hydrophilic interaction liquid chromatography (HILIC) was carried out on a Dionex UltiMate 3000 RSLC system using a ZIC-pHILIC column to analyse the extract. The metabolic adaptation to salt stress was investigated by systematically identify already known osmoprotectants summarized and publicly available in the DEOP database (<http://www.cbrc.kaust.edu.sa/deop/index.php>). The ability to produce and accumulate compatible solutes is one adaptation to osmotic stress that is conserved across not only across prokaryotes but across all domains of life. In total, 36 metabolites were identified which are linked to osmoprotective activity. In addition, the results indicate that the majority of putatively identified metabolites are part of amino acid or carbohydrate metabolism. The level of 75% of the metabolites found in these two parts of the metabolism were higher in MgSO₄ stressed samples. The control and stressed samples had a completely different response to X-ray irradiation. In the controls, the majority of these metabolites are less abundant after irradiation. In the stressed samples, the intensity of these metabolites is higher after exposure to X-rays.

The findings revealed that there is an increase in the production of osmoprotectants. These findings advance our understanding of the biochemical responses and pathways that may allow organisms to grow under Mars and Mars-like conditions.

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