Goldschmidt 2024 Abstract
https://doi.org/10.46247/gold2024.24593

High pressure experimentation for informing the search for life on icy ocean worlds

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Understanding the habitability and adaptability of life under extreme conditions is a crucial aspect for astrobiology, and is vital for developing strategies to prevent microbial contamination during space missions and ensuring the integrity of astrobiological discoveries. The current interest in the icy moons Titan, Enceladus, and Europa as targets for astrobiological studies, and the potential for Earth's deep subsurface to harbor clues about life on early Earth, makes high pressure microbiological research is particularly timely. While the pressures known to support life on Earth are 0.1- 125 MPa, Titan, Enceladus, and Europa are thought to have subsurface liquid oceans with pressures varying from 1.5-800 MPa (Weber et al., 2023; Sohl et al., 2014), and habitability is a major unknown. Experimentation in these pressure ranges is challenging, but essential to move the science forward with astrobiologically relevant studies of microbial adaptability.

This study presents an Adaptive Laboratory Evolution (ALE) experiment with the bacterium *Shewanella oneidensis* MR-1, with the goal of understanding its evolutionary adaptation to high pressure environments, towards informing habitability on icy ocean worlds. Several studies have treated *S. oneidensis* with high pressure, but none to date have used ALE to adapt the organism to grow and reproduce under pressures greater than 125 MPa.

Here, we present data from an ongoing experiment, where *S. oneidensis* MR-1 is subjected to incremental increases, simulating the pressure conditions of icy ocean world subsurface environments. The sample is currently growing at 38 MPa, well above its known pressure range, with an end goal of 150 MPa or higher. Through protein assays and different staining techniques, we show that the sample continues to grow and is viable while under pressure. We are presenting a comparative genomic and transcriptomic analysis. The results are expected to shed light on the mechanisms by which microorganisms can adapt to, and potentially thrive in, high pressure conditions, challenging our understanding of the limits of life.

Sohl, et al. (2014). *JGR: Planets*, *119*(5), 1013–1036. Weber, et al. (2023). *Life*, *13*(8), 1726.