Effects of Elevated Pressure on Microbial Growth: Using a Model Extremophile to Explore Habitability in Subsurface Ocean Worlds. G.C. Oliver[1], A. Cario[1], K. L. Rogers[1,2], [1]Department of Earth and Environmental Sciences, (oliveg@rpi.edu), [2]New York Center for Astrobiology, Rensselaer Polytechnic Institute, Troy, NY, USA.

Deep-sea hydrothermal systems have been proposed as a potential location for the emergence of life on Earth [1] and are analogous to potential habitable subsurface ocean environments on Europa [2] and Enceladus [3]. While deep-sea environments host a variety of geochemical and geothermal conditions, elevated pressures are common to all aqueous subsurface ecosystems [4]. Understanding how microbes adapt to and thrive in high-pressure environments is an essential link between microbial subsurface processes and planetary habitability. We are using a model extremophile, *Archaeoglobus fulgidus*, to determine how elevated pressures affect the growth, metabolism, and physiology of deep-sea and subsurface microorganisms. *A. fulgidus* cycles carbon and sulfur via heterotrophic and autotrophic sulfate reduction in various high temperature, ambient pressure and high-pressure niches on Earth including shallow marine vents, deep-sea hydrothermal vents, deep geothermal wells, and deep oil reservoirs [5,6].

Here we report the results of *A. fulgidus* growth experiments at optimum temperature, 83°C, and pressures up to 60 MPa for heterotrophic growth on lactate and sulfate, as well as preliminary results for autotrophic high-pressure growth. For heterotrophic conditions, exponential growth was observed over the entire pressure range tested, although lower cell densities were observed at 50 and 60 MPa compared to experiments conducted from 0.1-40 MPa. At pressures up to 40 MPa, cell density, and growth rates were comparable to ambient pressure controls. Additionally, high-pressure (0.1-50 MPa) batch cultivation stimulated biofilm formation, likely a stress response to elevated sulfide concentrations. This response has been directly observed, where *A. fulgidus* was identified thriving in biofilm structures in deep-sea hydrothermal systems [7]. These biofilms and other organic polymeric substances may serve as useful biomarkers and are ubiquitous in surface and subsurface environments on Earth [8]. For example, organic polymeric substances have been detected in subsurface samples in the Mars analog environments of Rio Tinto [9].

Traditional high-pressure batch cultivation requires decompression during subsampling, which could lead to loss of volatiles, potentially negatively impacting growth of strains that rely on dissolved gas species in their metabolism. For *A. fulgidus* growth on lactate and sulfate, subsampling decompression did not impact growth rates or cell density from 10-30 MPa, and only slightly impacted growth at 40 MPa. However, decompression did negatively impact autotrophic growth on H_2/CO_2 and thiosulfate during exponential growth likely due to decompressive loss of H_2 and CO_2 during subsampling. Nonetheless prior to decompression, autotrophic growth at 20 MPa was comparable to growth at ambient pressures suggesting that *A. fulgidus* is piezotolerant for both heterotrophic and autotrophic metabolisms.

In summary, these results show that *A. fulgidus* can successfully grow at pressures up to 60 MPa after multiple decompressions during heterotrophic growth and produces biofilm, which may be an advantageous adaptive strategy and potentially serve as a useful biomarker. Furthermore, decompression can inhibit growth of strains whose metabolisms depend on dissolved volatiles. These results have important implications for future life detection missions to subsurface ocean worlds such as Europa and Enceladus. For example, life detection via enrichments would necessitate growth chambers that reflect *in situ* pressures (<10 MPa on Enceladus seafloor and 100-200 MPa at Europa's seafloor). Putative high-pressure hydrothermal systems on those moons could realistically support metabolisms similar to those explored here, which depend on CO_2, sulfate, and simple organic compounds (e.g. lactate). Continuing exploration of the effects of pressure on growth of model extremophiles like *A. fulgidus* is an essential component of understanding habitability of Europa, Enceladus, and other subsurface ocean worlds.

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