Linking metabolism, elemental cycles and environmental conditions in the deep biosphere :

Growth of a model extremophile, *Archaeoglobus fulgidus*, under high-pressure conditions

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Background and Objectives

A majority of Earth's biosphere is hosted in subsurface environments[1,2] where global scale biogeochemical and energy cycles are driven by diverse microbial communities that operate on and are influenced by micro-scale environmental variables. While the subsurface hosts a variety of geochemical and geothermal conditions, elevated pressures are common to all subsurface ecosystems[3]. Here we are using a model extremophile, *Archaeoglobus fulgidus*, to investigate how elevated pressures affect the growth, metabolism, and physiology of subsurface microorganisms. Initial experiments focused on high-pressure growth of *A. fulgidus* growing under batch conditions via heterotrophic sulfate reduction[4]. We focused on the following questions :

- Can *A. fulgidus* grow under the high-pressure conditions found in many of the subsurface environments where it has been identified ?
- How do exponential growth rates and cellular yields respond to increasing pressures ?
- What are the morphological responses to high-pressure growth ?
- Do traditional high-pressure batch culture techniques, which usually require decompression for subsampling, affect observed growth rates ?

Results

How does pressure affect *A. fulgidus* growth ?

Physiological Growth from 1-400 bars

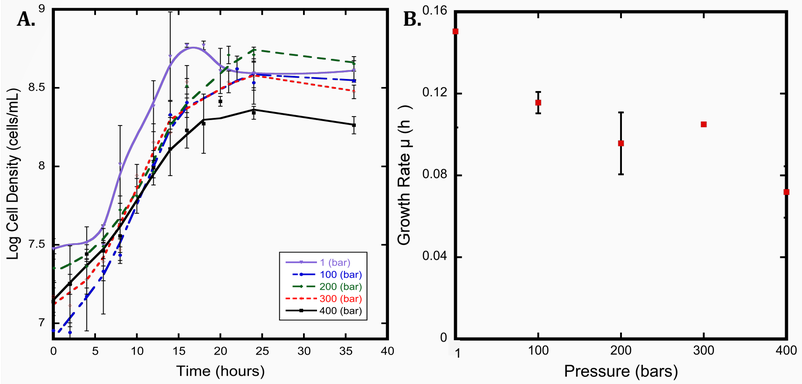

Figure 1. High-pressure growth conditions for *A. fulgidus* represented by the growth curves (A) and the correlated growth rates (B) over 1 to 400 bar pressure range.

Cell Densities impacted from 500-800 bars

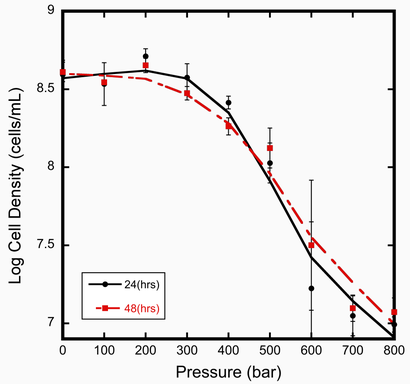

Figure 2. Maximal cell densities reached by *A. fulgidus* when grown over a range of pressures (1-800 bars) at 24hrs and 48 hrs

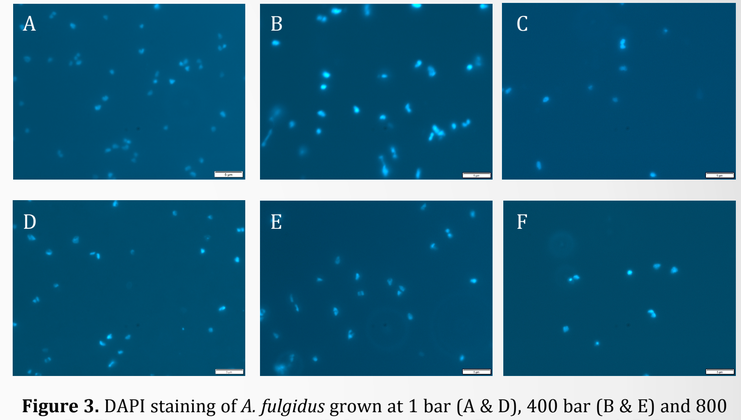

Figure 3. DAPI staining of *A. fulgidus* grown at 1 bar (A & D), 400 bar (B & E) and 800 bar (C & F) after 24hrs (A–C), 36hrs (D & E) and 48hrs (F) of incubation. A, B, D & E are 20X dilution; E & F have no dilution. Bar, 5 μm.

Methods

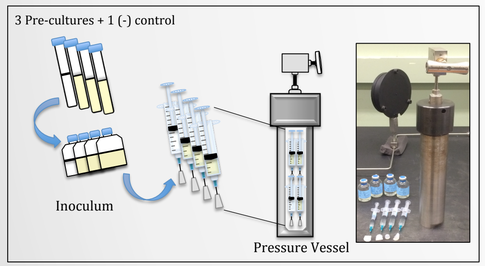

Diagram 1. High-pressure batch culture procedure and setup

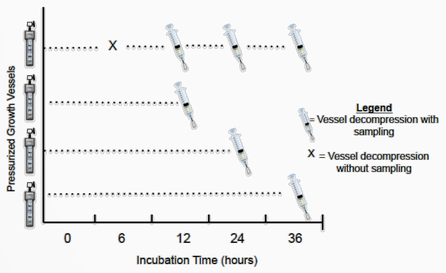

Diagram 2. Experimental schematic for subsampling cellular integrity tests

Here we performed batch culture growth (Diagram 1) of *A. fulgidus* at optimum temperature (83°C) for pressures up to 800 bar. Growth rates, cellular yields and cell morphologies are reported. DAPI staining method was used for cellular enumerations. All experiments were done in triplicate (at least) for statistical significance.

Traditional high-pressure batch cultivation of microbial species[5] usually requires short-periods of decompression (and cooling for thermophiles) when collecting samples for cell enumeration and other analysis. In order to test if these methods affected the observed growth rates we conducted a series of experiments (Diagram 2 and Table 1) in several high pressure vessels and compared growth rates for those cultures that were not decompressed to those that were.

High-Pressure Cultivation Experiments	Growth Pressure (bar)	Initial Inoculum (%)	Sampling interval (hrs)	Vessel sampling interval (hrs)	Total length of experiment (hrs)
Batch Culture Growth (Growth Curves and Maximum Cell Density)	1	5	2	8	36
	100	5	2	8	36
	200	5	2	8	36
	300	5	2	8	36
	400	5	2	8	36
	500	5	24	24	48
	600	5	24	24	48
	700	5	24	24	48
	800	5	24	24	48
High-Pressure Subsampling Cellular Integrity	100	5	12	12	36
	200	5	12	12	36
	300	5	12	12	36
	400	5	12	12	36

Table 1. Description of high-pressure experimental design and duration

Does decompression impact *A. fulgidus* growth ?

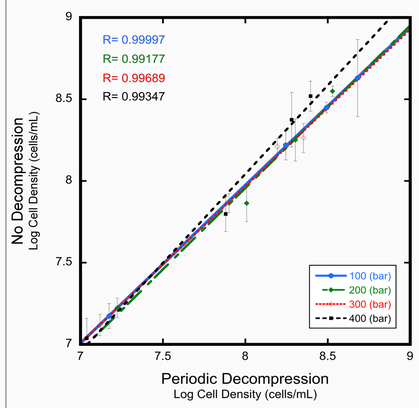

Figure 4. Cell density ratio of non decompressed cells and periodic decompressed cells, tested for *A. fulgidus* cells grown 36hrs over 100 to 400 bars.

Does pressure induce changes in *A. fulgidus* cell behavior?

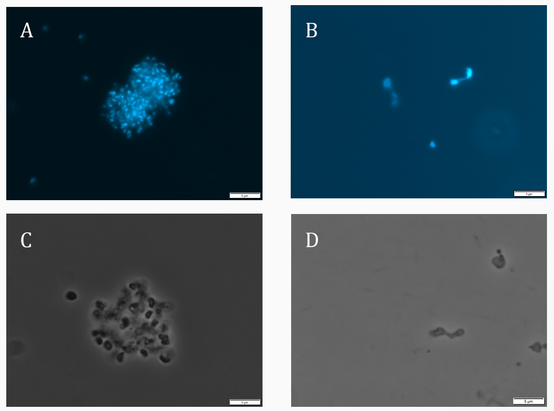

Figure 5. Pressure stimulation of cell flocculation (A & C) and morphological changes seen with DAPI staining (A) and phase contrast microscopy(C) of *A. fulgidus* grown under 100 bars after 36hrs (A & C) and 500 bars after 24hrs(B & D). Bar, 5 μm.

Conclusions

- Exponential growth rates for *A. fulgidus* were 0.15hr⁻-0.065hr⁻ ± 0.047 at pressures up to 400 bars
- Lower cell densities was observed at 500 bars and there was no growth from 600-800 bars
- Cellular aggregation occurred after 24 hours at pressures 100-500 bars
- Cell elongation was seen at pressures over 500 bars, which also negatively impacted growth
- Repeated subsampling decompressions required for batch experiments did not significantly impact exponential growth from 100-400 bars (cell density ratios ≈1)

Our data suggest that *A. fulgidus* continues carbon, sulfur and energy cycling at least up to 400 bars, representing a variety of subsurface environments.

The ability of subsurface organisms to drive biogeochemical cycles at elevated pressures is a critical link between the surface and subsurface biospheres and understanding how species-scale processes operate under these conditions is a vital part of the global scale biogeochemical models.

References

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