Habitability in the Piezosphere: Experimental investigation of the high-pressure growth of a model extremophile, *Archaeoglobus fulgidus*

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Background and Motivation

Extreme conditions of deep-sea hydrothermal systems have been proposed as a potential location for the emergence of life on Earth[1,2] and are analogous to potential habitable subsurface environments on Mars[2], Europa[3], and Enceladus[4]. While the subsurface on Earth hosts a variety of geochemical and geothermal conditions, elevated pressures are common to all subsurface ecosystems[5]. Here we are using a model extremophile, *Archaeoglobus fulgidus*, to investigate how elevated pressures affect the growth, metabolism, and physiology of subsurface microorganisms. These experiments focus on sulfate reduction by *A. fulgidus* from 1-800 bar. We targeted the following questions:

- Can *A. fulgidus* grow at the elevated pressure conditions of terrestrial and extraterrestrial subsurface environments?
- How do exponential growth rates and cellular yields respond to increasing pressures ?
- Do cellular morphologies vary with high-pressure growth?
- Does decompression affect the growth of high-pressure microbial cultures?

Habitability at depth on Europa

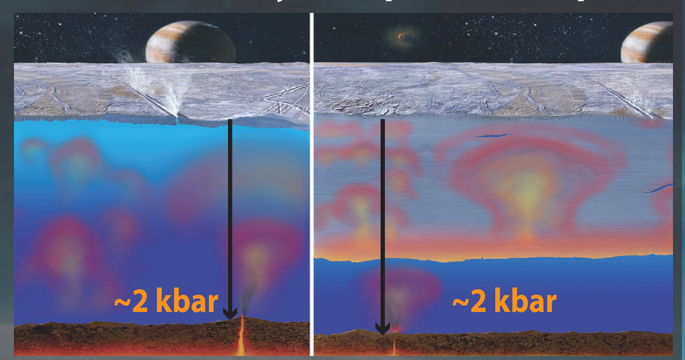

Figure 1: Two proposed cross section profiles of Europa's ocean, all potentially habitable deep-sea environments are at ~1.4-2 kbar[3].

Ocean-Core interface on Enceladus

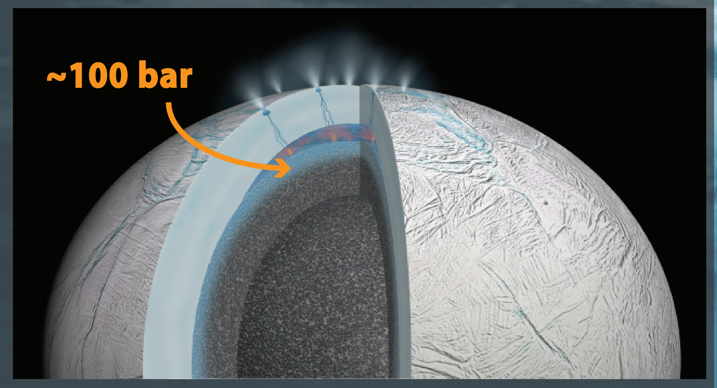

Figure 2: Proposed hydrothermal activiy on Enceladus from ~80-500 bar[4]. Credit Figure 1 & 2: NASA/JPL-Caltech

Methods

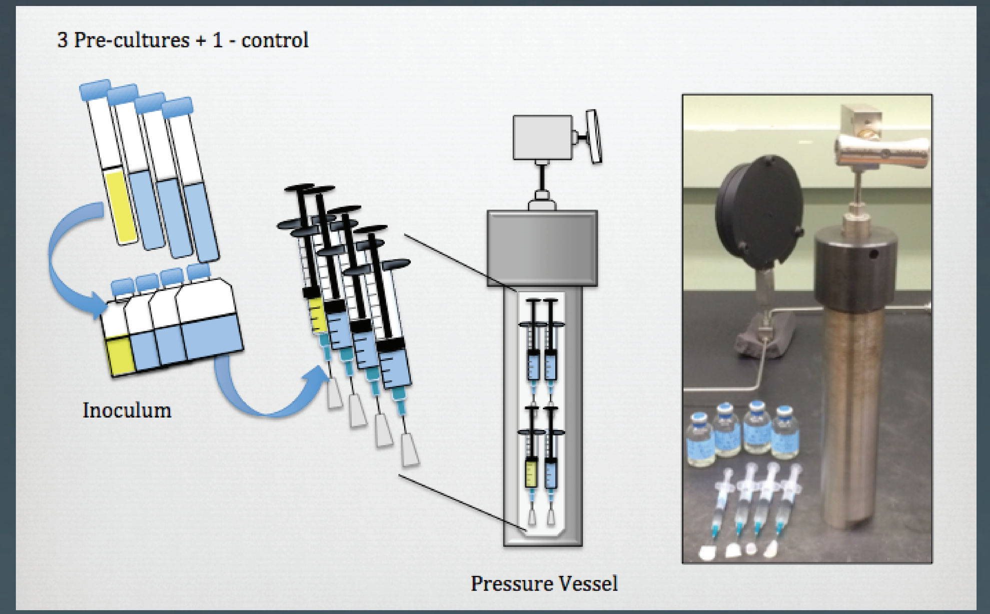

Figure 3: High-pressure batch culture procedure

We performed batch culture growth (Figure 3) of *A. fulgidus* at optimum temperature (83°C) for pressures up to 800 bar. Growth rates, cellular yields and cell morphologies, determined from DAPI-stained subsamples are reported for triplicate experiments.

Traditional high-pressure batch cultivation of microbial species usually requires short-periods of decompression[6] (and cooling for thermophiles) when collecting samples for cell enumeration and other analyses. In order to test if these methods affected the observed growth rates we conducted a series of experiments (Figure 4 and 5) in several high pressure vessels and compared cell densities for those cultures that were not decompressed to those that were.

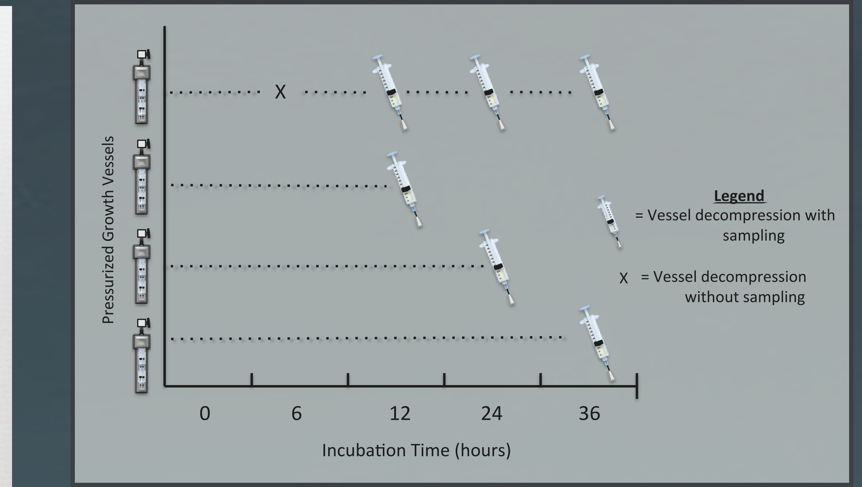

Figure 4: Experimental setup to test cellular growth rates with subsampling decompression.

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

Figure 5: High pressure experimental design

Results

How does pressure affect *A. fulgidus* growth?

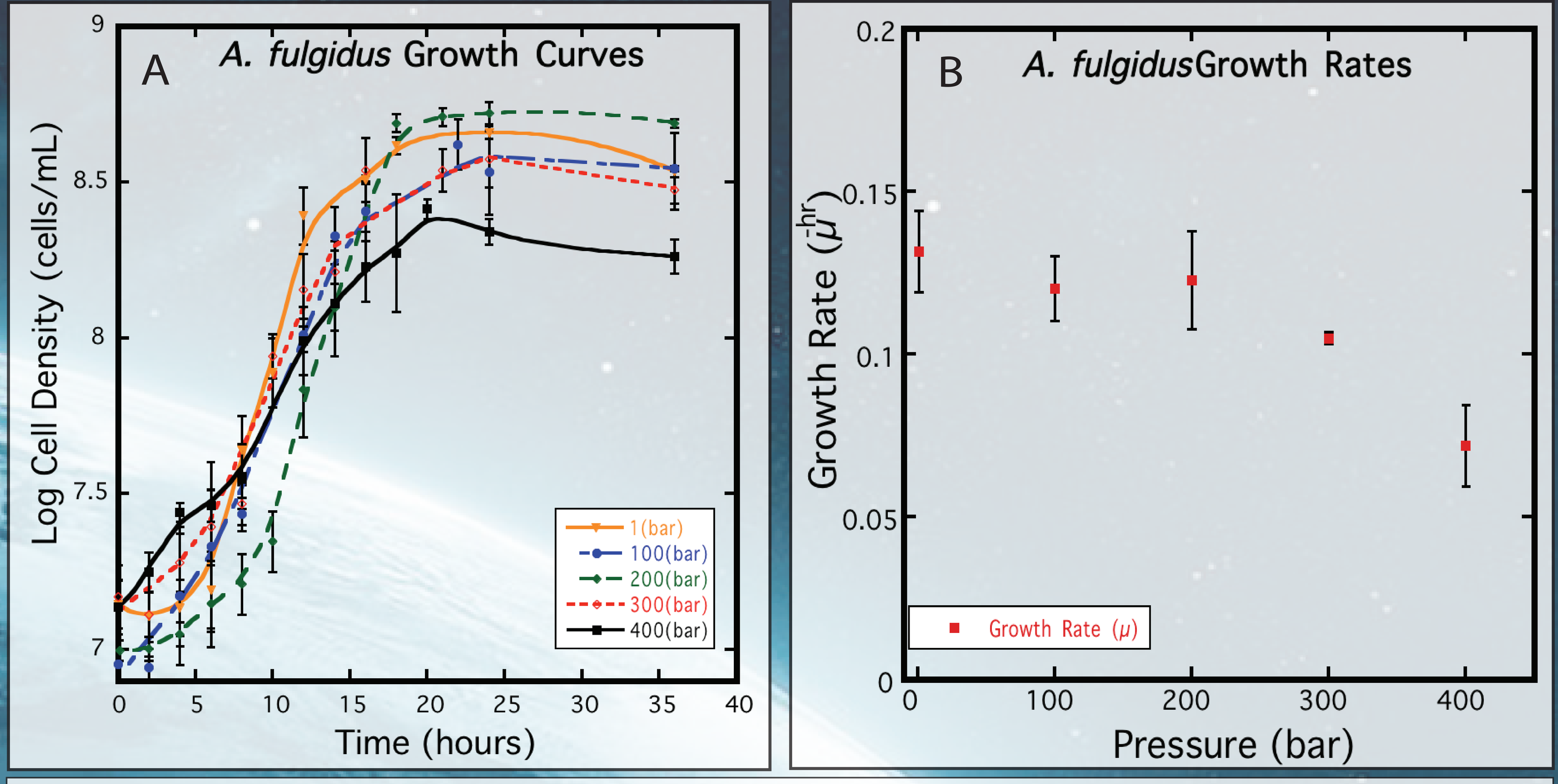

Figure 6: High-pressure growth of *A. fulgidus* from 1-400 bar. (A) Exponential growth curves and (B) growth rates (μ) as a function of pressure.

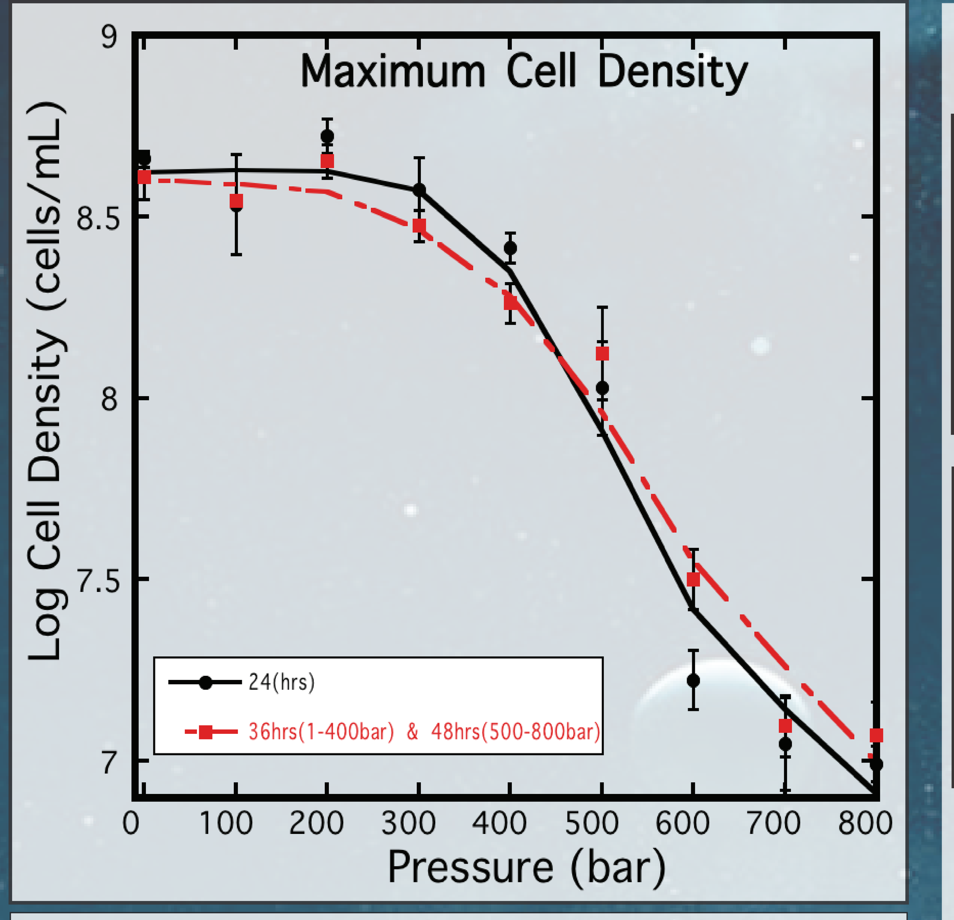

Figure 7: Maximal cell densities for *A. fulgidus* over a range of pressures (1-800 bar) at 24hrs and 48hrs.

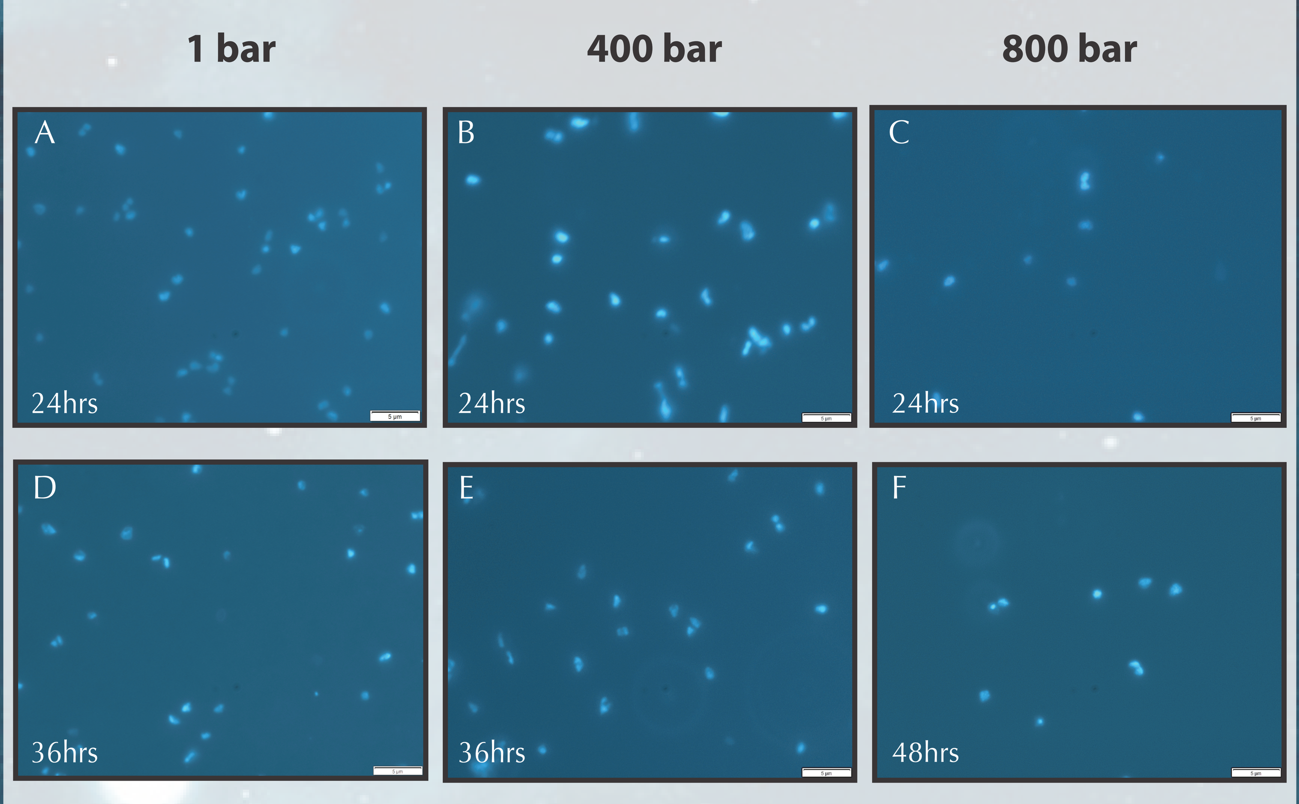

Figure 8: DAPI stained *A. fulgidus* grown at 1 bar (A & D), 400 bar (B & E) and 800 bar (C & F) after 24hrs (A-C), 36hrs (D & E) and 48hrs (F) of incubation. A, B, D and E are 20X dilution; E & F have no dilution. Bar, 5µm.

Does decompression impact *A. fulgidus* growth?

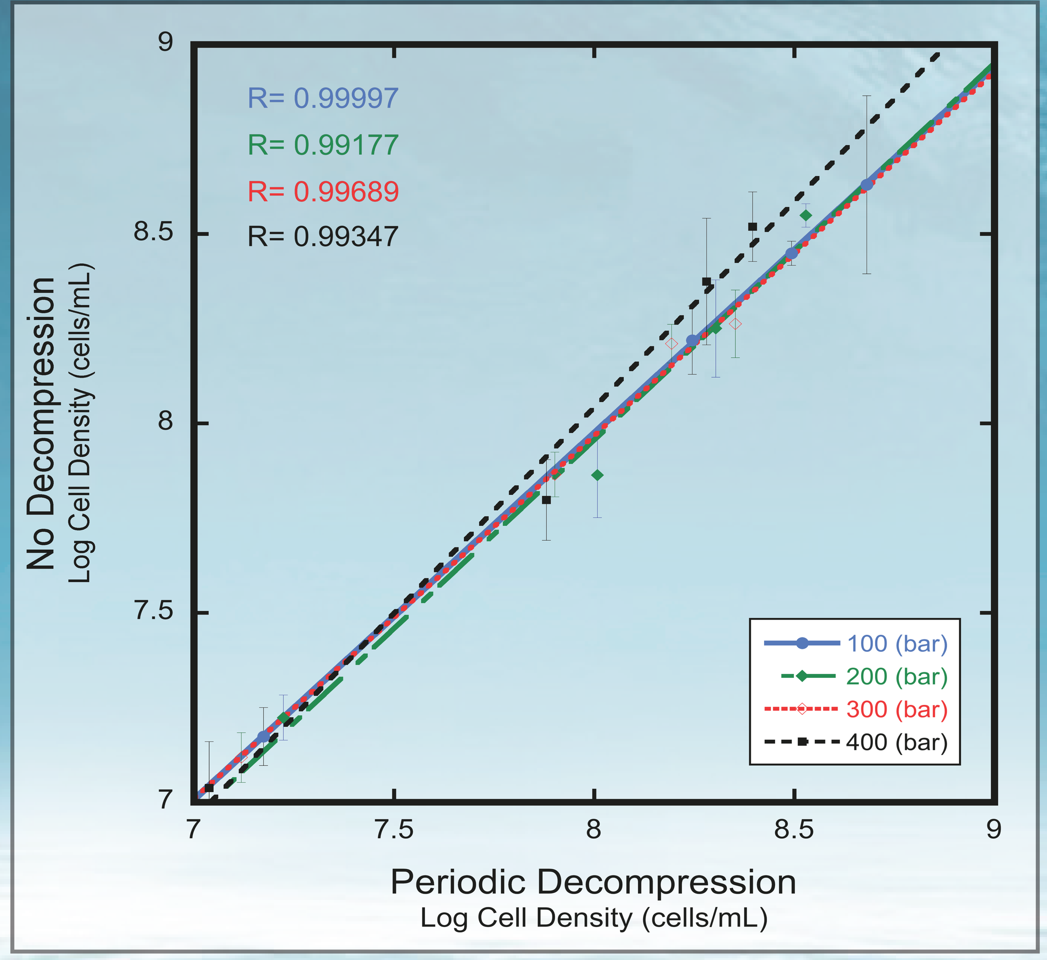

Figure 8: Cell density ratio of non-decompressed cells and periodically decompressed cells, for *A. fulgidus* grown 36 hours from 100-400 bar.

Does *A. fulgidus* cell behavior vary over a range of pressures?

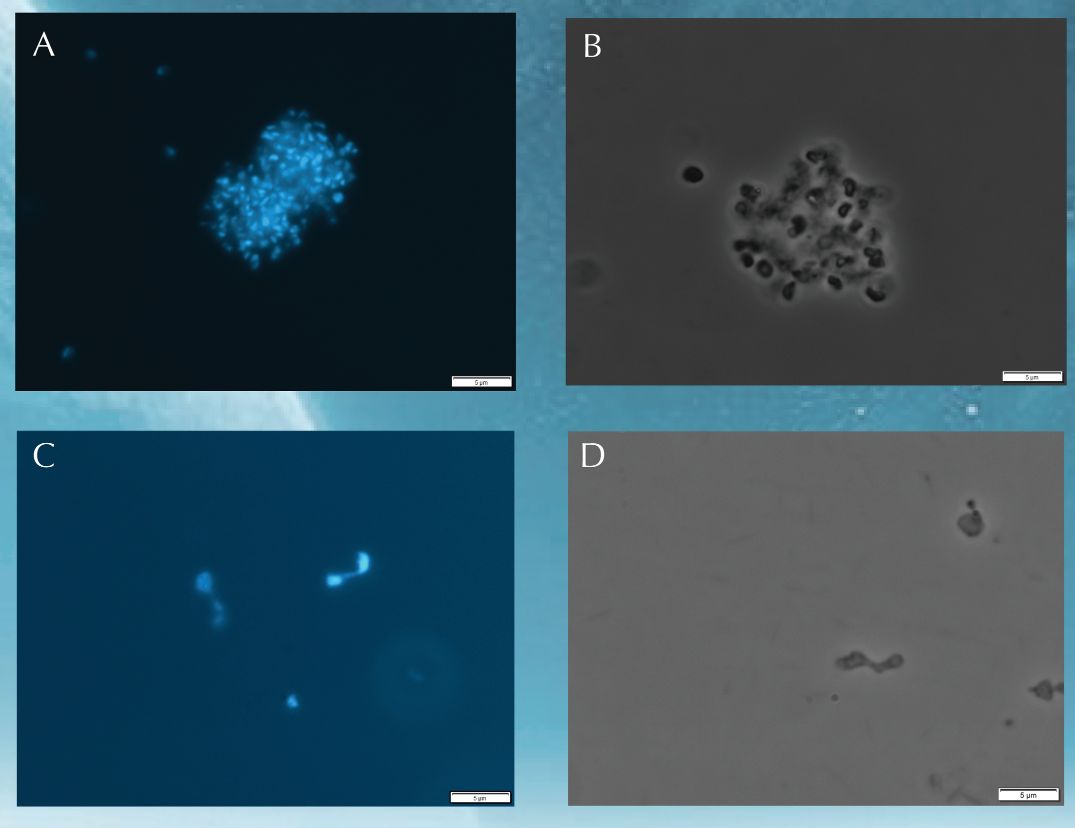

Figure 8. Cell flocculation (A & B) observed over *A. fulgidus* physiological range from 1-400 bar. Morphological changes seen with DAPI staining (C) and phase contrast microscopy (D) of *A. fulgidus* grown under 100 bar after 36hrs (A & B) and 500 bar after 24hrs(B & D). Bar, 5 µm.

Conclusions

- Exponential growth rates for *A. fulgidus* were 0.13 hr^{-1} - 0.071 hr^{-1} ± 0.048 at pressures up to 400 bar (~4000 meters below sea level depths).
- Lower cell densities were observed at 500 bar with no growth from 600-800 bar.
- Cell flocculation was observed from 1-400 bar and cell elongation was seen at pressures over 500 bar, which also negatively impacted growth.
- Repeated decompressions from subsampling did not significantly impact exponential growth from 100-400 bar (cell density ratios ~1).

Our data suggest that *A. fulgidus* continues carbon, sulfur and energy cycling uneffected by elevated pressures up to 400 bar with little to no affect of decompression on cell denisties. These pressure ranges represent a variety of deep-sea and subsurface environments on Earth. Today, extremophiles, like *A. fulgidus*, thriving in these high temperature and pressure regimes exhibit unique adaptive strategies that may lend insight into how microbial communities thrive at Earth's seafloor and how life might thrive in the subsurface of other ocean worlds throughout the solar system.

References

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