Filamentous microbial growth through anaerobic chemosynthetic processes from the crustal subseafloor of the Juan de Fuca Ridge flank.

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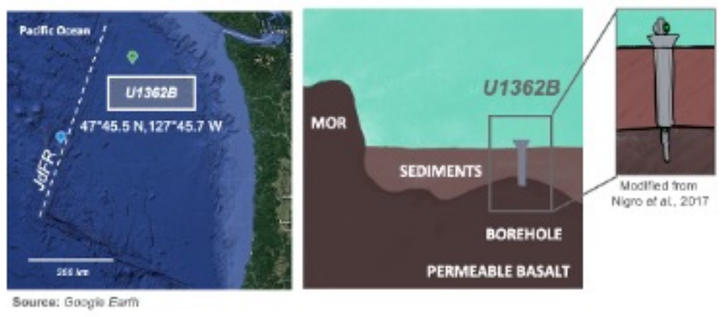
Abstract

Anoxic subseafloor crustal fluids (60-65 °C and pH 7.0-7.3) from Juan de Fuca Ridge (JdFR) flank were used in this study to enrich and potentially isolate anaerobic chemosynthetic hydrogenotrophs driving primary production in these environments. Selective enrichments using H_2 as primary electron donor (PED), CO_2 or NO_3^- as terminal electron acceptors (TEA), and CO_2 as the carbon (C) source were incubated at temperatures between 65 °C and 75 °C. Cellular morphologies and sizes associated with all cultures tend to vary with incubation times from small cocci to filamentous cells. Similar extensive filamentous, "fungi-like" morphologies containing "spore-like" cells develop at later growth stages under both CO_2- and NO_3^--reducing conditions, suggesting that the same microorganism might be capable of both anaerobic H_2-oxidation processes. Microbial transfers made every two weeks for the CO_2-reducing culture and three weeks for the NO_3^--reducing culture reflect overall doubling times between 4 and 5 days. Additional phylogenetic characterizations will help reveal whether observed morphological similarities are founded on phylogenetic relationships or whether they represent a broader lifestyle adaptation to their crustal subseafloor environment.

Methods

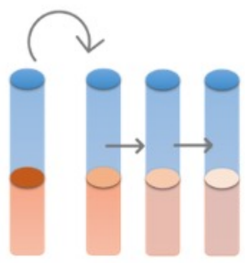
Inoculum
Subseafloor crustal fluids of borehole U1362B at 60-65°C and pH 7.3-7.5



1. Enrichment Conditions

Conditions	CO_2 Reduction	NO_3^- Reduction
PED	H_2	H_2
TEA	CO_2	NO_3^-
C-source	CO_2	CO_2
pH	7.0	7.0
% w/v NaCl	1.8	2.0
Temp	75°C	65°C

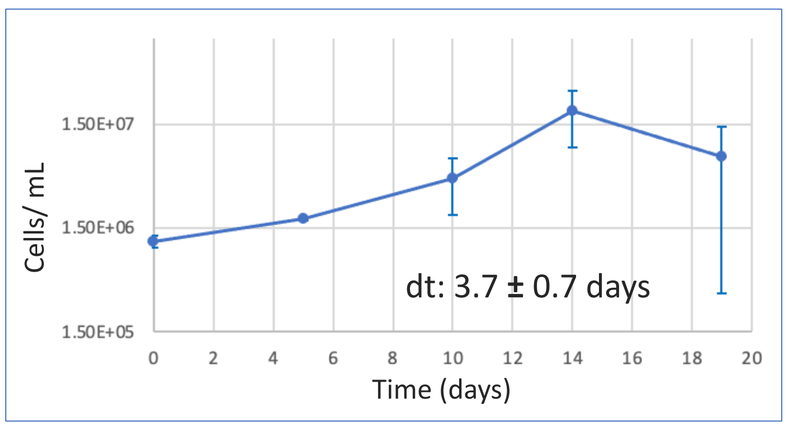
2. Serial Dilutions



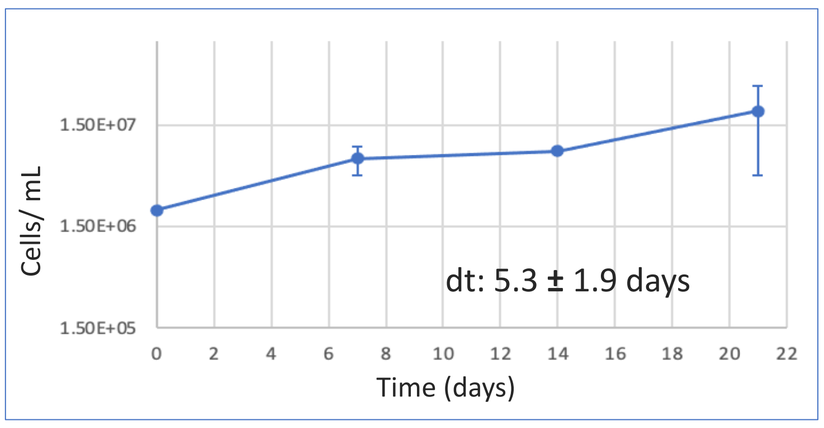
x6 CO2 Reduction
x5 NO_3^- reduction

Results

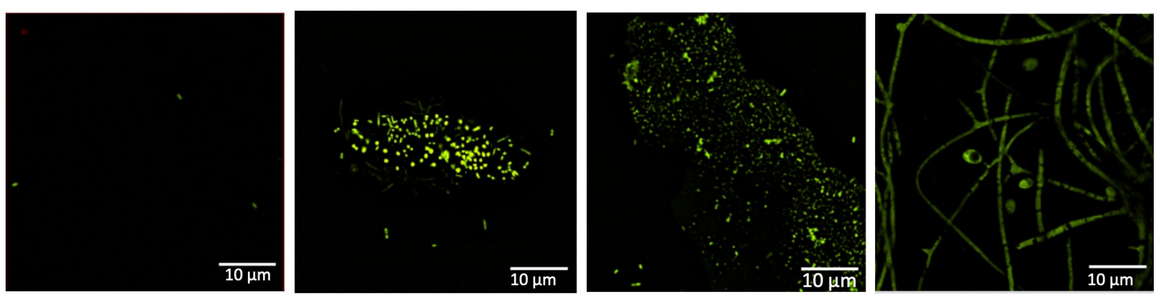
CO_2 Reducing Culture OE at 80°C

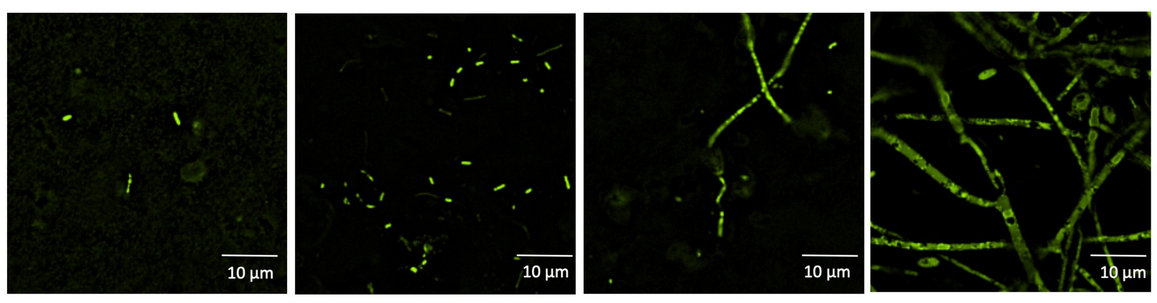


NO_3^- Reducing Culture 34 at 65°C

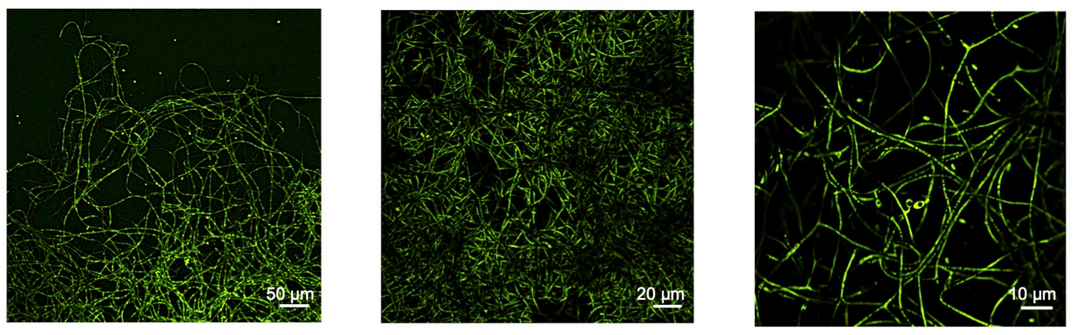


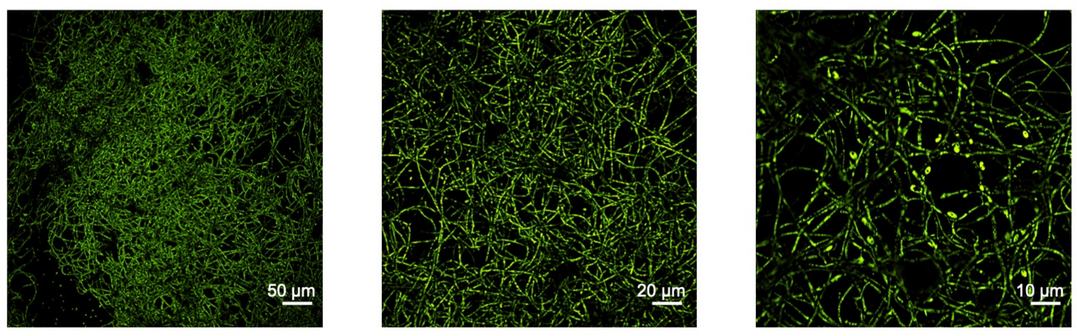
Doubling times of anerobic hydrogenotrophs from the Juan de Fuca Ridge flank ranged from 4-5 days. Highest cell densities were obtained on day 14 for CO_2 reducing culture OE (2.04E+07 cells/mL) and 21 days for NO_3^- 34 (2.05E+07 cells/mL), with cells in culture OE reaching the death phase between 14 and 19 days.





Acridine Orange (AO) stained cells displayed cocci morphologies (about 0.5-2 µm in length) in early batch growth. Rod and filamentous morphologies (4-10 µm in length) and the formation of filament networks (100s of µm in length) were observed in both cultures in later stages of growth. All microscopy images reflect 0.5mL of culture sample





Cultures were incubated for 14 days (CO_2 reduction) or 18-21 days (NO_3^-reduction) prior to sampling 0.5mL of culture for AO imaging. Extensive filamentous growth has been shown to form mat-like networks on a larger scale. When observing specific 4-10 µm long filaments, there appears to be brightly stained circular morphologies within a dimmer casing. Bright "spores" are seen both budding off the filaments or dispersed across the slide.

Discussion and Future Plans

We hypothesize that morphological similarities observed between CO_2 reducing culture OE and NO_3^{--} reducing culture 34 may either reflect phylogenetic relationships or an individual species capable of both CO_2 and NO_3^{--} reduction or broader lifestyle adaptations to the crustal subseafloor environment of the Juan de Fuca Ridge flank. Future SEM microscopy analysis will better capture the morphology of filaments and "spore"-like formations. While slow growth and low biomass production over time ultimately presents a challenge for DNA extraction required for phylogenetic analysis, molecular and phylogenetic efforts will aid in understanding the 16S rRNA gene identity of these microorganisms.

1. Baquiran, J.P.M., et. Al. (2016) *Front. Microbiol.* 7, 396; **2.** Rappé, M. et. Al. (2016) *ISME j.* 10(8), 2033-2047; **3.** Labonté, J. M. et. Al. (2017) *Front. Microbiol.* 8, 1434; **4.** Lin, H. T. et. Al. (2012) *Geochim. Cosmochim. Acta* 85, 213-227; **5.** Nigro, O. D. et. Al. (2017) *Mbiol* 8(2), e02129-16; **6.** Orcutt, B. et. Al. (2019) Cruise Resort for R/V Atlantis + ROV Jason Expedition AT42-11; **7.** Wee, S. Y. et. Al. (2021) *Appl. Environ. Microbiol.* 87(22), e01519-21;