Microbial physiologies of anaerobic primary producers from the crustal subseafloor of the Juan de Fuca Ridge flank.

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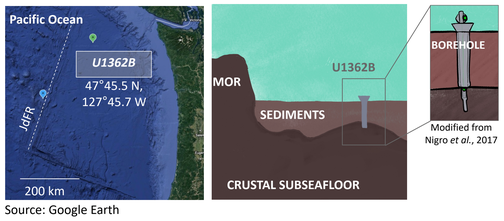
Abstract

Anoxic subseafloor crustal fluids (60-65 °C and pH 7.0-7.3) from Juan de Fuca Ridge (JdFR) flank were used 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 a terminal electron acceptor (TEA), and CO_2 as the carbon (C) source were incubated at temperatures between 65 °C and 80 °C. Two successful CO_2 reducing cultures were obtained after six consecutive serial dilutions at 75 °C, and three NO_3^- reducing cultures were obtained after five consecutive serial dilutions at 65 °C or 75 °C. Cellular morphologies and sizes tend to vary between cultures, with CO_2 reducing cultures growing as small cocci capable of biofilm formation and NO_3^- reducing cultures growing as small cocci that later develop into elongated filamentous cells and aggregates. Despite the higher free energy of reaction generally associated with the microbial oxidation of H_2 via NO_3^- reduction, in comparison to the microbial oxidation of H_2 via CO_2 reduction, CO_2 reducing cultures showed significantly faster doubling times while also reaching higher cell densities *in vitro*. Observed growth differences between the two CO_2 reducing cultures and three NO_3^- reducing cultures could indicate a broader lifestyle adaptation to low NO_3^- concentrations in the highly reduced subseafloor that favors CO_2 reduction over NO_3^- reduction. Current molecular phylogenetic endeavors will help reveal the phylogenetic identities associated with the anaerobic chemosynthetic cultures generated from subseafloor crustal fluids of the JdFR flank.

Sampling



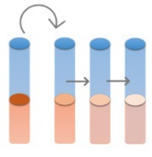
Source: Google Earth

Anoxic subseafloor crustal fluids (65 °C, pH 7.0-7.3) sampled from borehole U1362B[1-4] on May 2019[5] were used to enrich for CO_2 and NO_3^- reducing chemolithoautotrophs.

Enrichments

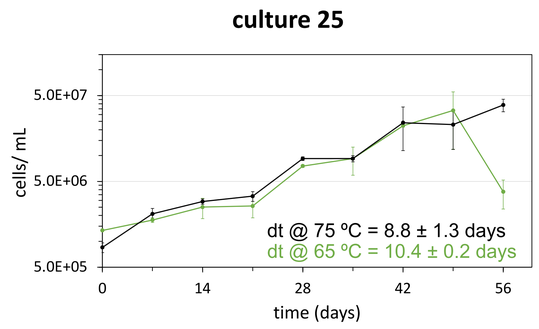
Conditions	CO_2 Reduction	NO_3^- Reduction
PED	H_2	H_2
TEA	CO_2	NO_3^-
C-Source	CO_2	CO_2
pH	7.0	7.0
% w/v NaCl	1.8	2.0
Temp	75-80°C	65-75°C
Base Media	DSM 141[6,7]	modified SME [6-8]

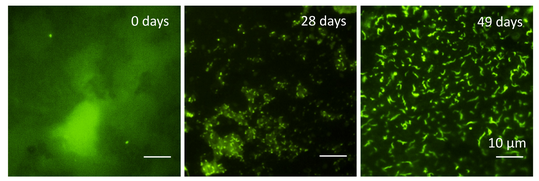
Serial Dilutions

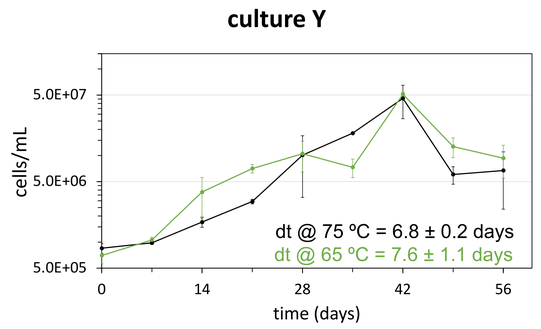


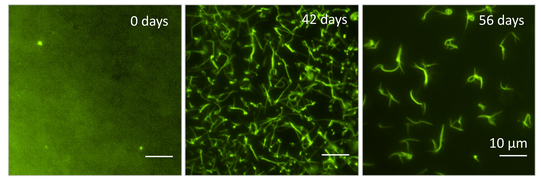
6x CO_2 reduction
5x NO_3^- reduction

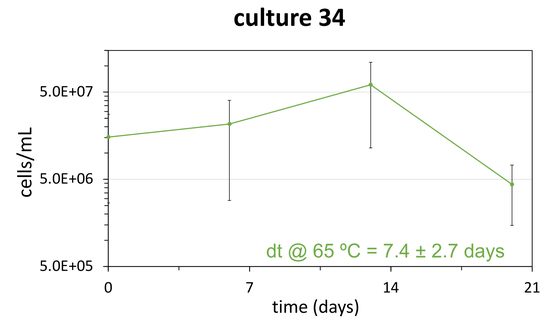
NO_3^- Reducing Cultures

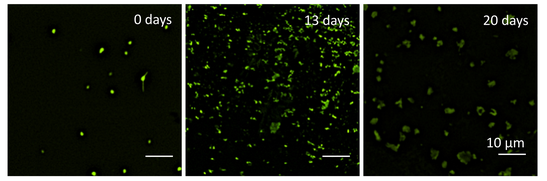






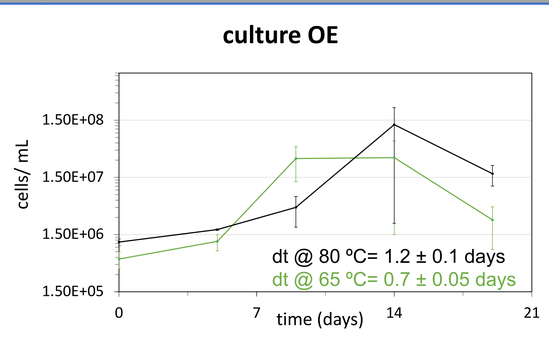


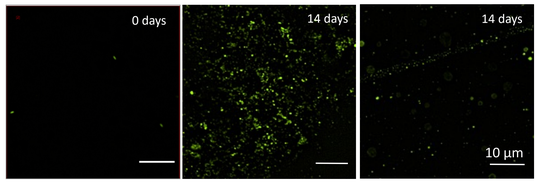


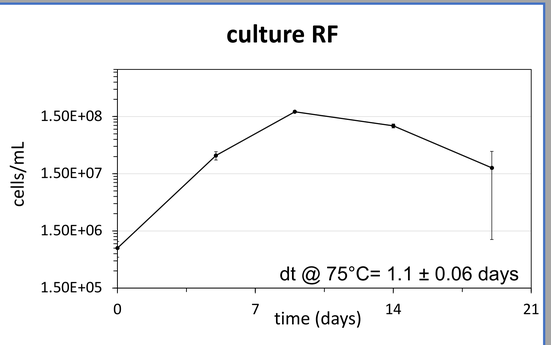


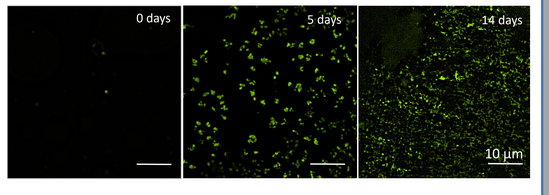
Cultures 25 and Y displayed similar cell doubling times under enrichment temperatures (75 °C) and *in situ* (65 °C) temperature conditions. Cell doubling times for all cultures ranged from ~ 7 - 10 days. Highest cell densities were reached between 42 days and 49 days for cultures Y and 25, and by 13 days for culture 34. Cells from all cultures had cocci shapes during early growth stages that appeared to elongate into filaments over time. Note: all microscopy images for all three cultures represent 0.5 mL of sample.

CO_2 Reducing Cultures









Culture OE displayed similar cell doubling times under enrichment temperatures (75 °C) and *in situ* (65 °C) temperature conditions. Cell doubling times for both culture OE and RF were ~ 1 day . Highest cell densities were reached between 9 days and 14 days for both cultures. Cells were small cocci that would aggregate over time and at times elongate during later stages. Note: all microscopy images for all three cultures represent 0.5 mL of sample.

Discussion

H_2-oxidizing CO_2-reducing cultures grew faster and to higher cell densities than H_2-oxidizing NO_3^--reducing cultures. Cell doubling times were not affected by temperature differences in CO_2- and NO_3^--reducing cultures. Growth of CO_2-reducing culture RF at *in situ* temperature conditions (65 °C) will be established in the future for comparison. Observed growth differences potentially reflect broader lifestyle adaptations that favor CO_2 reduction over NO_3^- reduction in the subseafloor crustal aquifer of the JdFR flank. Ongoing phylogenetic research efforts are focused on understanding the purity status of the cultures as well their genomic identities.

References

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Acknowledgments

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