## Cultivation of chemosynthetic life from the subseafloor crustal aquifer of the Juan de Fuca Ridge flank

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The sediment-covered subseafloor crustal aquifer of the Juan de Fuca Ridge (JdFR) flank contains hot, anoxic H2-rich crustal fluids that support microbial life [1]. Here, we used 65 °C anoxic crustal fluid collected from borehole U1362B (during R/V Atlantis cruise AT42-11 in May 2019) to enrich for chemosynthetic primary producers of the crustal subseafloor. Microbial enrichments, established at temperatures between 60 °C and 80 °C, selected for autotrophic microbial growth via the oxidation of H<sub>2</sub> coupled to the reduction of  $NO_3^-$ ,  $SO_4^{-2}$ , Fe(III), AsO<sub>4</sub><sup>3-,</sup> or CO<sub>2</sub>. Through these efforts, we generated 11 putative isolates representing all of the anaerobic respiration processes we enriched for, after five to six consecutive dilution-to-extinction series. These cultures grow much slower, and many display significantly different morphologies, than members of the same metabolic functional groups from deep-sea vent systems at mid ocean ridges (MOR). Illumina short-read sequencing approaches to date have revealed one pure culture (enriched under  $AsO_4^{3-}$ reducing conditions) closely related to Thermaerobacter marianensis (isolated from sediment of the Challenger Deep in the Mariana Trench; [2]). Near complete genomes related to the genome of Thermus scotoductus (isolated from hot tap water in Iceland; [3]) were obtained from cultures enriched under both NO3- and SO42-reducing conditions. Other 16S rRNA gene sequences in co-culture with our Thermus spp. suggest that we may still have mixed cultures within some of our putative isolates, as well as potential contaminants contained in our sequences. Results to date reveal notable differences associated with the cultivation and phylogenetic identification of H2oxidizing chemolithoautotrophs from the subseafloor crustal aquifer of the JdFR flank, in comparison to that of deep-sea vent microorganisms performing the same metabolisms at MORs.

[1] Nigro O. D. et al. (2017), *mBio* 8(2), e02129-16; [2] Takai K. et al. (1999) *IJSEM* 49(2), 619-628; [3] Kristjansson J.K. et