Microbiology of metal-reducing isolate *Thermaerobacter sp. s*train 36 from the Subseafloor Ocean Crust

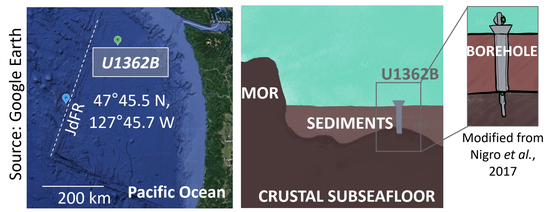
Maya Kreger[1,2],Jessica Choi[1,3], Jacob Cote[4], Abhijit Manna[1], Arkadiy Garber[5], Michael Rappé[6,], Ileana Pérez-Rodríguez[1]

[1]Department of Earth and Environmental Science, University of Pennsylvania; [2]Department of Biology, University of Pennsylvania; [3]Department of Ecology and Evolutionary Biology, University of Michigan; [4]Perelman School of Medicine Cell and Molecular Biology Graduate Group, University of Pennsylvania; [5]Biodesign Center for Mechanisms of Evolution, Arizona State University; [6]Hawai'i Institute of Marine Biology, University of Hawai'i at Manoa

Abstract

Anoxic subseafloor crustal fluids from sediment-covered ocean crust in the flank of the Juan de Fuca Ridge (JdFR) were collected and used to isolate anaerobic chemosynthetic microorganisms fueled by rock-derived energy sources within the oceanic crust. Here, we present results of *Thermaerobacter* sp. strain 36, which represents the first confirmed isolate obtained directly from the subseafloor crustal aquifer at JdFR. Strain 36 grows as a thermophilic primary producer through the microbial oxidation of H_2 coupled either to As(V) or Fe(III) reduction. Under these conditions, laboratory growth is slow and reaches low cell densities. Cells are pleiomorphic, meaning that they change from small cocci to short rods and elongated filaments during growth. Phylogenetic analysis of the 16s rRNA gene sequence shows that the closest relative of strain 36 is *Thermaerobacter marianensis*, a bacterium isolated from sediments of the Challenger Deep at the Mariana Trench. Whole genome analyses are currently underway to better understand the relationship between strain 36 and *T. marianensis* and their role in deep marine crustal environments. We hypothesize that both organisms represent a species capable of long-distance dispersal as spores.

Study System



Anoxic subseafloor crustal fluids (65 °C, pH 7.0-7.3)[1-5] were sampled from borehole U1362B during cruise AT42-11 on May 2019.

Enrichments and Isolations

Crustal fluids were enriched for H_2-oxidizing, As(V) reducing primary producers at 60°C and 1.5% w/v NaCl, using a modified version of DSM 1210 medium[6]. Strain 36 was isolated under the same conditions through 6 rounds of serial dilutions.

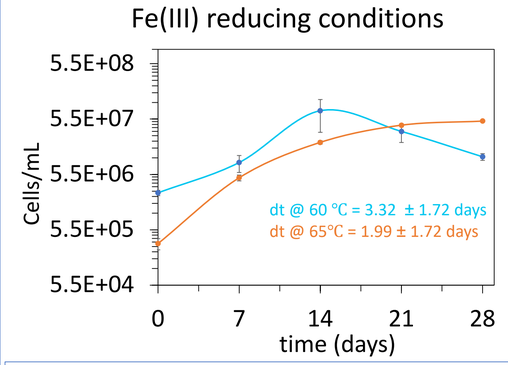
Whole Genome Isolation and Analysis

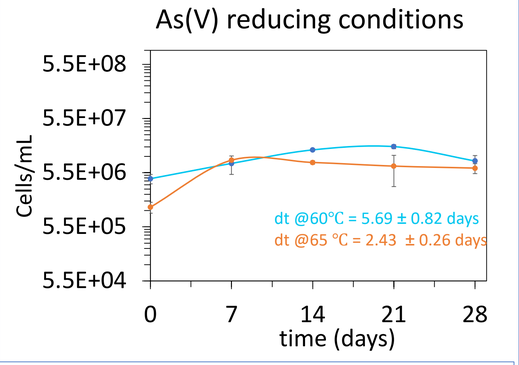
A non-commercial DNA extraction protocol [7] was applied to biomass generated under As(V) reducing conditions. Illumina short-read sequences were generated, assembled, and evaluated for genome quality (CheckM, SprayNPray)[8, 9], phylogenomic analyses (MEGA, GToTree, SprayNPray) [9, 10], and gene annotations (BAKTA, EMAPPER, Pseudofinder, FeGenie) [11, 12]. Results are currently being used for evolutionary and functional comparisons between the genomes of strain 36 and its closest cultured relative, *T. marianensis* [13].

Physiological Analysis

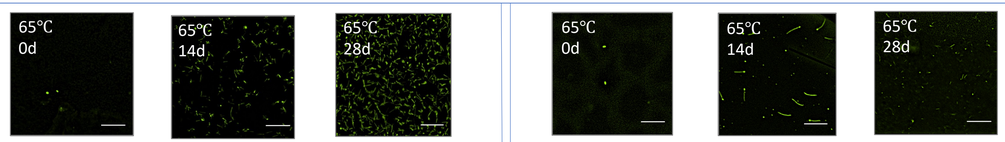
- Duplicate microbial growth curves were performed at 60°C (enrichment temperature) and 65 °C (*in-situ* temperature) under As(V) and Fe(III) reducing conditions.
- Growth quantification was performed via direct cell counts under epifluorescence (Olympus BX53) microscopy.
- Additional morphological analysis is being performed using scanning electron microscopy (QUANTA 600 SEM).

Microbial Metabolism and Physiology Results



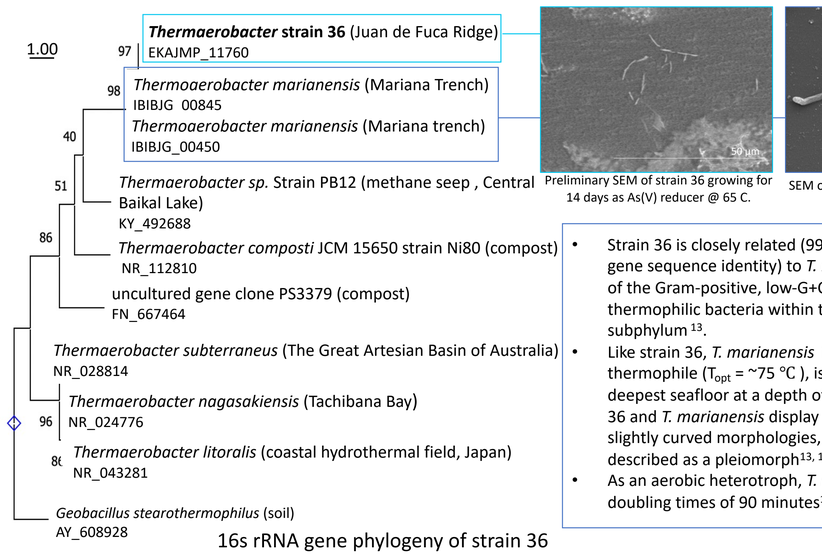


Strain 36 displayed shorter doubling times at *in-situ* temperature conditions of 65°C versus enrichment temperature conditions of 60 °C. However, the microorganism grew faster and to higher cell densities under Fe(III) reducing conditions. Regardless, all cell doubling times show slow growth at the scale of days.



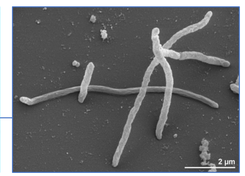
Epifluorescence microscopy images show morphological changes in strain 36 from small cocci to a mix of elongated filaments and cocci starting ~ 14 days of incubation under all growth conditions. Scale bars represent lengths of 10 µm.

Phylogenetic Analysis



16s rRNA gene phylogeny of strain 36

Preliminary SEM of strain 36 growing for 14 days as As(V) reducer @ 65 C.



SEM of *T. marinensis* growing as aerobic heterotroph @ 65 C[14].

- Strain 36 is closely related (99 % shared 16S rRNA gene sequence identity) to *T. marianensis,* a member of the Gram-positive, low-G+C-content anaerobic thermophilic bacteria within the *Bacillus-Clostridium* subphylum [13].
- Like strain 36, *T. marianensis* is a deep-ocean thermophile (T_{opt} = ~75 °C), isolated from the world's deepest seafloor at a depth of 10,897 m [13]. Both strain 36 and *T. marianensis* display rod-shaped, straight to slightly curved morphologies, but *T. marianensis* is not described as a pleiomorph[13, 14].
- As an aerobic heterotroph, *T. mariensis* displays doubling times of 90 minutes[13].

Genomic Comparisons

	strain 36	*T. marinensis*
genome sequence	In ~ 80 contigs	single unfragmented sequence
coding density	75-85 %	78.2 %
GC content	72	72.5
genome size	2.8 Mbp	2.8 Mbp
number of total genes	2359	2382
number of protein coding genes	2305	2382
hypothetical proteins	369	277
number of CRISPR arrays detected	1	1
number of sporulation related genes	79	96
number of motility related genes	85	72
low affinity oxygen reductase	2 Loci (5 genes total)	2 Loci (5 genes total)
CO dehydrogenase	3 copies	3 copies
As(V) reductase	present (1)	present (1)
Fe(III) reductase	No markers detected	No markers detected

Discussion

Interestingly, one of the two 16S rRNA gene sequences in the genome of *T. marianensis* appears to be more closely related to the 16S rRNA gene of strain 36–although they all phylogenetically represent the same species. Their potential ability to sporulate may play a key role in their dispersal to different seafloor and subseafloor environments. Further research will be required to understand growth rate differences between the anaerobic chemolithoautotrophic growth of strain 36 and the aerobic heterotrophic growth of *T. marianensis*, as well as their morphological deviations. Overall, our results expand our understanding of the strategies and adaptations relevant to life in the oceanic crust.

References

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Acknowledgements

We thank Beth Orcutt and team for leading the expedition of the AT42-11 in May 2019, as well as the team of the R/V Atlantis and ROV Jason in their key role in sample acquisition. for their for their work on this project. We thank Olivia Nigro and Stephanie Carr for supporting our participation in the expedition, and John Carr for his help maintaining live cultures. This research was made possible through funding via the Deep Carbon Observatory to M.R and I.P.-R., Penn Start-up funds to I.P.-R. and from the Center for Undergraduate Research and Fellowships' Grants for Faculty Mentoring Undergraduate Research (GfFMUR) to I.P.-R.