JdF Subsurface Aminicenantes

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Project
» Microbial activity in the crustal deep biosphere (Slow Life in Crust)

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Abstract
After decades studying the microbial "deep biosphere: in subseafloor oceanic crust, the growth and life strategies in this anoxic, low energy habitat remain poorly described. Using new single cell genomics data combined with previously published genomic data, we reveal the life strategies of two distinct lineages of uncultivated Aminicenantia bacteria from the basaltic subseafloor oceanic crust of the eastern flank of the Juan de Fuca Ridge (JdFR). This dataset represents the new single-cell amplified genomes (SAGs) generated for this project from samples collected in 2019 on expedition AT42-11. All SAG data for this project can be found on NCBI under BioProject ID PRJNA842252 under accession numbers: JAMZRZ000000000 (JDF1 composite genome); JAMZSA000000000 (AH-873-B07); JAMZSB000000000 (AC-708-M15); JAMZSC000000000 (AC-708-I09); JAMZSD000000000 (AC-335-O07); JAMZSE000000000 (AC-335-L06); JAMZSF000000000 (AC-335-K20); JAMZSG000000000 (AC-335-G13); JAMZSH000000000 (AC-335-B20); JAMZSI000000000 (AC-335-A11); JAMZSJ000000000 (AC-334-K16); JAMZSK000000000 (AC-334-E05). All MAG data for this project can be found on IMG. All data are also published at DOI 10.1038/s41396-023-01454-5.

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Coverage

Location: Juan de Fuca Ridge flank, CORK Borehole Observatories installed at IODP Holes U1362A and U1362B

Methods & Sampling

Sample collection: Borehole observatories accessing subseafloor crustal fluids were installed on the eastern flank of the JdFR in 2004 and 2010 during IODP Expeditions 301 and 327, respectively (Fisher et al. 2011; Fisher et al. 2005). These observatories penetrate ~ 240 m of sediment and ~110-290 m of underlying basement to enable crustal fluid collection from 8-190 m below the sediment-basement interface. Crustal fluids in this region are consistently 62-64°C, anoxic, and sulfate-replete (~18 mmol sulfate per kg water; Wheat et al. 2013). New crustal fluid sampling occurred in May 2019 during expedition AT42-11 on R/V *Atlantis* with ROV *Jason II,* both operated by Woods Hole Oceanographic Institution. Single cell genomic data was generated from cells collected from borehole observatory U1362B using established syringe sampling approaches (Carr et al. 2019; Wheat et al. 2011). Briefly, the top plug on the wellhead of U1362B was removed to freely vent crustal fluid from the top of the observatory

(Fisher et al. 2011). After the borehole dead volume had been flushed, crustal fluid was collected using a sterilized syringe (Wheat et al. 2011). Once recovered, the fluid was distributed in a nitrogen-flushed glove bag into cryovials, amended with 5% glycerol and 1X-Tris-EDTA buffer (final concentrations), and immediately flash-frozen with liquid nitrogen before long term storage at -70°C . In addition to the new sample, we examined existing single cell genomic and metagenomic data generated from samples collected from borehole observatories in International Ocean Discovery Program (IODP) Holes U1301A, U1362A, and U1362B in 2011 (AT18-07) using methods described elsewhere (Carr et al. 2019; Jungbluth et al. 2016; Fisher et al. 2012; Jungbluth et al. 2017).

Genomic sequencing, assembly, and phylogenomic analysis: Nineteen JdFR *Aminicenantia* metagenome assembled genomes (MAGs) and single-cell amplified genomes (SAGs) were identified across three independent sampling expeditions that occurred over a nine-year period. Two new metagenome assembled genomes (MAGs 3300037598_45, 3300037558_26) were sequenced, assembled, and binned from 2010 and 2011 metagenomes by the Joint Genome Institute (Jungbluth et al. 2013; Clum et al. 2021). Four MAGs from 2011 sampling were produced previously (Jungbluth et al. 2016; Jungbluth et al. 2017). Single cell amplified genomes (SAGs) from 2011 sampling were created as previously described from Holes U1362A and U1362B (Jungbluth et al. 2016; Carr et al. 2019). This study examines ten of these 2011 SAGs from U1362B (IDs with AC-334- and AC-335-) and two SAGs from U1362A (AC-708-). One new SAG was generated from the 2019 Hole U1362B fluid sampling (AH-873) following the same cell sorting, lysis, amplification, sequencing, and assembly approach as described elsewhere (Carr et al. 2019), and based on the standard workflow of the Single Cell Genomics Center at Bigelow Laboratory for Ocean Sciences (East Boothbay, Maine, USA; Stepanauskas et al. 2017). Nine SAGs within the *Aminicenantia* order JdFR-78 are nearly identical (average pairwise nucleotide identity, ANI, >99%), prompting the generation of a nearly complete composite genome assembly from these SAGs named JDF1.

Genome annotation and assessment of viral interactions: JdFR *Aminicenantia* SAGs and MAGs were annotated with KOFAMSCAN using KEGG Orthology Hidden Markov Model (KO HMM) version 4.3, -mapper option using default settings (Kanehisa and Sato, 2020). Single KEGG Orthology IDs were reported for each open reading frame (ORF) that met default thresholds (Aramaki et al. 2020). Annotations were input into KEGGDecoder and KEGG Mapper - Reconstruct Pathway visualization tools (Kanehisa and Sato; Graham et al., 2018).

Data Processing Description

Genome annotation and assessment of viral interactions: JdFR *Aminicenantia* SAGs and MAGs were annotated with KOFAMSCAN using KEGG Orthology Hidden Markov Model (KO HMM) version 4.3, -mapper option using default settings. Single KEGG Orthology IDs were reported for each open reading frame (ORF) that met default thresholds. Annotations were input into KEGGDecoder and KEGG Mapper - Reconstruct Pathway visualization tools.

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Related Publications

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Methods

Aramaki, T., Blanc-Mathieu, R., Endo, H., Ohkubo, K., Kanehisa, M., Goto, S., & Ogata, H. (2019). KofamKOALA: KEGG Ortholog assignment based on profile HMM and adaptive score threshold. Bioinformatics, 36(7), 2251–2252. https://doi.org/10.1093/bioinformatics/btz859
Methods

Booker, A. E., D'Angelo, T., Adams-Beyea, A., Brown, J. M., Nigro, O., Rappé, M. S., Stepanauskas, R., & Orcutt, B. N. (2023). Life strategies for Aminicenantia in subseafloor oceanic crust. The ISME Journal, 17(9), 1406–1415. https://doi.org/10.1038/s41396-023-01454-5
Results

Carr, S. A., Jungbluth, S. P., Eloe-Fadrosh, E. A., Stepanauskas, R., Woyke, T., Rappé, M. S., & Orcutt, B. N.

(2019). Carboxydotrophy potential of uncultivated Hydrothermarchaeota from the subseafloor crustal biosphere. The ISME Journal, 13(6), 1457–1468. https://doi.org/10.1038/s41396-019-0352-9
Methods

Clum, A., Huntemann, M., Bushnell, B., Foster, B., Foster, B., Roux, S., Hajek, P. P., Varghese, N., Mukherjee, S., Reddy, T. B. K., Daum, C., Yoshinaga, Y., O'Malley, R., Seshadri, R., Kyrpides, N. C., Eloe-Fadrosh, E. A., Chen, I.-M. A., Copeland, A., & Ivanova, N. N. (2021). DOE JGI Metagenome Workflow. MSystems, 6(3). https://doi.org/10.1128/msystems.00804-20 https://doi.org/10.1128/mSystems.00804-20
Methods

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Graham, E. D., Heidelberg, J. F., & Tully, B. J. (2018). Potential for primary productivity in a globally-distributed bacterial phototroph. The ISME Journal, 12(7), 1861–1866. https://doi.org/10.1038/s41396-018-0091-3
Methods

Jungbluth, S. P., Amend, J. P., & Rappé, M. S. (2017). Metagenome sequencing and 98 microbial genomes from Juan de Fuca Ridge flank subsurface fluids. Scientific Data, 4(1). https://doi.org/10.1038/sdata.2017.37
Methods

Jungbluth, S. P., Bowers, R. M., Lin, H.-T., Cowen, J. P., & Rappé, M. S. (2016). Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt. The ISME Journal, 10(8), 2033–2047. doi:10.1038/ismej.2015.248
Methods

Jungbluth, S. P., Grote, J., Lin, H.-T., Cowen, J. P., & Rappé, M. S. (2012). Microbial diversity within basement fluids of the sediment-buried Juan de Fuca Ridge flank. The ISME Journal, 7(1), 161–172. https://doi.org/10.1038/ismej.2012.73
Methods

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Methods

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Methods

Wheat, C. G., Jannasch, H. W., Kastner, M., Hulme, S., Cowen, J., Edwards, K. J., … Glazer, B. (2011). Fluid sampling from oceanic borehole observatories: design and methods for CORK activities (19902010). Proceedings of the IODP. doi:10.2204/iodp.proc.327.109.2011
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

Microbial activity in the crustal deep biosphere (Slow Life in Crust)

Coverage: Juan de Fuca Ridge flank CORKs, 47N/127W

NSF Award Abstract:
The marine deep biosphere is the habitat for life existing under the sea floor. The zone has remarkably low energy sources creating a paradox of how life can persist there. Resolving this energy paradox is a grand challenge in deep biosphere research. The Juan de Fuca Ridge flank off the coast of Washington, USA, is an accessible, low energy environment making it an attractive location for addressing this challenge. A series of experiments will be conducted on the seafloor at the Juan de Fuca Ridge flank, using established subseafloor observatories that access the crustal deep biosphere, to provide the first direct in situ measurement of microbial activity in the crustal subsurface. This project will provide essential information about the ability of life to survive under conditions that we are not able to replicate in the laboratory, but that are increasingly important for understanding microbial community interaction in the environment. This information can then be used in models of global microbial activity for estimating the impact of this biosphere on elemental cycling, transforming our understanding of microbial processes within this vast subseafloor habitat. To communicate these discoveries to the public, the project will include a ship-to-shore outreach program during the cruise. In addition public lectures will be presented, and an interactive display of deep-sea video footage will be set up for the annual public Open House at the Bigelow Laboratory for Ocean Sciences in Maine. Diverse undergraduate students and a postdoctoral researcher will be recruited to participate in the research and public outreach activities.

This project proposes to leverage existing subsurface infrastructure on the eastern flank of the Juan de Fuca Ridge with advances in single-cell based molecular and geochemical approaches to make fundamental new discoveries about the activity of life in the deep crustal biosphere. During a two-week research cruise, the research team will incubate crustal fluids in situ and in the laboratory with labeled substrates for tracking single-cell activity, coupled with radioisotope tracer activity and potentiostat measurements, with the objective of determining in situ and potential rates of activity and cellular physiology. The research will also identify which metabolisms active microorganisms utilize under in situ and laboratory conditions, the rates of these processes, and the microorganisms involved. The results are expected to provide explicit hypothesis testing of microbial activity and in situ microbial growth rates from the crustal deep biosphere to transform understanding of microbial activity in the crustal deep biosphere and generate critical information about the ability of life to survive under low energy conditions.

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Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1737017

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