

Symbiotic diversity and mineral-associated microbial ecology in marine microbiomes

Thesis by
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In Partial Fulfillment of the Requirements for
the degree of
Doctor of Philosophy

The Caltech logo is displayed in a bold, orange, sans-serif font. The letters are thick and closely spaced, with the 'C' and 'h' being particularly prominent. The logo is centered within a light yellow rectangular background.

CALIFORNIA INSTITUTE OF TECHNOLOGY
Pasadena, California

2020
(Defended August 3, 2020)

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ACKNOWLEDGEMENTS

This thesis would not have been possible if not for the opportunities afforded by my thesis advisor Victoria Orphan. Thank you, Victoria, for your inspiring drive to push the boundaries of microbial ecology, and for your boundless energy to take on any project no matter how challenging. It has been a privilege to pursue my doctorate in the Orphan Lab and under its aegis dive three thousand feet beneath the western Pacific on *Alvin* and drill nineteen thousand feet beneath the eastern Pacific on *Chikyu*. I cannot be thankful enough for your tireless work that made this thesis possible.

I would like to thank Woody Fischer for his ability to bring fresh, exciting ideas into any research question and for always pushing me to become a better scientist. The weekly meetings of the Geobiology Reading Group were a highlight of my academic career at Caltech and profoundly expanded my perspective on the breadth of disciplines within Geobiology. I also am thankful for Woody's advice and guidance in the project that became Chapter 4 of this thesis and for his thoughtful comments on the other chapters. I'd like to thank both Woody and John Grotzinger for leading expeditions to the Turks and Caicos Islands and for assembling such an incredible team to explore the microbiology and sedimentology of Little Ambergris Cay.

I would further like to thank George Rossman and Jared Leadbetter for serving on my thesis committee. George's infectious enthusiasm for mineralogy made my teaching assistantship in his Optical Mineralogy lab course a pleasure, and his advice and technical knowledge made the mineralogical analyses in Chapter 2 of this thesis possible. My first microbiology course was Jared's Microbial Physiology, where I first gained an appreciation for the incredible diversity of microbial life and gained an understanding of microbial biochemistry that was indispensable for my thesis work.

So much of the data presented here could not have been collected if not for the deep well of technical knowledge available in the Orphan Lab. First and foremost I would like to thank Stephanie Cannon for her unrelenting work in curating, managing, and disseminating the technical expertise collected over many years by former and current Orphan Lab members. It cannot be understated the extent to which so much of the work in this thesis would not have been possible but for the methods developed by Orphan Lab members Anne Dekas, Elizabeth Trembath-Reichert, Kat Dawson, Hang Yu, Ranjani Murali, Benjamin Harrison, Grayson Chadwick, Shawn McGlynn,

Roland Hatzenpichler, Alice Michel, Sean Mullin, Fabai Wu, David Case, Alexis Pasulka, Abigail Green-Saxena, Masataka Aoki, and so many others.

I would further like to thank my friend, colleague, and fellow member of Team SRB, Ranjani Murali, for her many hours of lab work and discussions that contributed to Chapter 1 of this thesis. Ranjani's patience and insights were invaluable throughout the investigation of SEEP-SRB1g, particularly in our nanoSIMS analysis, which constituted an especially exciting and dynamic portion of this project.

I cannot thank enough my friends for providing support and generally being great throughout the years. Thanks to my friends from GPS, Elizabeth Trembath-Reichert, Lewis Ward, Cecilia Sanders, John Magyar, John Naviaux, Peter Martin, and many others who were always excited, supportive, and kind, and often up to get Ernie's. Thanks to my hausmates Austin Chadwick, Joe Bowkett, Alistair Hayden, Mike Citrin, and Dan Johnson for all the great times. I'm also deeply thankful to Judith Wu for her unconditional love and support.

I'd also like to thank my undergraduate thesis advisor, Bob Gaines of Pomona College, for his enthusiasm for geology, Earth history, and geomicrobiology throughout my college career that inspired me to pursue graduate studies in the earth sciences.

Finally, I could never have completed this thesis without the immeasurable support of my dad, my mom, and my brother. Thank you.

ABSTRACT

This thesis investigates ecological interactions in the seafloor between microbial taxa (Chapters 1 and 2) and between these microorganisms and their mineral hosts (Chapters 2 through 4). In seafloor sediments, electron acceptors are often limited, forcing microorganisms inhabiting these sediments to acquire symbiotic partners and/or perform extracellular electron transfer to insoluble electron acceptors. Seafloor methane seeps present an endmember case wherein extremely reducing fluids charged with methane advect through sediment. In these benthic ecosystems, anaerobic methanotrophic archaea (ANME) form symbiotic partnerships with sulfate-reducing bacteria (SRB), but it remained unclear if certain ANME exhibit a preference for certain SRB partners. In Chapter 1, I present results documenting such a pattern of partnership specificity in ANME-SRB consortia. In Chapter 2, I further examine these patterns in rare ANME taxa through development and application of a density-separation protocol refined from published work. This protocol exploits the co-association of microbial taxa on mineral surfaces to aid in the detection of novel symbioses, and further is useful to detect microbial interactions with certain minerals. In Chapter 3, I focus on the interaction between ANME-SRB consortia and authigenic silicates that have been observed on consortium exteriors, finding evidence to support that the precipitation of these silicates is actively mediated by ANME-SRB. In Chapter 4, I perform geochemical modeling benchmarked by synchrotron X-ray analysis to examine the imprint of extracellular electron transport by metal-reducing microorganisms on Precambrian manganese-rich sedimentary rocks.

PUBLISHED CONTENT AND CONTRIBUTIONS

Metcalfe, K.S., et al. (2020) “Experimentally-validated correlation analysis reveals new anaerobic methane oxidation partnerships with consortium-level heterogeneity in diazotrophy.” *bioRxiv*, doi: 10.1101/2020.04.12.038331. Resubmitted with minor revisions to *The ISME Journal*.

K.S.M. conceptualized the project and performed correlation analysis, analysis of flow cytometry data, comparative genomics of SRB (in collaboration with R.M.), designed SEEP-SRB1g FISH probe, prepared SIP incubations (with R.M.), processed nanoSIMS data, and wrote the manuscript.

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I n t r o d u c t i o n

The geochemical dynamism of Earth's surface—unique among known terrestrial planets—is inextricably tied to the emergence and radiation of microbial life. The expansion of microbial metabolic diversity has shaped the course of Earth history, for example causing a step change in atmospheric oxygen concentrations [1], creating a pathway for the transformation of N₂ into biomass [2], and modulating the production and consumption of the powerful greenhouse gas CH₄ [3]. Microbial life in all its variegated forms directs the flow of electrons between diverse donors and acceptors, and in so doing maintains the biogeochemical cycles necessary for Earth's habitability. The numerous biochemical means by which these microorganisms transmit electrons between substrates present many opportunities for discovery, but the experimental study of these mechanisms is made more challenging by the difficulties associated with acquiring pure cultures of environmental microorganisms, the vast majority of which have not been cultured [4].

It has been proposed that the cultivation of environmental microorganisms is challenging due to the reliance of many microbial taxa upon symbiotic partnerships with other microorganisms [5]. Many microbial metabolisms important for the functioning of biogeochemical cycles are facilitated by a symbiotic relationship between microbial taxa, such as that between anaerobic methanotrophic archaea (ANME) and sulfate reducing bacteria (SRB) responsible for the anaerobic oxidation of methane (AOM) in seafloor sediments worldwide [6, 7]. In this and in many microbial symbioses, physical attachment of partner taxa facilitates the transfer of nutrients [8–10], reducing equivalents [11], and/or electrons [12–14] between partners. Study of these symbiotic relationships *in situ* has

uncovered significant diversity in the sequence identity of partner taxa [15–17], but how this diversity may influence the character of these symbioses remains largely an open question.

Evidence from the study of several different microbial symbioses indicates that the nature of a symbiotic relationship can vary between different pairings of partner taxa. In the classic mutualism between reef-building coral hosts and their *Symbiodinium* sp. endosymbionts (colloquially termed ‘zooxanthellae’), coral access energy from sunlight via *Symbiodinium* sp. and in turn provide habitat to endosymbionts [18]. Study of coral hosts from a range of habitats has demonstrated that hosts exhibit preference for specific lineages of *Symbiodinium* sp. [19] which appears to have physiological implications for different host-symbiont pairings. The membership of host-symbiont pairs has been observed to predict host tolerance to thermal stress [20–22] and endosymbiont transcriptional profiles [23, 24]. Lichen, a microbial symbiosis between filamentous fungi and endosymbiotic green algae or cyanobacteria, have also been shown to exhibit specificity between partner taxa [25].

For other symbiotic relationships between microorganisms, such as that between ANME and SRB, the specificity with which certain taxa co-associate remains unclear, but may have significant implications for the physiologies of different pairs of symbiotic partners. Extensive study of ANME-SRB consortia has documented a diversity of different ANME-SRB partnerships by fluorescence *in situ* hybridization (FISH)-based microscopy [16, 17, 26] and flow cytometry [27]. However, these results largely served to catalog the ANME-SRB pairings observed in Nature but did not synthesize these datasets to examine the extent to which certain SRB lineages may preferentially associate with certain ANME.

In Chapter 1, I present evidence from an ecological survey of Costa Rica methane seep sediments for a highly specific partnership between certain ANME and SRB partners, and further present stable isotope probing evidence indicating that this specificity is important for symbiotic function.

While the results in Chapter 1 were successfully extracted from a complex dataset of many hundreds of methane seep sediment samples, detection of partnerships between more rare ANME subtypes was not possible, requiring the application of techniques to parse microbial communities at higher resolution. In Chapter 2, I present results from the application of a density-separation protocol refined from previously-published techniques [28]. By exploiting the intimate physical associations between sediment-dwelling microorganisms and minerals, I amplified the correlation signal between ANME and SRB taxa co-associating on mineral surfaces by separating minerals in methane seep sediment samples by density. These techniques circumvent the so-called ‘hairball’ [29] of computationally-inferred ecological interactions resulting from correlation analysis of complex sediment microbiomes. The results I present in Chapter 2 further document interactions between certain ANME-SRB consortia and certain minerals common in marine sediments, providing avenues for future study of these microbe-mineral interactions.

Such interactions between microorganisms and minerals form an interface between the geosphere and biosphere that has served to preserve a record of microbial life on Earth. AOM, mediated by microbial metabolism, drives the precipitation of carbonate minerals at sites of methane seepage [30]. Microscopy study of ANME-SRB consortia, however, documents authigenic silicate minerals associated with consortia [31, 32]. In Chapter 3, I

investigate the growth of these phases in AOM incubations from which almost all sediment had been removed, finding strong evidence for their growth from media significantly undersaturated with respect to previously-measured equilibria for precipitation of amorphous silica and clays. Together with evidence from seep carbonates, I infer that ANME-SRB consortia may mediate the precipitation of authigenic silicates, representing a means by which ANME-SRB consortia may be preserved in the rock record.

Microbial metabolism has also left an imprint on the rock record by directing redox transformations of mineral electron acceptors [33]. In Chapter 4, I present coupled synchrotron X-ray spectroscopy and geochemical modeling results that indicate Mn(III) mineral phases found in abundance in Precambrian manganese-rich sedimentary rocks were likely stabilized through microbially-mediated reduction of primary Mn oxides, rather than through abiotic means.

Spanning a range of different geomicrobiological questions, this thesis provides the groundwork for further exploration of the diverse microbial symbioses and microbe-mineral interactions that have shaped Earth's biogeochemistry. In particular, I hope future work further explores the hypothesized patterns of partnership specificity in ANME-SRB consortia presented in the first and second chapters of this dissertation. Although Chapter 1 presents a compelling argument that the partnership between ANME-2b and SEEP-SRB1g is highly specific, the degree of specificity or promiscuity in other ANME-SRB partnerships merits further quantitative exploration by FISH experiments. The partnership between ANME-2c and SEEP-SRB1a presents a particularly interesting case, in which ANME-2c, a clade consisting of multiple genera, may exhibit preference either for SEEP-SRB1a or SEEP-SRB2. Evidence for this flexibility comes from both correlation analysis

and FISH experiments performed on cold seep sediment samples in this thesis (Ch. 1) as well as FISH experiments on samples of sedimented hydrothermal vents of the Guaymas Basin in the Gulf of California [34] that show ANME-2c to preferentially associate with SEEP-SRB1a or SEEP-SRB2 in Costa Rica cold seep sediments and with SEEP-SRB2 in Guaymas Basin sedimented hydrothermal vent sites, although it will require further analysis to determine how these patterns of association relate to diversity within the ANME-2c clade. This pattern differs substantially from the high degree of preference exhibited by ANME-2b or ANME-2a for SEEP-SRB1g or SEEP-SRB1a partners, respectively. Exploring the relationship between the SRB taxon preferred by ANME-2c and the environmental parameters of the sediment from which consortia were sampled will likely yield insight into the dynamics of ANME-2c partnership specificity. Network analysis also implies that ANME-2c form intimate associations in hot seep sediments with members of the candidate phylum Atribacteria (data not shown) thought to be involved in hydrocarbon degradation [35], but the nature of this association is entirely unknown. Additionally, ANME-2c are members of consortia of diverse morphotypes (G. Chadwick, pers. comm.) and which may relate to patterns of partnership specificity. ANME-1, representing a separate order of methanotrophic archaea with multiple genera, also exhibits similar flexibility in its partnerships with SRB, as ANME-1 has been observed to associate with SEEP-SRB2 in cold seep sediments (Ch. 1) and members of the HotSeep-1 in methane-rich hydrothermal vent sediments [34]; further work could characterize the nature of ANME-1 partnerships *in situ* and further shed light on the parameters that may contribute to ANME-1 forming partnerships with different SRB partner taxa.

The discoveries described in this thesis point toward a more holistic understanding of the symbiotic relationship that lies at the heart of AOM. Previous work has primarily focused on the biochemistry of anaerobic methane oxidation within ANME, as these microorganisms presented striking similarities to methanogenic archaea. However, relatively less emphasis has been placed on investigating in detail the role of partner SRB. Although the scope of this thesis was constrained to sketching the contours of symbiotic diversity in AOM, it is my hope that future research will explore the co-evolution of ANME and their SRB partners, and in so doing shed new light on the enigma of the anaerobic oxidation of methane.

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