Quantitative Chemical Mass Transfer in Coastal Sediments during Early Diagenesis: Effects of Biological Transport, Mineralogy, and Fabric: Phase III

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LONG-TERM GOALS

Program goals were to provide a better quantitative and mechanistic understanding of chemical processes that occur in fine-grained coastal and continental margin sediments. Our long-term goal is to understand how the chemical and textural characteristics of sediments comprised of different clay mineral suites might exert a selective pressure on the development of natural consortia of bacteria.

OBJECTIVES

The overall goal of this project is to evaluate the mechanism controlling the selection of different physiological groups of bacteria by defined clay minerals typically found in sea floor mineral assemblages, as well as quartz, the most common sedimentary mineral. Specifically our objectives were to: (1) create reproducible anaerobic microbial consortia associated with different mono-mineralogic substrates and to assess how stable they are over time; (2) determine changes in the textural and physical properties of mono-minerals as a result of microbial activity; (3) evaluate whether controlled mixtures of minerals and microbial communities can be used to create geomicrobiological assemblages with predictable ecological and physiological properties.

APPROACH

We hypothesize that defined minerals can select specific physiological groups of microorganisms due to differences in their structure and composition, and thereby produce mineral/microbial assemblages with predictable properties.

We are investigating two major groups of anaerobes, sulfate and metal (iron)- reducing bacteria, because they are abundant in marine environments, well adapted to environments with large gradients or changing in physical-chemical environmental conditions, and have a great capacity to oxidize and reduce a large variety of electron donors and acceptors.

We have conducted our experiments under anaerobic conditions using natural sediment samples as a source for the enrichment cultures. Sediment samples were collected from two sites (sandy and clay) in the San Diego Bay, near the Scripps Marine Facility.

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To investigate the interactions between marine bacteria and sedimentary minerals common in marine environments, we used pure, fine-grained separates of smectite, illite, chlorite, kaolinite and quartz. Anaerobic consortia were allowed to develop in a basal medium amended by one of the minerals, one carbon source (lactate, acetate, formate) and one electron-acceptor (sulfate or Fe(III)). The samples were amended with additional carbon source and electron acceptor once a week as we monitored the activity of microorganisms by measuring metabolic products of sulfate or iron reduction, such as hydrogen sulfide or ferrous iron. After approximately 1 month, each sample was transferred to fresh medium. Stable consortia were obtained after 3 transfers.

We have applied several powerful molecular biology tools, such as T-RFLP (terminal restriction fragment length polymorphism) or cloning and sequencing genes encoding 16S rRNA, to assess the community diversity associated with each mineral. Combined these approaches can be used to rapidly compare which microorganisms are specifically found within each mineral/electron-donor/electron-acceptor set.

WORK COMPLETED

Ninety serum bottles with anaerobic (sulfate-and iron-reducing) enrichments, with approximately 1g of each monomineralogic substrate in 10 ml basal medium amended by a given electron-donor/acceptor pair, were established for long-term experiments to confirm the reproducibility of our previous findings. The experiments were run at 18°C over three months by periodically adding neccesary e-donor/acceptor and otherwise leaving each bottle undisturbed, until transferring to new bottles (transfers occurred 3 times).

We conducted the molecular biology experiments (DNA extractions and PCR, followed by T-RFLP analysis) to evaluate the similarities or differences of the microbial populations associated with each mineral for a given growth condition.

Using Genetic Profile and Excel software programs to analyze T-RFLP data, we have generated microbial profile for one of investigated group of microorganisms, sulfate-reducing bacteria, associated with clay minerals.

RESULTS

Previous experiments with the five minerals, quartz, smectite, illite, chlorite and kaolinite and two enrichments from a sandy sediment sample collected near the SIO beach, one iron-reducing and one sulfate-reducing, with lactate as the carbon source, have suggested that there is a definite preference of different microbial populations for different mineral substrates. For example, we found that several species of sulfate-reducing bacteria belonging to the genus *Desulfomicrobium (D. escambium, D.hypogenium,D. baculatum*) were associated with only kaolinite and chlorite. *Desulfovibrio* spp. predominated on quartz and smectite. In contrast, species of iron-reducing bacteria did not show such a strong correlation between the defined types of minerals.

To confirm the reproducibility of the results, we have repeated this preliminary experiment in greater detail, starting with two sediment samples from the San Diego Bay, one sandy and one clay, again using the five minerals and iron and sulfate as electron-acceptors, but also using 3 different electron donors. The matrix of this long-term experiment is shown in Table 1.

Table 1. San Diego Bay Enrichments

	CLAY			SAND		
Mineral	Lactate	Acetate	Formate	Lactate	Acetate	Formate
Soluble Fe(III) as the electron acceptor						
Kaolinite	+	+	+	+	+	+
Illite	+	+	+	+	+	+
Chlorite	+	+	+	+	+	+
Quartz	+	+	+	+	+	+
Smectite	+	+	+	+	+	+
SO_4^{2-} as the electron acceptor						
Kaolinite	+	+	+	+	+	+
Illite	+	+	+	+	+	+
Chlorite	+	+	+	+	+	+
Quartz	+	+	+	+	+	+
Smectite	+	+	+	+	+	+
Insoluble Fe(III) oxides as the electron acceptor						
Kaolinite	+	+	+			
Illite	+	+	+			
Chlorite	+	+	+			
Quartz	-	-	-			
Smectite	+	+	+			

+ indicates positive growth; - no growth; blank, not tested

All carbon sources, lactate, formate or acetate, were provided at a concentration of 10-20mM, and sulfate was added at 20 mM. For iron-reducing enrichments, two forms of iron were used: 10mM $FePO_4$ as a soluble form or approximately 30mM ferrihydrite (FeOx) as the insoluble form. The latter form of iron is more environmentally relevant and it is important to investigate how the association between the iron oxides and the sedimentary minerals affects the activity of the microbial consortia.

Results presented in the table 1 showed that bacteria developed well on all minerals, carbon sources and electron-acceptors, except for quartz and iron oxide.
We also observed large variations in the rate of iron reduction during bacterial growth for each of the 5 minerals, ranging from quartz (slow Fe reduction) to smectite (rapid Fe reduction).

We determined the optimal approach for extracting DNA from each mineral/bacteria assemblage, using several molecular biology kits designed for DNA extractions from soil. We used T-RFLP analyses to determine and visualize significant changes in microbial structure profile associated with different mineral structure. These data allowed us to extrapolate the effect of mineral structure on bacterial structure community.

Figure 1 shows the structure of sulfate-reducing community from San Diego Bay associated with clay minerals. Each peak on electropherogram represents as individual bacterial species. Using two restriction enzymes (Hae III and Rsa I) for T-RFLP analyses, we observed that smectite, chlorite and illite possessed significant diversity of fragmented DNA patterns whereas kaolinite did not.

Figure 1. Set of FeOx-reducing enrichments grown on acetate. Note the obvious difference in color due to differences in the transformation of iron. FeOx was least reduced (still mostly orange in color) when present with quartz and most reduced (forming a black color) with smectite.

Sulfate Reducers/Clay Site

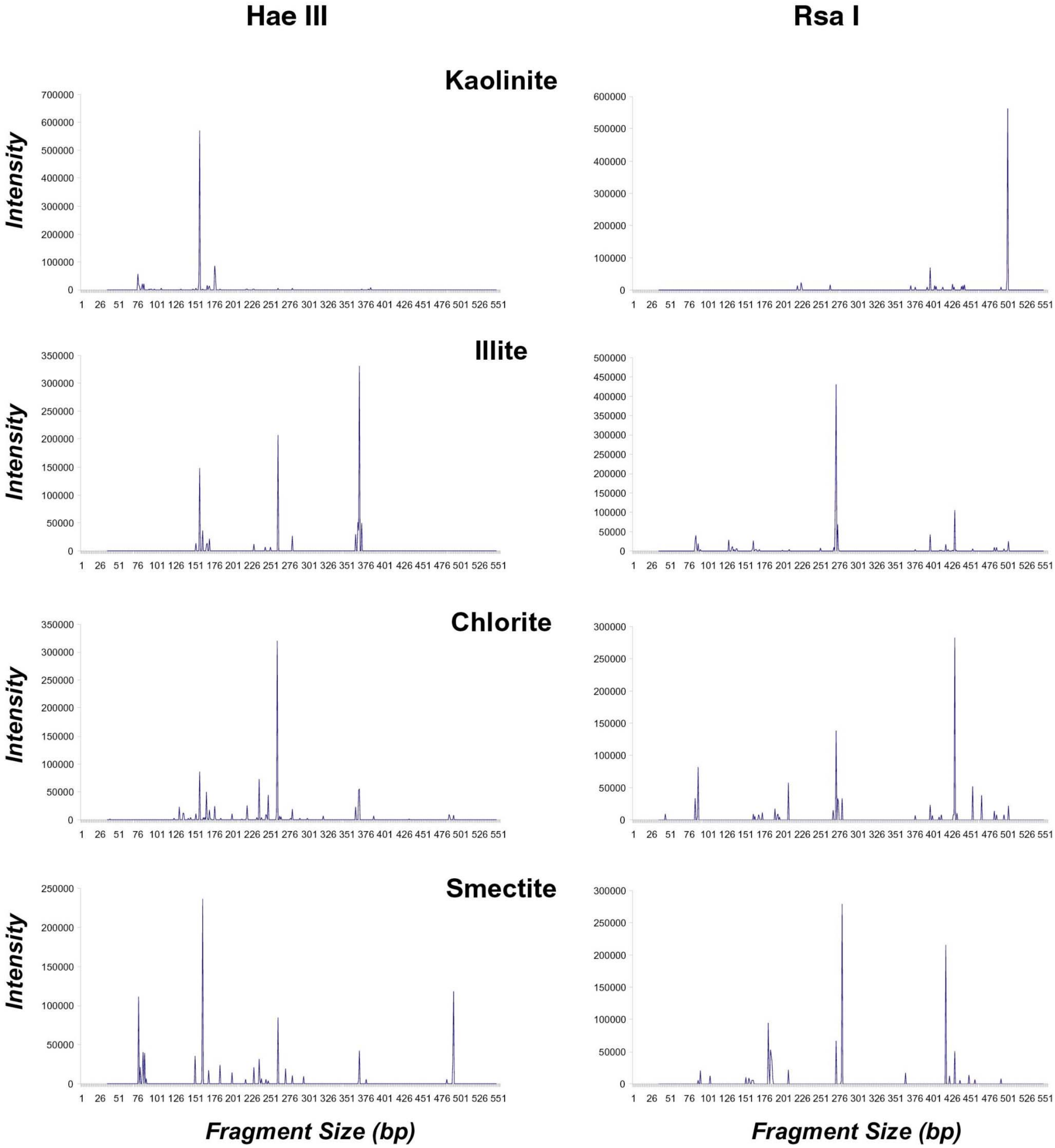

Figure 2. Fragment size pattern of bacterial community associated with clay minerals

We also created a library of 35 clones, random representatives from smectite/sulfate-reducing assemblage, to confirm the diversity and identity of bacterial species associated with other minerals. We have found a dominancy of *Desulfovibrio* sp. for smectite and all of other rested minerals. We are currently trying to identify the unique peaks associated with each type of mineral through further clone analysis.

IMPACT/APPLICATIONS

It is important to predict and assess the how feedbacks between sediment mineralogy and microbial community structure affect the cycling of metals, carbon and nutrients between sediments and the ocean. These experiments will provide vital information on the effects of minerals on microbial community composition, specifically under sulfate- and iron-reducing conditions. It will further our understanding of how bacteria physically interact with the dominant mineral species in sediments. Results of such a study can be used to design more sophisticated experiments to deconvolute the effects of complex microbial consortia on the physical and chemical properties of sediments and on the cycling of carbon and nutrients between sediments and the ocean.

TRANSITIONS

This project started as a purely geochemical study of biogeochemical fluxes in fine-grained sediments with Dr. Miriam Kastner as the PI and Dr. Barbara Ransom as the Co-PI at SIO. My laboratory began collaborating with Dr. Ransom in FY01 and the scope and impact of this work expanded into the fields of molecular biology and microbial remediation of toxic metals and organic compounds. When Dr. Ransom left SIO I agreed to become the PI on this grant in order to complete the microbiological work we had initiated and for which exciting preliminary results were obtained.