

Mineralogical controls on surface colonization by sulfur-metabolizing microbial communities

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When characterizing microbial diversity and the microbial ecosystem of the shallow subsurface the mineral matrix is generally assumed to be homogenous and unreactive. We report here experimental evidence that microorganisms colonize rock surfaces according to the rock's chemistry and the organism's metabolic requirements and tolerances. We investigated this phenomenon using laboratory biofilm reactors with both a pure culture of sulfur-oxidizing *Thiothrix unzii* and a mixed environmental sulfur-metabolizing community from Lower Kane, Cave, WY, USA. Reactors contained rock and mineral chips (calcite, albite, microcline, quartz, chert, Madison Limestone (ML), Madison Dolostone (MD), and basalt) amended with one of the two inoculants. Biomass of attached microorganisms on each mineral surface was quantified. The 16S rRNA of attached microbial communities were compared using Roche FLX Titanium 454 next generation pyrosequencing.

A primary controlling factor on taxonomy of attached microorganisms in both pure and mixed culture experiments was mineral buffering capacity. In mixed culture experiments acid-buffering carbonates were preferentially colonized by neutrophilic sulfur-oxidizing microorganisms (~18% to ~27% of microorganisms), while acidophilic sulfur-oxidizing microorganisms colonized non-buffering quartz exclusively (~46% of microorganisms). The nutrient content of the rock was a controlling factor on biomass accumulation, with neutrophilic organisms selecting between carbonate surfaces of equivalent buffer capacities according to the availability of phosphate. Dry biomass on ML was $17.8 \pm 2.3 \text{ mg cm}^{-2}$ and MD was $20.6 \pm 6.8 \text{ mg cm}^{-2}$; while nutrient poor calcite accumulated $2.4 \pm 0.3 \text{ mg cm}^{-2}$. Biomass accumulation was minimal on non-buffering nutrient-limited surfaces. These factors are countered by the competitive exclusion of some populations. A pure culture of *T. unzii* preferentially colonizes carbonates while a very closely related *Thiothrix spp* is excluded from these same rock samples in a mixed culture.

Diversity analysis reveals that ML, MD, and calcite have >98% of sequences belonging to shared OTUs. The carbonates have <3% of sequences belonging to OTUs shared with any silicate mineral surface with the exception of basalt (~85% similarity). These four surfaces were host to the least diverse microbial communities, suggesting that competitive exclusion of microorganisms not adapted to these surfaces is a controlling variable on taxonomy. Furthermore, the microorganisms on basalt reveal a unique association between *Thiothrix unzii* (often found in mid-ocean ridge environments) and basalt, where it excludes other sulfur oxidizers and accumulates the highest non-carbonate biomass in both pure ($3.5 \pm 1.0 \text{ mg cm}^{-2}$) and mixed culture ($5.4 \pm 1.4 \text{ mg cm}^{-2}$) experiments. This association suggests that adaptations to specific rocks may be retained even when the organism is displaced from an ancestral rock/mineral surface habitat. Combined, these variables (buffering capacity, nutrient availability, competitive exclusion, tolerance of surface geochemistry, and latent adaptations) affect biomass density, local diversity, and global diversity of the attached communities on mineral and rock surfaces and suggest that different populations are more tolerant of, and more competitive on, specific rock/mineral types.

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