MINERALOGICAL CHARACTERIZATION OF CALCIUM CARBONATE POLYMORPHS BIOLOGICALLY PRECIPITATED DURING HETEROTROPHIC BACTERIAL GROWTH.

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Introduction: Carbonates are interesting terrestrial minerals since they can be produced abiotically as well as by biological activity [1]. Heterotrophic bacteria can precipitate calcium carbonate ($CaCO_3$) during growth [2] although the role of bacteria in nucleation and crystal growth is unclear. Calcium carbonate precipitation is likely biologically-induced, resulting from metabolic activity, rather being directly genetically controlled (biologically-mediated) [3]. Calcium carbonate precipitation by photoautotrophic cyanobacteria and by sulfate reducing bacteria has been given a great deal of attention in the past. Comparatively little work has focused on carbonate precipitation by chemoheterotrophic bacteria. Given the complexity and variety of biomineralization processes, there are likely mineralogical differences between $CaCO_3$ precipitated by different microbes, and studies to date have not focused on characterizing these similarities and differences. In this study we have focused on characterizing the mineralogy of $CaCO_3$ precipitated by a variety of heterotrophic microbes and have demonstrated that bacterial heterotrophy can result in the production of a variety of $CaCO_3$ polymorphs and an abundant variety of crystal morphologies, including bacterial encapsulation. There is compelling evidence that carbonates exist on the Martian surface [4] and carbonates are present in the ALH84001 meteorite, though, their biotic origin is controversial [5]. Definitively distinguishing biotic carbonate from abiotic carbonate is not currently possible [1]. This work was conducted to generate data that will aid in distinguishing biogenic from abiotic $CaCO_3$.

Results and Discussion: Eighteen heterotrophic microbes capable of precipitating $CaCO_3$ when grown on B4 media supplemented with calcium acetate or calcium citrate were selected, after an initial screening, to have their respective precipitates characterized microscopically and spectroscopic ally. The microbes were each identified by 16S rDNA sequencing: as a eukaryotic yeast *Rhodotorula mucilaginosa* (1), *Rhodococcus sp.* (5), *Arthrobacter sp.* (8), *Agromyces sp.* (1), *Actinobacter sp.* (1), and *Ralstonia sp.* (1). Interesting trends were observed. Many isolates (8/18) demonstrated that larger mineral structures were composed of agglomerations of individual bacterial cells encrusted in microcrystals. *Actinobacter sp.* isolate F4A9 was one such example (Figure 1). Interestingly, encapsulation was not observed for *Rhodococcus sp.* or *Ralstonia sp.* while it was a common feature in *Arthrobacter, Agromyces, and Actinobacter* isolates. Cyanobacteria have demonstrated mechanisms to avoid cell encapsulation [6], while a similar mechanism may exist in *Rhodococcus* or *Ralstonia*, it does not appear to function in these other genera.

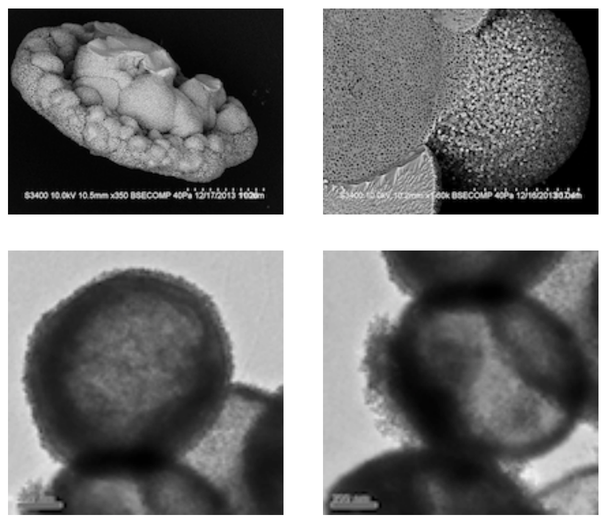

Figure 1 – The upper left panel shows a SEM image of a large $CaCO_3$ mineral structure. The image in the upper right panel is a closer look at the same structure, and suggests that it is composed of several bacterial cells that have been encrusted in $CaCO_3$. The lower panels show TEM images of the same sample, demonstrating bacterial encrustation of individual cells. Lattice fringe of these crystals was conducted to conclusively demonstrate their crystalline nature (not shown).

Other isolates that did not become encrusted in microcrystals, such as the yeast, *Rhodotorula mucilaginosa*, examined in this work, caused the precipitation of very regular crystals, which were not associated with the cell wall but rather formed at the interface between the bacteria and the growth media (Figure 2).

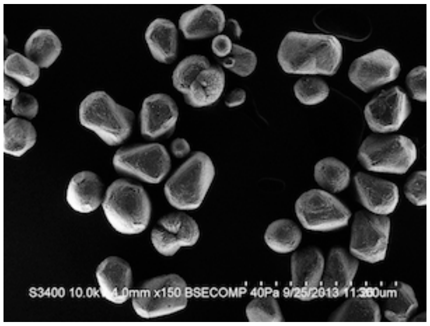

Figure 2- SEM image of the regularly shaped crystals formed during *Rhodotorula mucilaginosa* growth.

Micro-XRD revealed that calcite was the major $CaCO_3$ polymorph precipitated by each of the microbial isolates, although, aragonite was also detected in the *Actinobacter sp.* isolate F4A5 sample and vaterite was detected in the *Arthrobacter sp.* isolate JG9 sample (Figure 3). Lattice fringe imaging identified each crystal as aragonite. This apparent discrepancy may arise from aragonite being formed at the outermost younger areas of the crystal (where the lattice fringes were measured) and calcite making up the bulk of the rest of the precipitate.

Arthrobacter sp. isolate JG9 was also the only mineral to have a red coloration, which may have been due to the presence of ferric iron (likely as fine-grained hematite) as detected by EDX (not shown). In addition, this precipitate had two very distinct mineral morphologies: rhomboid to disphenoid like aggregates with epitaxial rhombs on surface and spherical aggregates with a pitted texture (Figure 4). These two morphologies are also commonly observed in the $CaCO_3$ precipitated by *Myxococcus xanthus* [7], which is also known to precipitate a mixture of calcite and vaterite [7].

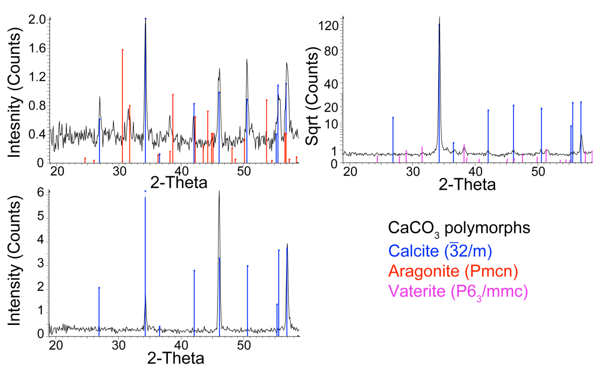

Figure 3 – The upper left panel shows micro-XRD spectra demonstrating that *Actinobacter* isolate F4A5 contained a mix of aragonite and calcite. The upper right, that *Arthrobacter sp.* JG9 contained a mix of vaterite and calcite and the lower panel shows spectra for *Actinobacter* F4A9 that is representative of the spectra observed for other isolates.

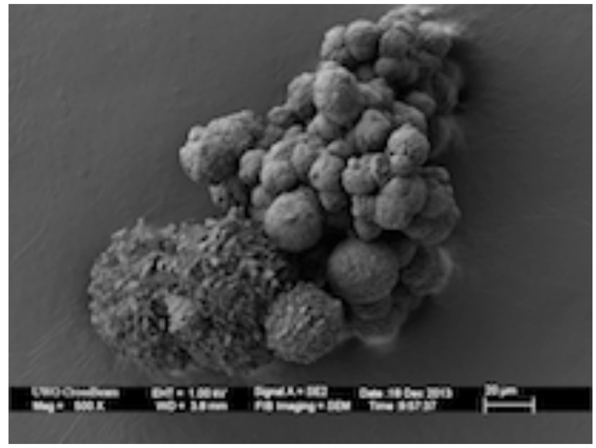

Figure 4 – SEM image of the two morphologies of calcite and vaterite present in *Arthrobacter sp.* isolate JG9 samples.

Conclusions and Implications: Heterotrophic bacterial growth resulting in $CaCO_3$ precipitation appears to be common among the microbial isolates examined in this study. Though media and temperature were consistent throughout the precipitation process, each $CaCO_3$ polymorph was observed and several morphologies were also encountered, indicating that specific attributes of the bacterial cell, responsible for precipitate nucleation and growth, is affecting the resultant mineral. Bacterial encrustation was a common phenomenon observed in this study, and though it may not be common in cyanobacteria [6], it could be a useful biosignautre. Based on this work a combination of crystal structure, chemistry, micro- to nano-scale imaging and possibly isotopic characterization will be necessary to establish the biogenicity of any carbonates recovered from extraterrestrial environments. Future work will examine the utility of various spectroscopic techniques (Raman, UV-induced fluorescence, reflectance) for distinguishing and characterizing biotic and abiogenic carbonates.

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References: [1] Thompson, S.P. *et al.* (2014) *Icarus* **229**, 1-10 [2] Boquet, E., Boronat, A. & Ramos-Cormenzana, A. (1973) *Nature* **246,** 527–529 [3] Dupraz, C. *et al.* (2009) *Earth Sci. Rev.* **96,** 141–162 [4] Sutter, B. *et al.* (2012) *Icarus* **218**, 290-296 [5] McKay, D. S. D. *et al.* (1996) *Science* **273,** 924–930 [6] Thompson, J. B. & Ferris, F. G. (1990) *Geology* **18,** 995 [7] Gonzalez-Munoz, M. T. *et al.* (2010) *Geological Society, London, Special Publications* **336,** 31–50