Quantifying the contribution of viruses and phage to nutrient cycling with NanoSIMS

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Project goals: The LLNL Biofuels SFA is developing advanced methods to support biofuel research in particular, and the study of complex microbial communities in general. We are currently working on algal biofuel ponds, and we are motivated to extend our methods to microbial activity associated with biofuel crops. We are intersted in the nutrient cycles that underpin the productivity of these systems, as well as pathogens that undermine them. Our approach is to combine genomic methods with stable isotope probing and high spatial resolution secondary ion mass spectrometry imaging. This approach enables sequence identity to be linked to function in complex systems so that we can test genome-derived and other hypotheses. Here we extend our approach to the characterization of viruses.

URL: http://bio-sfa.llnl.gov/

Viruses and phage are thought to have significant roles in nutrient cycling in virtually all ecosystems through their effects on microbial and eukaryotic populations. In the environment, most of our ecological knowledge about phages comes from aquatic systems where they are known to kill at least 40% of the standing bacterial stock and sustain up to 55% of bacterial production through liberation of C. Its also known that algal viruses significantly affect C cycling in natural algal blooms, and in soils, we hypothesize that phage are disproportionately active in the rhizosphere, where microbial activity is higher compared to the bulk soil. Viruses and phage hijack the protein and nucleic acid synthesis systems of their host cells to replicate themselves, resulting in the eventual rupture of the cells. The nutrients from the lysed cells and newly produced viral or phage particles then are presumably available to the microbial community for reuse, but this remains untested. Our goal is to develop quantitative methods for characterizing the viral and phage role in these systems using a combination of methods centered on stable isotope probing and high spatial resolution imaging mass spectrometry ("nanoSIP").

To develop this new approach, our first goal was to assess feasibility of viral particle analysis in the LLNL Cameca NanoSIMS 50, a high spatial resolution imaging mass spectrometer we use for nanoSIP. In a series of pure cultures experiments, we determined that individual viral particles yield sufficient data to not only extract single particle isotopic information, but also to characterize nucleic acid isotopic enrichment (Fig. 1). We found that for larger virions such as *Vaccinia*, analytical precision can be as good as 10% for individual virions. For phage and smaller viruses, isotopic enrichment of individual particles is detectable at approximately twice natural isotopic abundance. These results have established the analytical methods for data extraction from viral particles and the analytical bounds for virus and phage quantitation.

In a second set of experiments, we examined the potential to quantify the degradation and uptake of viral particles by consumers in its native community. We also sought to determine viral C and N partitioning among consumers. For these experiments, we used EhV, a virus that infects the

coccolithophore *Emiliania huxleyi*. We isotopically labeled EhV by producing it in ^{13}C- and ^{15}N-labeled *E. huxleyi*. Then, the ^{13}C- and ^{15}N-labeled EhV was provided to the native microbial community without the algal host. Incubations were carried out for 40 hours and 27 days to characterize the degradation and uptake of the viral particles over time. In NanoSIMS imaging, viral particles and microbes can be differentiated based on size and morphology. The time-zero sample contains a high abundance of isotopically enriched submicron particles (EhV), whereas EhV-sized particles are much less abundant in the 40-hour sample, and in their place, there is a high abundance of isotopically enriched micron-scale microbes (Fig. 2). Isotopic enrichment levels in the microbes is many times higher than natural isotopic compositions, consistent with incorporation of the EhV or its degradation productions. The nanoSIP data also show that the microbes have a range of relative C and N uptake from EhV, consistent with differences in microbial need and processing capability.

While these nanoSIP results are interesting unto themselves, significant additional work remains before can achieve our goal of quantifying the role of virus and phage in nutrient cycling microbial systems. Towards that end, we are looking to include other methods, such as rapid phage isolation, enrichment, identification, visualization, and quantification, and to utilize nucleic acid sequence data to characterize and monitor phage and host dynamics in natural systems. We intend to apply these new tools in our future SFA work to gain a more mechanistic and holistic view of whether virus and phage-mediated lysis of algal, bacterial and fungal cells is a critical controller of the ultimate fate of algal/root C.

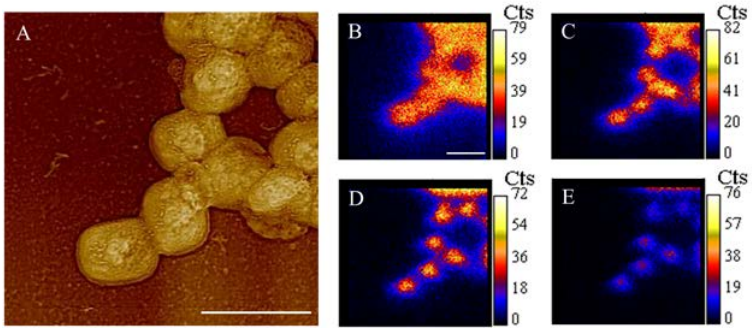

Fig. 1. Correlated AFM (A) and NanoSIMS (B - E) $^{12}C^{14}N^-$ images of depth-profiled vaccinia virions. The viral particles were produce with ^{15}N-labeled thymidine, which labeled their DNA. Isotopic data extracted from this time series shows the isotopic label is collocated with the DNA, where it is expected. Scale bars: 500 nm.

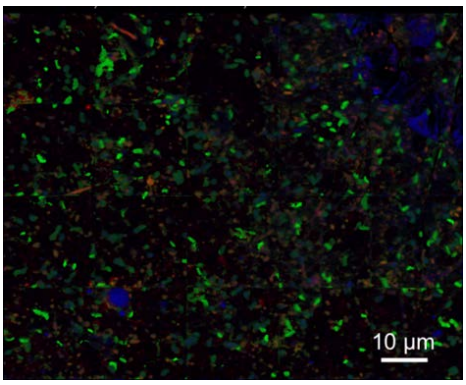

Fig. 2. Color addition mosaic image of nanoSIP experiment on an aquatic microbial community incubated in stable isotope (^{13}C & ^{15}N) labeled *E. huxleyi* viruses for 40 hours. The data show that the viral particles were degraded and incorporated by the microbes, and further show that some microbes incorporated more C or N from the viral particles. RED = ^{13}C enriched; GREEN = ^{15}N enriched; BLUE = unenriched organic matter. Cells that incorporate ^{13}C and ^{15}N tend to appear green with this color scale. The mosaic is 80 x 100 microns.

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