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Major in Biology

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The Effect of Starch Copper Oxide Nanoparticles on Differential Growth of Microbial Populations in Aquatic Environments

Throughout my Summer Undergraduate Research Fellowship (SURF) program, I got to perform my own research project with my mentor, Dr. Matt Hoch, and in doing so I developed into a stronger researcher. My research involves the effects of starch copper oxide nanoparticles (SCuONPs) on the composition and function of microbial communities in rice crop water. This is important because mineral copper oxide nanoparticles (CuONPs) are currently used as herbicides and fungicides in agriculture, but they can be harmful to the environment. SCuONPs are potentially a cleaner, more effective alternative, but their effects on microbial communities are not known. I hypothesized that bacterial taxa in the rice crop water will respond differently to SCuONPs, with some stimulated by organic components and others inhibited by CuO.

To test this hypothesis, rice crop water was collected, distributed into four replicates for each treatment, then incubated under ambient condition for a total of seven days. The treatments used were a control (unamended), low concentration of mineral-only CuONPs (30 µM Cu), low concentration SCuONPs (30 µM Cu), and high concentration SCuONPs (300 µM Cu). Every day, bacterial count samples were preserved in 2% formaldehyde and α-glucosidase activity was measured by fluorometry to assess starch degradation. On day one, day four, and day seven, samples were assayed for bioorthagonal noncanonical amino acid tagging (BONCAT) to assess levels of protein synthesis and filtered for subsequent metagenomic DNA extraction and 16SrDNA sequencing to determine bacterial taxa. The density of SYBR Green stained bacteria and BONCAT fluorescence intensity were determined flow cytometry (FCM). High and low BONCAT intensity populations were then collected by fluorescence activated cell sorting (FACS), filtered, and DNA extracted. All DNA extracts were sent for metagenomic 16Sr DNA sequencing. Sequence analysis will use QIIME2 workflows to assess the relative population composition of the total microbial communities, as well as populations with lower and higher growth, based on BONCAT-FACS.

Prior to performing the main experiment to test my hypothesis, I had to develop and test all my methods using pure cultures of bacteria and aquatic bacterial communities in John Gray Pond water. Most challenging was the BONCAT procedure, whose application to natural bacterial communities is still fairly new. The BONCAT procedure was first tested on Staphylococcus epidermidis cultures, using stationary phase cells as a control and cells stimulated in growth by amending to 10 mM glucose for 4 hours. The BONCAT procedure did indeed work, as the population amended with glucose showed increased activity compared to the control as determined by epifluorescence microscopy and FCM cell-specific fluorescence (Fig. 1).

For the main experiment, both the bacterial density (Fig. 2) and DNA extract concentrations (not shown) followed similar trends among treatments over time. The high concentration of SCuONP treatment showed significant cell death and growth inhibition, and the mineral CuONP treatment did not reach the maximum bacterial density in the control and low SCuONP treatments by Day 4 (Fig. 2). There was a decrease in cell density after four days in the control and lower CuONP treatments, which is consistent mortality due to viral lysis or heterotrophic protists feeding on the bacteria rather than Cu toxicity alone. (Fig. 2). The flow cytometer plots of forward scatter versus SYBR Green used for counting cells (Fig. 3) were generally similar in the Control and low mineral CuONP treatments, but there was some selection of higher DNA content cells in the low SCuONP treatment, possibly in response to the added organic content. However, a toxic response was evident in the high SCuONP treatment, consistent with CuO production of reactive oxygen species damaging DNA.

The α-glucosidase activity was measured each day to observe any starch degradation (Fig. 4). The low SCuONP treatment had significantly greater enzyme activity at Day 2 through Day 7 than any other treatment. The initial lag in activity for this treatment may be explained by catabolic repression due to the presence of glucose in the SCuONP preparation. The lowest enzyme activity was in the high SCuONP treatment and is consistent with both prolonged catabolic repression due to 10-fold greater glucose than in the low SCuONP treatment and CuO toxicity driven cell loss.

BONCAT analysis by FCM was performed on Days 0, 4, and 7. The Day 0 BONCAT assays and negative controls (Fig. 5) demonstrate the efficacy of the assay for protein synthesis and minimal non-specific staining. All of Day 4 and 7 BONCAT samples were analyzed, and two to three levels of cells-specific protein synthesis (growth rate proxy) were cell sorted by FCM-FACS (Fig. 6). Both treatments with the organic SCuONP had a population of fast-growing populations (P3 gate) whose appearance was consistent with the 10-fold difference in concentrations. Specifically, this population appeared first in the low organic SCuONP treatment at Day 4 then disappeared by Day 7. Whereas in the high organic SCuONP treatment it sustained

itself through Day 7, when there was also a loss of all low activity (P1 gate cells). All cell sorts were filtered, DNA extracted, and are at this time in the process having the 16SrDNA of the metagenomes sequenced for bacterial taxa analysis.

When I took a microbiology course required for my biology major my freshman year of college, I developed an interest in microbiology. Through this research fellowship, I was able to delve into that interest in a way that I would not have gotten to do otherwise. I also came into college with my mindset that research was not an option for me, as I did not see myself enjoying it. After finishing my SURF project, my perspective has changed on research, and I now see that I could potentially enjoy doing more research in the future. I also learned many new techniques and skills during my research fellowship. Through this opportunity, I learned how to operate multiple instruments that I had not used before, including a flow cytometer, epifluorescence microscope, and spectrophotometer. I also got very adept at using pipets, and I got to use a repeat pipet, which I had not gotten to use before my SURF project. Lastly, through this project, I was given the opportunity to perform a fairly new technique, BONCAT, and I had to get in contact with one of the authors of the paper to do so.

Although the SURF program finished in August, I was not completely done with analysis all BONCAT results, cell sorting and DNA extractions for sequencing and bacterial community analysis. In early fall I began collaborating with Michael Shannon (BS Biology Major) for FCM-FACS of BONCAT samples. All cell sorts were then filtered, DNA extracted, and are at this time in the process having the 16SrDNA of the metagenomes sequenced for bacterial taxa analysis. We will specifically know which bacterial taxa were inhibited by CuO versus benefited from the organic constituents of SCuONPs.

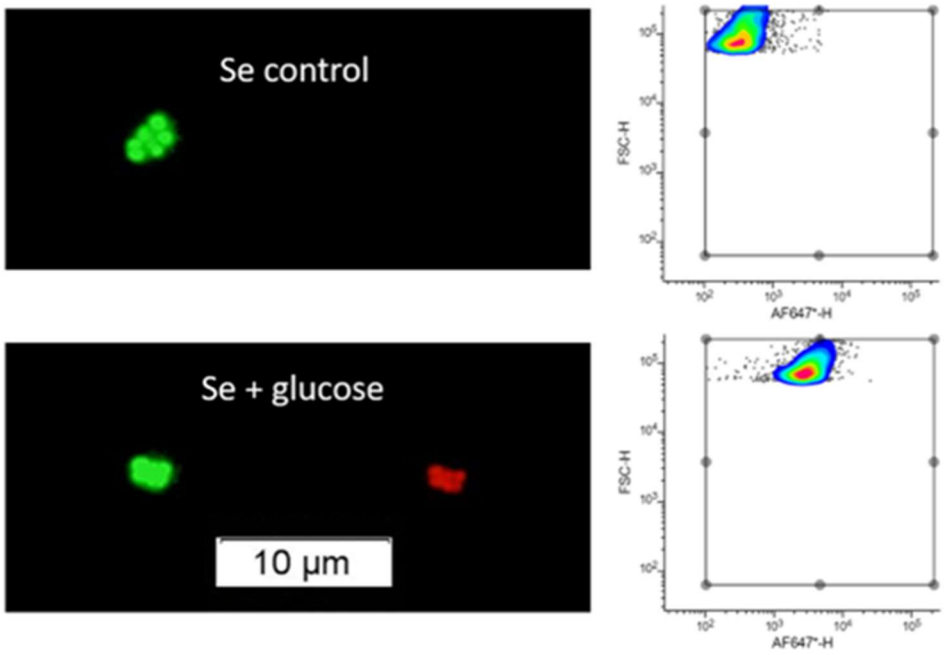

Figure 1. *Epifluorescence microscopy of SYBR Green stained and BONCAT active (red) S. epidermidis stationary phase control (top left) versus culture amended with glucose (bottom left); Flow cytometry results of forward-scatter (relative cell size) versus BONCAT AlexaFluor 647 fluorescence for control (top right) and glucose amended (bottom right) the S. epidermidis cultures.*

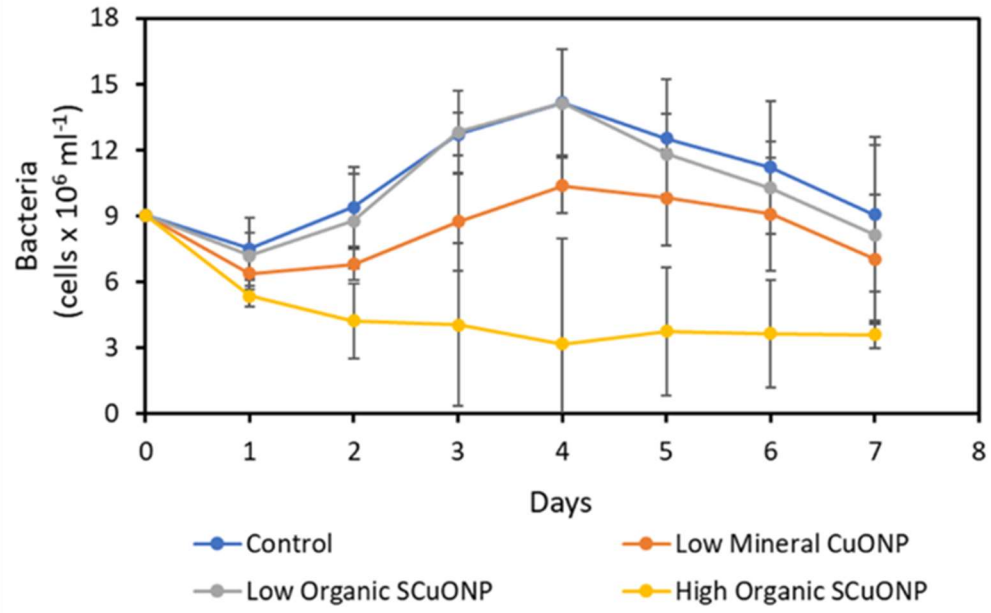

Figure 2. *Mean (± SD) daily bacterial density of each treatment determined by SYBR Green staining and FCM.*

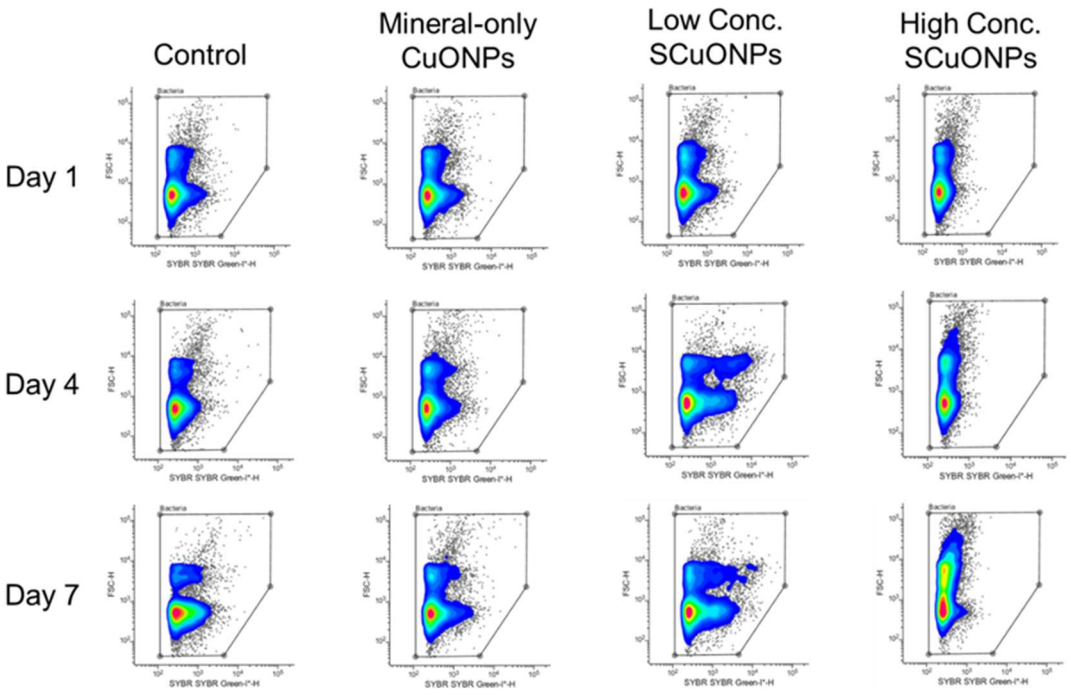

Figure 3. *Bacterial count flow cytometry plots of forward-scatter versus SYBR Green cell-specific fluorescence of DNA for replicate #4 of each treatment on days 1, 4, and 7.*

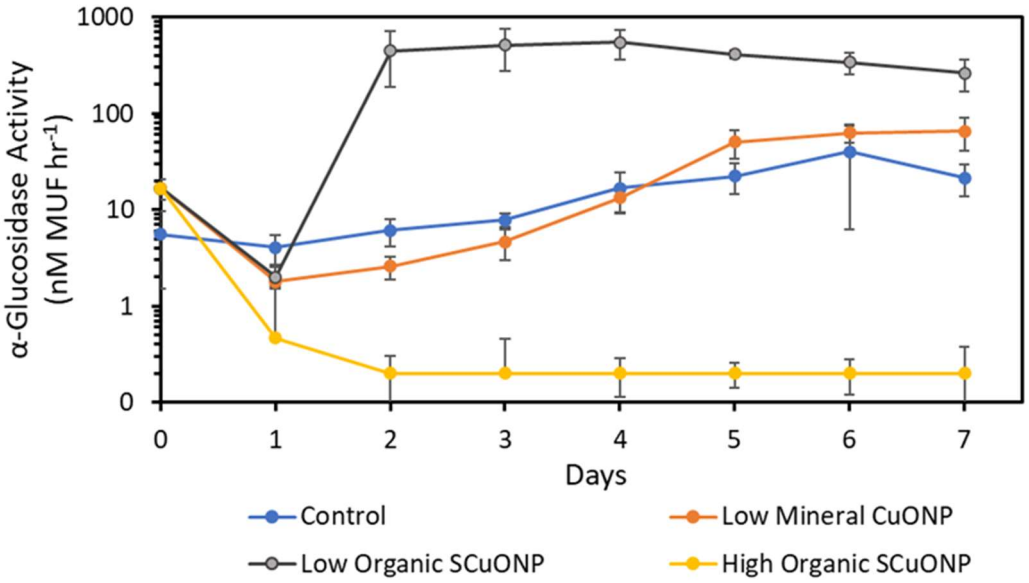

Figure 4. *Mean (± SD) daily α-glucosidase activity of each treatment.*

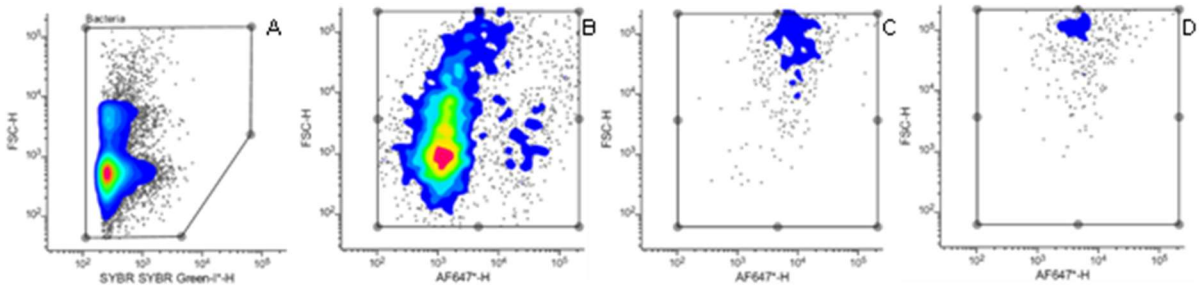

Figure 5. *Flow cytometry plots of the Day-0 rice crop water control for SYBR Green bacteria count (A) and AlexaFluor 647 dye fluorescence of the normal BONCAT assay involving incubation with the methionine analog, L-homopropargylglycine (+HPG; B), incubation with both +HPG and chloramphenicol, a prokaryotic-only protein synthesis inhibitor (C), and incubation without HPG to assess non-specific binding of AlexaFluor 647 dye (D). The PMT setting differed for FCM between SYBR and AF647 plots.*

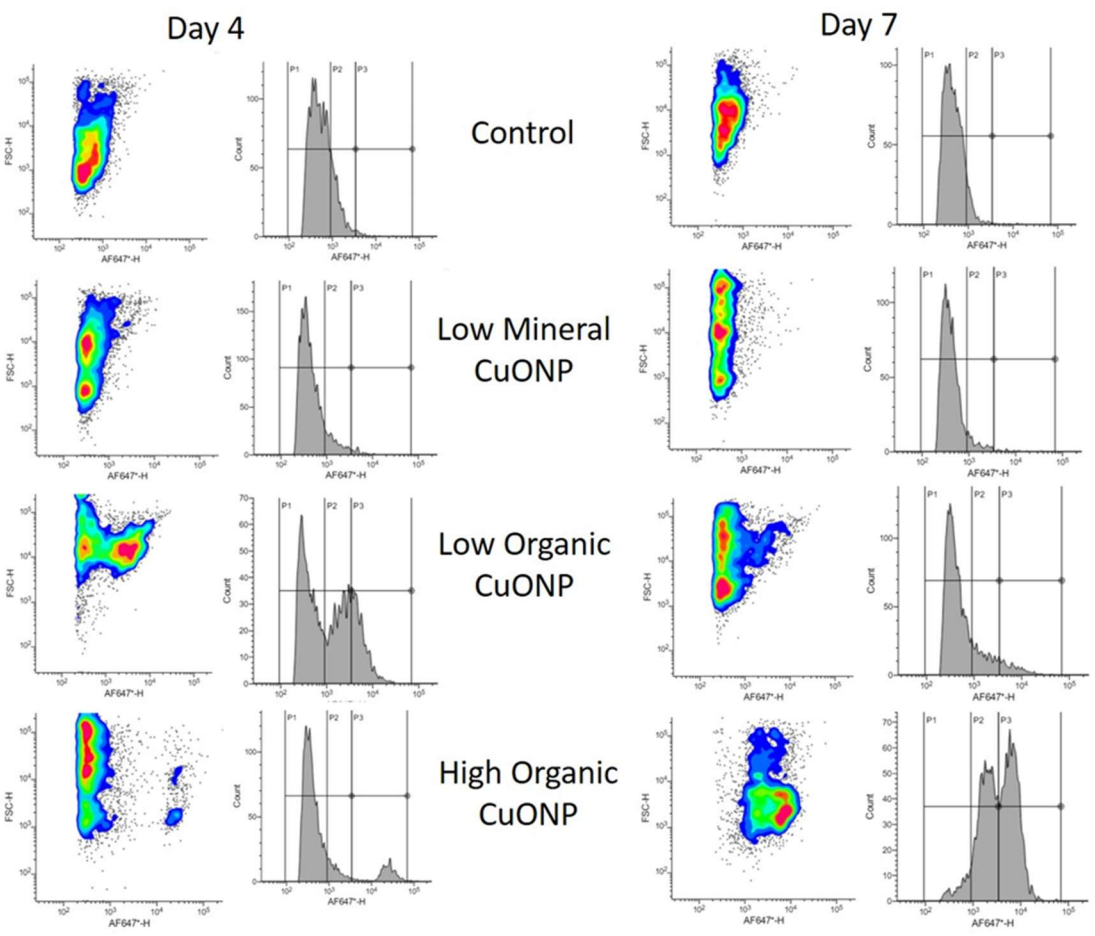

Figure 6. *Flow cytometry plots of FCM versus BONCAT-AF647 fluorescence and the histogram of the latter with three sorting gates, P1, P2, P3, of increasing cell-specific protein synthesis of control and three treatments for Day 4 and Day 7 samples.*