

152. NanoSIMS Isotope Imaging to Investigate Algal-Bacterial Interactions in Biofuel-Producing Communities

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Project Goals: The LLNL Biofuels SFA seeks to support robust and sustainable microalgae fuel production through a systems biology understanding of algal- bacterial interactions. We hypothesize that by understanding the factors that control cellular physiology and biogeochemical fluxes in and out of algal cells, particularly through the phycosphere, we can advance the efficiency and reliability of algal biofuel production. Our research includes studies of probiotic traits of phycosphere-associated bacteria, systems biology studies of model algae, and genome-enabled metabolic modeling to predict the interspecies exchanges that promote algal growth, lipid production and healthy co-cultures. Our overall goal is to develop a comprehensive understanding of complex microbial communities needed to advance the use of biological properties for practical energy production.

To better understand the mechanisms by which probiotic bacteria promote growth, health, and/or lipid-producing capabilities of unicellular microalgae, we are currently investigating algal-bacterial interactions in two experimental systems: 1) simple co-cultures incubated in the laboratory consisting of one bacterium and one algal species, and 2) complex, natural communities (man-made algal ponds or natural aquatic ecosystems) with a diverse array of bacteria, algae, and other organisms. One of the key approaches we have used, NanoSIP (stable isotope probing followed by NanoSIMS isotope imaging analysis), allows us to monitor the transfer of metabolites specific to one partner in algal-bacterial consortia.

To examine exchange of metabolites in a highly controlled algal-bacterial system, we conducted a co-culture experiment using the microalga *Chlorella vulgaris* and the plant growth-promoting bacterium *Azospirillum brasilense* 1 and investigated C and N transfers between them using NanoSIP. The two isolates were grown separately, labeled with ¹³C and/or ¹⁵N, and then co-incubated inside alginate beads for four days. We used the NanoSIMS 50 at LLNL to examine the transfer of metabolites from one partner to the other. Labeling of bacteria with heavy isotopes provided the unequivocal identification of bacteria attached to algal cells, the former being often hidden due to their small sizes and altered shapes when attached. Using this approach, we have demonstrated the reciprocal transfer of carbon and nitrogen in this partnership. Our results show that physical attachment between algae and bacteria resulted in higher levels of transfer in most cases, but attachment was not necessary for transfer to occur.

In our first investigations of algal-bacteria symbiosis in a natural system, we have examined the cell-specific metabolism of microalgae and bacteria collected from a natural springtime bloom in the coastal Eastern Atlantic. Mixed communities were incubated for 12 hours in the presence of ¹³C-bicarbonate and ¹⁵N leucine, the former serving as a marker for photosynthetic carbon fixation and the latter for heterotrophic bacterial carbon production. Again we used NanoSIP, this time to measure autotrophic and heterotrophic activity. This approach allows us to compare cell-specific metabolism of algal and bacterial populations, and also enabled us to identify algal and bacterial cells attached to one another, which appears to be a relatively common phenomenon. NanoSIP data for over 4,000 cells indicate that mixotrophy was uncommon in the bloom samples analyzed. NanoSIP was able to detect increased bacterial metabolism in response to increases in temperature. NanoSIP enables cell-specific

measurements of activity for both algae and bacteria and allows the quantification of C and N transfer between cells, providing valuable insight into symbiotic relationships that occur in constructed as well as natural ecosystems.

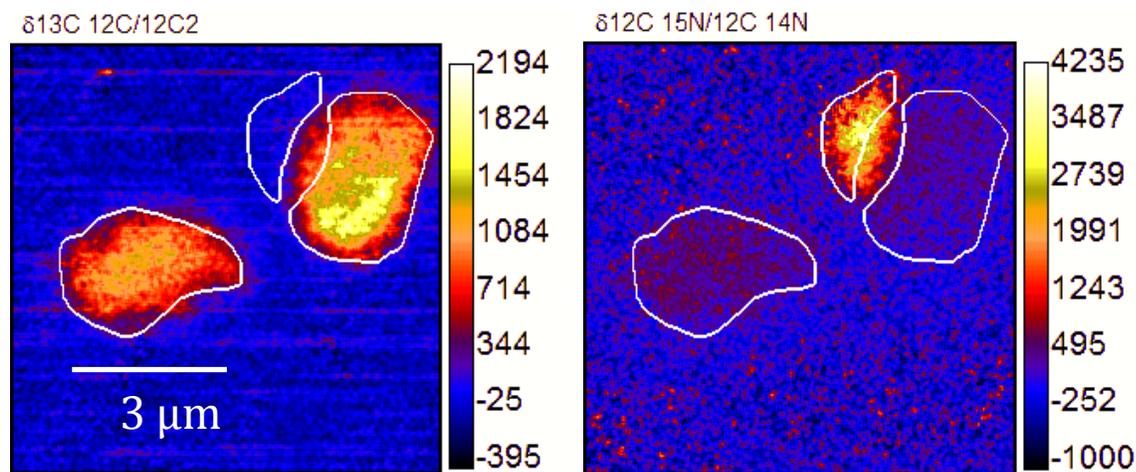


Figure 1. NanoSIP images showing autotrophy and heterotrophy in a mixed assemblage. The $\delta^{13}\text{C}$ image (left) shows 2 algal cells that took up the ^{13}C tracer, indicating autotrophy. The $\delta^{15}\text{N}$ image (right) shows a bacterium (attached to one of the algal cells) that incorporated the ^{15}N -leucine tracer, indicating heterotrophy.

Publications

1. Gonzalez, L. E. & Bashan, Y. Increased Growth of the Microalga *Chlorella vulgaris* when Coimmobilized and Cocultured in Alginate Beads with the Plant-Growth-Promoting Bacterium *Azospirillum brasilense*. *Appl. Environ. Microbiol.* 66, 1527-1531 (2000).

This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 and supported by the Genome Sciences Program of the Office of Biological and Environmental Research under the LLNL Biofuels SFA, FWP SCW1039.