

Taxonomic composition of simplified bacterial communities conferring growth and biomass enhancements to biofuel-producing algae

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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.

Mutualistic algal-bacterial interactions may arise when bacteria provide metabolically beneficial substances to their algal partners in exchange for energy-dense organic compounds. We seek to understand these positive exchanges by establishing closely interacting algal-bacterial cultures, simplifying the resultant algal-attached bacterial communities through enrichments, and monitoring the algae for elevated growth and biomass characteristics. After initial screening and downselection, we have analyzed the bacterial community composition of nearly 100 samples to correlate the presence/absence of specific bacteria with measured of algal health, identify putative modes of metabolite transfer from bacterium to alga, and inform upcoming genome sequencing endeavors which will inform metabolic modeling of the effects of individual beneficial bacteria.

We used two model biofuel-producing microalgal strains, *Phaeodactylum tricornutum* (*Pt*) and *Nannochloropsis salina* (*Ns*), to enrich for growth-promoting bacteria from established algal raceway ponds in Texas and the coastal Pacific Ocean. Enrichments were evaluated for increases in chlorophyll fluorescence (an estimator for health and growth). Eventually, 13 *Ns* and 36 *Pt* enrichments were established and their productivity was evaluated under 24 h light and 12 h light-dark cycles. The DNA of bacterial communities of the algal phycospheres versus the total community were collected, the 16S rRNA gene partially sequenced and compared.

Results indicate enhanced algal growth and yield in many enrichments, highlighting the successful establishment of beneficial bacterial communities. We confirmed bacterial attachment to algal phycospheres via fluorescence microscopy, scanning electron microscopy, and NanoSIMS imaging. Light cycle affected the prevalence of beneficial interactions in both the *Ns* versus the *Pt* enrichments, although in different directions. As expected, bacterial community richness of enrichments was significantly reduced relative to source communities. In all simplified communities, the majority of bacteria belonged to the *Rhodobacteraceae*. At the

genus level, *Phaeobacter* were abundant regardless of algal host. In *Pt* cultures *Labrenzia*, *Loktanella* and *Hyphomonas* were abundant members, whereas the *Ns* cultures were enriched in *Marivita*, *Marinobacter* and *Algoriphagus*. These abundant taxa were not always correlated with a probiotic effect. Interestingly, two *Loktanella* species appeared to have opposing effects depending on the host, with *L. vestfoldensis* being beneficial to *Ns* and detrimental to *Pt*, while *L. rosea* had the opposite effect.

The positive growth effects observed in these enrichments suggest that maintenance of algal-bacterial mutualisms may assist with establishing robust algal biofuel cultures. The high proportion of *Rhodobacteraceae* in the enrichments is an encouraging observation since several sub-groupings of this lineage are major contributors to algal health in natural ecosystems. For example, it has been demonstrated that some species release growth-enhancing hormones¹ or vitamins² to an algal cohort and, in some cases, antibiotics to kill algicidal bacteria³. Future work will further characterize these relationships using experimental and bioinformatic approaches and identify the chemical and ecological mechanisms underpinning these complex symbioses.

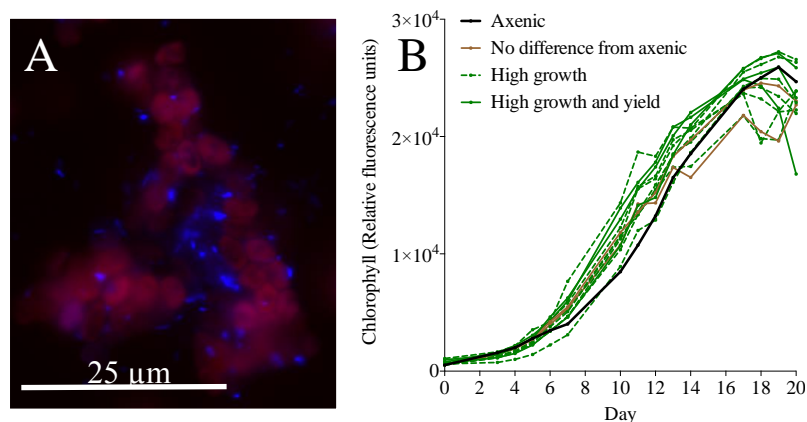


Figure 1. (A) Fluorescence micrograph of *N. salina* (red) with associated bacteria (blue). (B) Growth and yield enhancement measurements of *N. salina* enrichments.

Citations

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