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Final Technical Report for subcontract number B612144

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Disclaimer

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Final Technical Report for subcontract number B612144

Award Title: A Syatems Biology Approach to Microbial Symbioses: How Algal-Bacterial Interactions Control Resource Allocation in Biofuel-Producing Communities

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I) Summary of research originally proposed.

The original statement of work stipulated that the Subcontractor shall perform bacterial and algal cultivation and manipulation, microbe isolation, preparation of samples for sequencing and isotopic analysis, data analysis, and manuscript preparation. The Subcontractor shall work closely with Dr. Mayali and other LLNL scientists, and shall participate in monthly SFA meetings (either in person or by telephone). The Subcontractor shall deliver a final report at the conclusion of the work.

II) Summary of accomplishments made during this grant period.

Six strains of heterotrophic bacteria isolated in the Marcu lab were provided to LLNL and Stanford University. These were frozen stocks and plated cultures of *Novosphingobium* (1 strain), *Methylobacterium* (1 strain), *Arthrobacter* (2 strains) and *Pigmentiphag*a (2 strains). Equally, *Chlamydomonas reinhardtii* axenic strain cc-1690 was provided on solid medium to both institutions.

The P strains were unknown at the start of this project, and the Marcu lab did culturing, PCR and sequenced the 16S RNA for P1, P2a and P2b. MegaBlast indicated 100% similarity of both P1 and P2a to Pigmentiphaga litoralis (Fig1). However, culturing of these isolates indicated a different appearance and pigmentation of the bacterial colonies. This was confirmed by the difference in the spectral fingerprints at 440 and 630 nm.

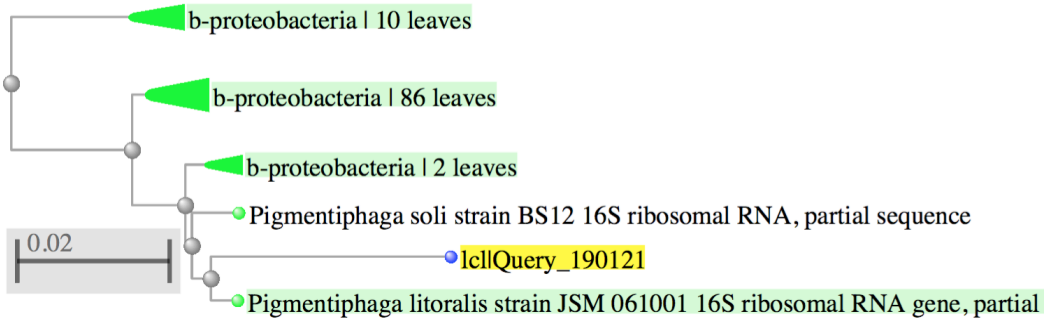

Fig1. P1 and P2a. Two morphotypes of Pigmentiphaga litoralis

Sequencing of P2b isolate, obtained from growth in rich medium, demonstrated that tge genus is the same as for B3, Arthrobacter sp. (Fig2).

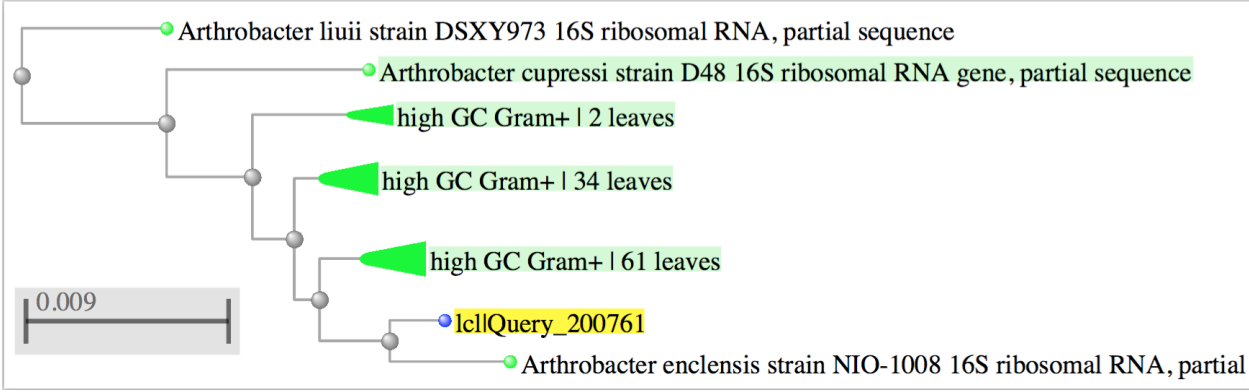

Fig. 2 B3 and P2b – Two morphotypes of Arthrobacter, with distinct spectra

These two isolates also differed in their abstrobance spectra at 600 and 700 nm. Moreover, they induce a different response in Chlamydomonas in co-cultures, as B3 was initially identified as biomass-producing only, while P2b was part of the consortium that induced both biomass and lipid production. These differece were presented during the SFA meetings to direct the work by the Stanford group in selecting strains that induce lipids, and for the computational group to be able to compare behavior in co-culture from the same genus of bacteria that may have minimal differences in sequence but different outcomes phenotypically.

To identify and select optimal bacterial partners the strains were categorized in biomass producing and lipid and biomass producing, based on previous data collected prior to the start of this subcontract. This background information (Table 1) was shared with the collaborators at LLNL and Stanford U.

	Biomass	**Biomass + Lipid**
Bacteria	- *Novosphingobium sp.* (B1) - *Bacillus sp* (B2) - *Arthrobacter sp,* (B3) - *Methylobacterium sp.*(B4)	- *Pigmentiphaga litoralis* (P1, P2a) - *Arthrobacter sp.* (P2b)
Medium	Bold's or TAP	P49
Increase in photosynthetic yield	+	+
Algal protection from exogenous H_2O_2	+	+
Modulation of ROS levels	+	+
Growth enhanced	+	+
Lipid/surfactant production	-	+
Associations (prelim. observations)	• B1 - association with alga seems optional (but when close to algal cells bacteria clump) • B2 - external, induces algal lysis • B3 - direct attachment to phycosphere? • B4 - tight association, not clear if outside or inside the phycosphere	• P1 - optional, but direct contact with algae shows bacteria in clumps • P2b - tight, clumping (maybe lysis)
Co-cultures	• demonstrated with all 4 together in consortium, and each individual strain at a time.	• demonstrated with all 3 together, not with individual strains

Table I. *Chlamydomonas* co-cultures for biomass or lipid/surfactant production

The preliminary data obtained in the laboratory at NASA Ames was presented at the February 2015 DOE JGI meeting. Even though obtained with prior SETI and NASA funding and additional support from the Wellesley College, the data was presented under the umbrella of the LLNL SFA, to demonstrate one of the paths forward for the *Chlamydomonas* part of the project.

Background information on the Chlamydomonas-bacterial interactions was provided to Dr. Jeniffer Pett-Ridge for the interim SFA report, and to support Dr. Jiao's LDRD proposal 2015 entitled: "Cell-Cell Interactions in Mixed Communities: the Role of Biosurfactants Produced by Bacteria in Enhancing Algal Biomass Production". This proposal ended up not being selected for funding. Similarly, updated information on tree phylogeny of the bacterial strains was provided to Dr. Mayali Xavier for the JGI CSP proposals, which was awarded in July 2015. Sequencing of genomes of the bacteria isolated by the SETI PI is part of the JGI CSP proposal.

Deliberate reconstitution of lipid induction in *Chlamydomonas* was essential for the progress of this project and to provide direction for the Stanford collaborators. This was accomplished in the NASA Ames laboratory, to support the original observation on enhanced lipid production. This demonstration was done with multiple bacterial strains and the data was provided to the Stanford lab.

For the gene expression part of the proposal, primer sequences that were originally designed for specific genes of interest and were shared with all collaborators. Given a change in direction of the project towards global transcriptomics, and the dependency of this approach on the work performed at Stanford, the LLNL PIs and SETI Co-I agreed on a different approach, parallel with gene expression, using proteomics. in collaboration with Drs. Rhona Stuart and Xavier Mayali. Dr Marcu proposed to aim this project at distinguishing the mechanism of algal protection by bacteria. She provided the background data obtained in her laboratory previously; as well as supporting literature and context, and new scientific questions of interest as outlined below. The data indicated that both *Novosphingobium* and *Pigmentiphaga* protect *Chlamydomonas* from exogenous hydrogen peroxide (Fig3).

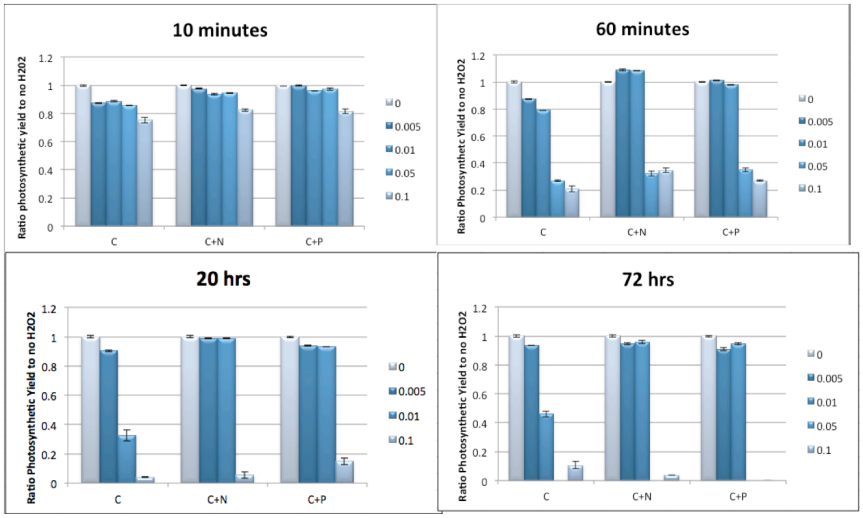

Fig. 3 The photosynthetic efficiency of Chlamydomonas in the presence of hydrogen peroxide is preserved in the presence of heterotrophic bacteria. (C+N = Chlamydomonas and Novophingobium, C+P= Chlamydomonas in co-culture with Pigmentiphaga).

Proposed mechanism of protection could be: a) algal stress proteins; b) extracellular bacterial catalases degrading hydrogen peroxide. In order to assess whether the latter is a possibility, an experimental approach was proposed to use a bacterium without catalase activity.

Additionally, the proteomic study would identify whether protein secretion from bacteria and/or *Chlamydomonas* could be part of the protection mechanism.

Another goal of this proteomic project targeted the component of the algal/bacterial proteome involved in the early protection (within minutes) vs the sustained protection (hours/days). This is essential to be able to distinguish whether the early protection is involved in the long-term phenotype of biomass and lipid production.

This proteomic approach was designed to compare future transcriptomics data with the proteome data, and made use of the fact that transcriptome information for effects of hydrogen peroxide on *Chlamydomonas* alone, without bacterial partners, already exists in the literature.

Additionally, a large amount of data already exists for the proteome, phosphoproteome and subcellular proteome of *Chlamydomonas* alone, to which *the Chlamydomonas*/bacteria data could be compared.

The proteome project was pending demonstration of feasibility at PNNL based on protein fractions provided by LLNL (Dr. Rhona Stuart) prior to the end of the funding of this subcontract.

In addition to experimental data and design, Dr. Marcu provided all the background information and supported numerous meetings at Stanford U. to further develop the project on lipid induction in *Chlamydomonas* by bacteria; contributed to the SFA team meetings with background and updated information on the *Chlamydomonas* project for the entire SFA team; and recruited two postdoctoral fellows, a student and a SETI volunteer for the project. The limited funding and the decision time to hire a postdoctoral fellow prevented the hires. (see details in Section III).

III) Summary of risks or obstacles, plus mitigation strategies.

The major obstacle over the duration of this project was unclear communication between team members.

Miscommunication between LLNL and NASA civil servants prior to the involvement of Dr. Marcu in this project, led to a number of issues that negatively affected Dr. Marcu, her research, collaborations, and resources available to her, including funding. Having to relocate laboratory twice and change division affiliation over the duration of the LLNL subcontract affected the time of performance for this subcontract.

Other obstacles, that are mostly part of working in a large team, included delayed and unclear communication and decisions amongst team members. In 2016 SETI recruited 2 postdoctoral fellows with expertise in *Chlamydomonas* work; hiring was delayed due to funding issues. In January 2017 Dr. Marcu recruited for the project a former student, who generated most of the original data that constituted the basis of this project, and a volunteer through the SETI volunteer program. Both hires ended suddenly in February 2017 when the decision to not renew the subcontract was made.

Even though the work at SETI provided all the original data for the part of the SFA related to the bacterial interaction with *Chlamydomonas*, the decision to fund SETI for only 1 year, and

other partners for 3 consecutive years, resulted in additional instability and pressure for the SETI PI, and the inability to plan long-term.

IV) Publications produced

D. YoungSmith, M. R. Brann, X. Mayali, O.E. Marcu (2015) Protection from Oxidative Stress in Bacterial Co-Cultures. DOE Genomic Science Contractors-Grantees Meeting XIII, Tysons, VA.

149. Protection from Oxidative Stress in Algal-Bacterial Co-Cultures

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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.

As part of a new SFA project focused on algal-bacterial interactions, the LLNL Biofuels SFA has recently initiated collaborations with O. Marcu's group at the SETI Institute. The Marcu lab is focused on laboratory co-cultures of the green alga Chlamydomonas reinhardtii grown in co-culture with bacterial strains that enhance the growth and lipid production of the microalga. The bacteria were isolated from the topsoil of Mojave Desert and are pre-adapted to the oxidative stress imposed by the surface exposure to UV radiation and desiccation. In the presence of these bacteria, the Chlamydomonas cultures show an initial slight increase in the levels of reactive oxygen species (ROS), but increased growth and photosynthetic efficiency. Remarkably, in the presence of a secondary stress induced by heat, the ROS levels in algae decrease as compared to controls, suggesting that the bacteria provide priming and protection against oxidative stress. Bacteria also protect the algae from hydrogen peroxide-induced stress, most likely through the activity of extracellular bacterial catalase, and from the stress induced by the removal of copper. Monitoring of ROS levels in the intracellular compartment versus supernatant suggests a role for the extracellular matrix in buffering their toxicity. Co-cultures that show increased lipid production can be reconstructed in laboratory conditions.

Currently the work focuses on the multiple bacterial consortia in co-cultures and their physical association with the algae, gene expression under stress conditions, the lipid metabolism and metal transport, and the role of the algal extracellular matrix in mediating the association and response to stress, for short- and long-term growth of algal cultures with enhanced productivity.

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