Identification and characterization of performance-enhancing bacterial symbionts of open algal cultures

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Introduction

To grow algae successfully on a large industrial scale in open ponds or bioreactors, a healthy bacterial flora will need to be established and maintained. Various clades of bacteria are known to grow in close proximity to algae, however the effects of different bacteria on algal growth and metabolism are poorly understood. Understanding these relationships will allow for the manipulation of the microbial community structure, and allow continuous propagation of large algal cultures which can be used to produce substantial amounts of biofuels of minimal land.

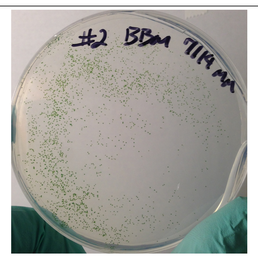
Goals of current study

- Cultivate photoautotrophic microbial communities (algae and heterotrophic bacteria) from nature in minimal media.
- Isolate and identify algae and bacteria in these microcosms using microscopy, metabolic studies, and 16S/18S rDNA sequencing.
- Analyze lipid content of microbial communities in comparison with pure cultures currently under study.
- Assess algal growth in pure cultures inoculated with isolated bacteria using *Chlorella sorokiniana* UTEX-1230.
- Analyze plated colonies of dominant algal species on BBM media as compared to BBM media supplemented with sterilized supernatant from mixed cultures

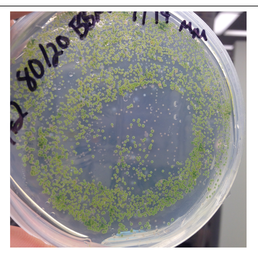
Establishment of algal cultures

9 sites in and around Lincoln, NE were selected for initial establishment of mixed algal microcosms. Soil and freshwater samples were inoculated into Bolds Basal Medium (BBM), a minimal mineral nutrient medium, and grown for 1-2 weeks until a green algal bloom was established. These cultures were then passaged every 5-7 days by diluting the culture 1:100 by volume into fresh BBM medium. After the 5th passage, a "master mix" culture was established.

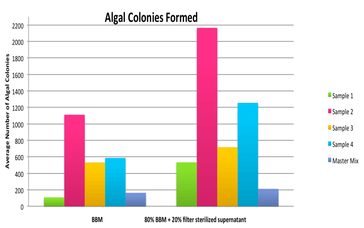
Culture supernatants stimulate algal growth



BBM



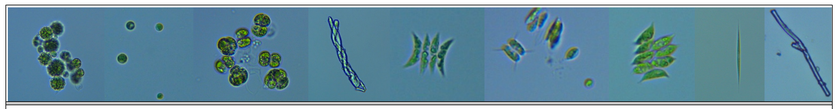
BBM with supernatant



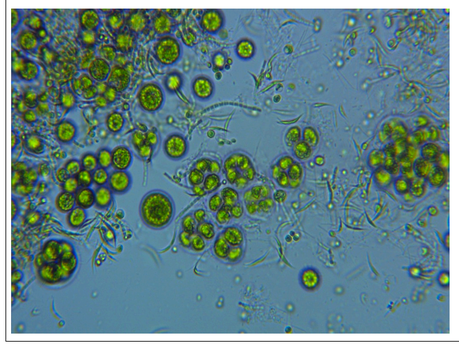
Photoautotrophic consortia containing *Chlorella*-like algae as the dominant algal species were plated for single colonies on minimal medium (BBM), or BBM containing 20% (v/v) supernatant from a stationary phase cultures.

Results indicate that the presence of spent-medium enhances the growth and colony forming capacity of these algae.

Establishing algal cultures and identifying algal types



- Chlorella1,2,3, Diatom, scenedesmus, ankistrodesmus



Between the 9 original samples collected, the major algal type was chlorella. Diversity was highest during the beginning of the project. *Scenedesmus* was the second most prevalent after several passages. *Ankisrodesmus* and different cyanobacteria were present after several passages at greatly reduced amounts.

Major bacterial type present in communities: PPFM

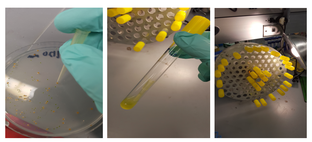
BBM with 0.5% MeOH

BBM

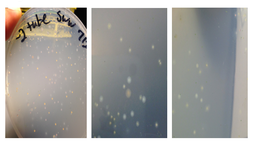
Pink pigmented facultative methylotrophs (PPFM) are α-proteobacteria that are plant commensal bacteria, and produce phytohormones that enhance plant growth. PPFMs consume methanol produced during plant metabolism. PPFM-like bacteria in algal microbiomes were identified as abundant pink colonies, and further demonstrated to preferentially use methanol as a carbon source.

Bacterial enhancement of *C. sorokiniana* UTEX-1230

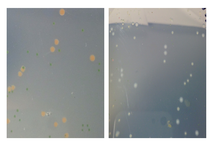
We sought to determine whether bacterial isolates from environmental cultures could enhance growth and/or photosynthetic capacity in the model industrial algal strain *C. sorokiniana* UTEX-1230. Standardized cultures of UTEX-1230 were inoculated with isolated bacterial colonies, and growth and chlorophyll content followed for 16 days. A number of bacterial isolates enhanced both parameters. Identification and further testing of these bacteria is currently underway.



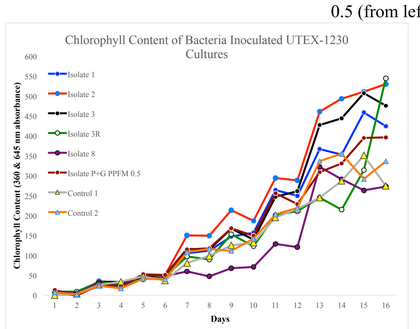
Experimental set-up

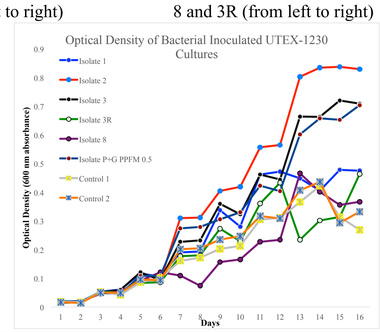


3 best performance enhancing bacterial isolates: 2, 3, and GP 0.5 (from left to right)



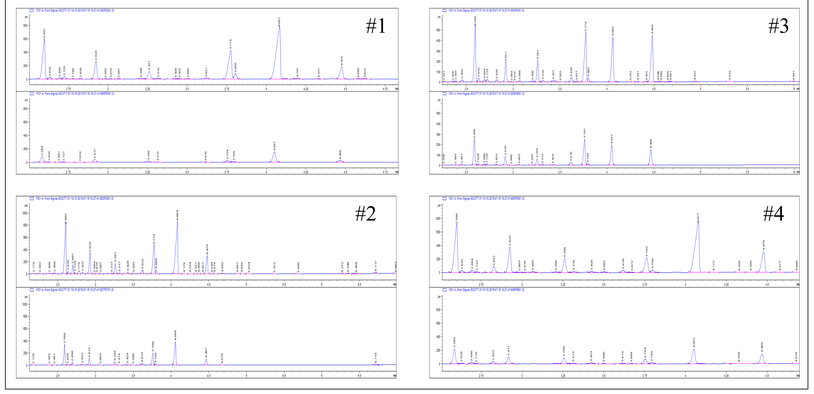
2 worst performance enhancing bacterial isolates: 8 and 3R (from left to right)





Fatty acid analysis

The fatty acid composition of the mixed environmental cultures was determined and compared to known values for UTEX-1230. The predominant fatty acids in each culture are palmitate (16:0), oleate (18:1), linoleate (18:2), and linolenate (18:3). These profiles are consistent with the the observation of *Chlorella* and/or *Scenedesmus* as the major algal species in these cultures.



Conclusions

- We have successfully established parameters for the initiation and propagation of photoautotrophic algal consortia to use as raw-materials in the identification of useful bacterial and algal strains.
- Culture media were established which allow the isolation and characterization of algal and bacterial partners in these communities.
- Bacterial isolates that enhance the growth of a model algal strain were identified, providing evidence that a rationally constructed microbial community may be more productive than pure algal cultures.
- Algal community structure changes over time under our conditions, with *Chlorella* and *Scenedesmus* spp. becoming the dominant types.

Current and Future Direction

- Sequencing16S rDNA of bacterial and ITS regions of algal isolates is in progress.
- Comparative analysis of wastewater samples before and after exposure to bacterial & algal isolates to determine which species work best at remediating carbon, nitrogen, and phosphorus prior to environmental discharge.
- Subjecting high growth and high fatty-acid content samples to further testing to enhance lipid production for more efficient bio-diesel production.
- Begin to study the chemical cross-talk between bacterial and algal isolates to screen for plant growth hormones in order to determine if algal growth/lipid productivity/ wastewater remediation can be enhanced by a specific set or type of bacterial exudate(s).
- Establish a mechanistic basis for algal growth enhancement by bacterial strains. Are the bacteria producing growth factors, vitamins, providing complementary metabolic pathways, removing toxic waste products, or some combination of these possibilities?

Acknowledgements

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