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Natural Products to Protect Algal Biofuel Ponds

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Background

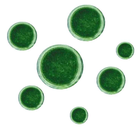
- Algal biofuel production cost can be reduced through elimination of pest-induced "pond crashes"[1,2]
- Current methods to eliminate pests include regular additions of pesticides, incurring a consistent cost[3]
- The cultivation of microalgae with unique bacterial consortia results in the death of their rotifer predators,[4] and potentially additional pests such as chytrids and phagotrophic algae
- Protective molecules produced in microalgae-bacterial consortia co-cultures can be identified with metabolomics tools

healthy pond

crashed pond

Crop

Biofuel Microalga



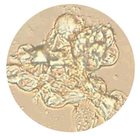
Microchloropsis salina

Pests

Rotifer

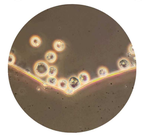
Brachionus plicatilis

Chytrid



Rhizophydium sp.

Phagotrophic Alga



Poterioochromonas malhamensis

- **Hypothesis:** Select bacteria in our consortia produce small molecules that protect microalgae from predators and pathogens.
- **Objective**: Identify protective small molecules produced by bacteria isolated from our unique consortia.

Experimental Design

Metabolomics

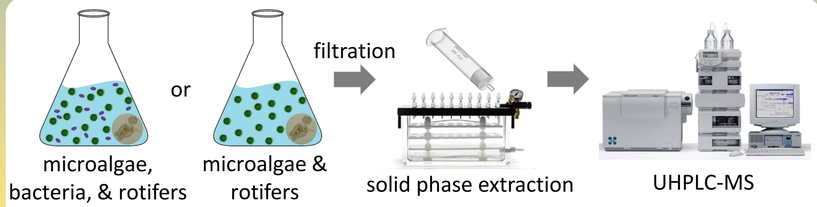


Figure 1: Workflow for identifying small molecules produced by protective bacteria in microalgae-bacterial co-cultures. Metabolites are extracted from filtered algal culture conditioned media and analyzed using spectroscopic methods (UHPLC-MS and ^{1}H NMR) prior to molecule identification.

Bacterial Extractions for Bioassay Screening

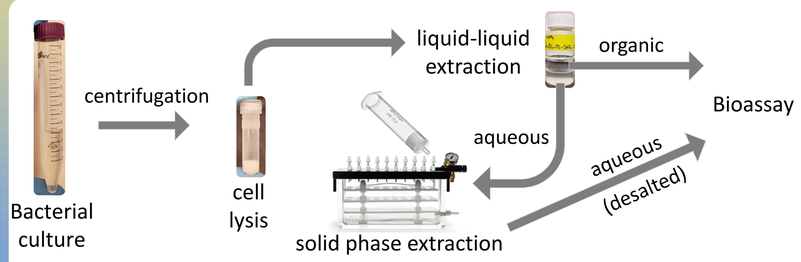
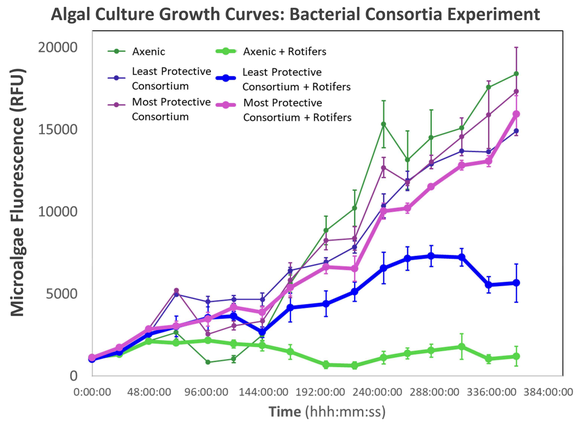


Figure 2: Workflow for generating bacterial isolate chemical extracts. Extracts will be tested against chytrids and phagotrophic algae.

Results

Exometabolome Extracts of Different Algae-bacterial Co-cultures Have Different Chemical Profiles



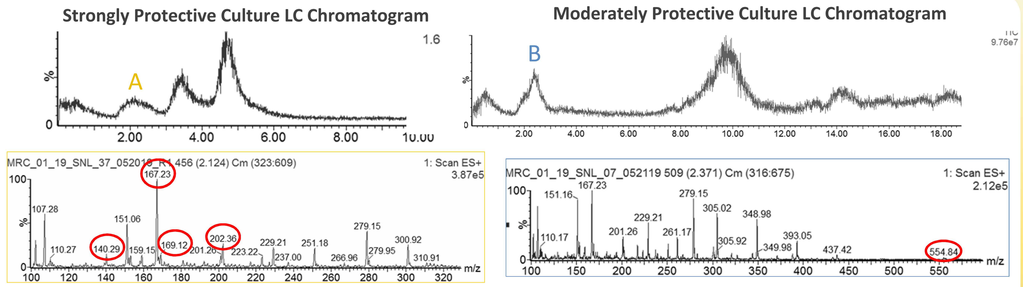


Figure 3: *Left*) Algal culture growth curves for bacterial consortia experiment. *Above left*) Liquid chromatography mass spectrometry (LC-MS) chromatogram for an algal-bacterial (**strongly protective**) culture conditioned media extract. The mass spectrum for peak A (gold outline) shows m/z signals for distinct metabolites (red circles). *Above Right*) LC-MS chromatogram for an algal-bacterial (**moderately protective**) culture conditioned media extract. The mass spectrum for peak B (blue outline) shows an m/z signal for a distinct metabolite (red circle).

Descriptive Statistics Indicate that Strata-X is the Best Extraction Method for Profiling Algal Culture Exometabolomes

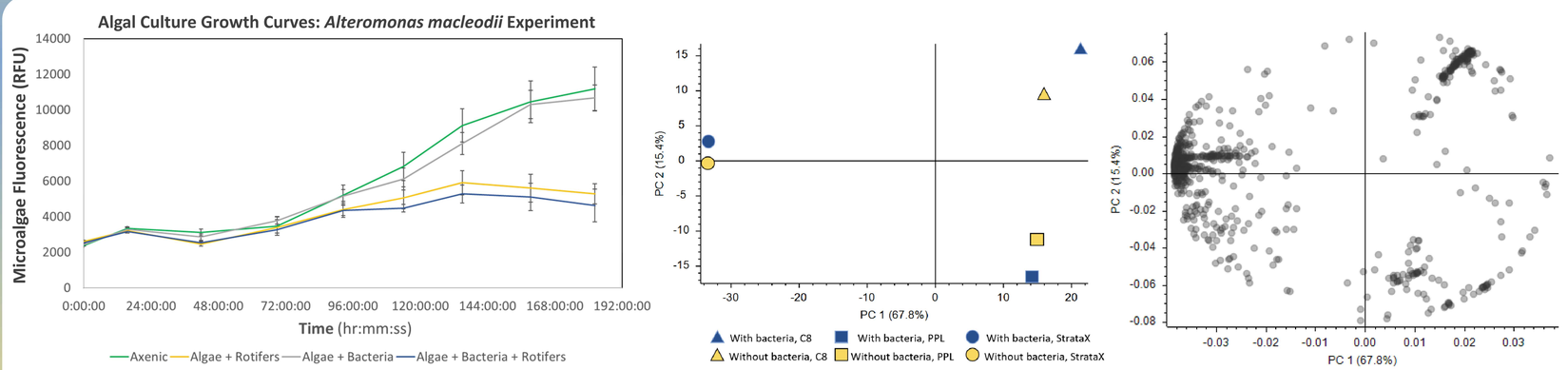
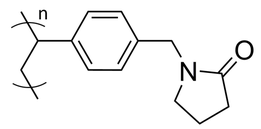


Figure 4: *Left*) Algal culture growth curves for *Alteromonas macleodii* experiment. This bacterium was selected to determine whether it could protect algae from rotifers like the bacterial consortia. *Middle*) Principal component analysis of reversed phase C18 LC-MS data acquired for algae + rotifers (gold) and algae + bacteria + rotifers (blue) conditioned media extracts. Three different solid phase extraction methods are represented by triangles (C8), squares (PPL), or circles (Strata-X). *Right*) Loadings plot where each point represents a molecular mass with a corresponding retention time. These points are responsible for the separation and grouping of treatment groups.

Conclusions

- Exometabolome extracts of algal cultures grown with moderately and very protective bacterial consortia have different chemical profiles
- Extracted molecules appear to be polar organic metabolites
- Metabolite concentrations are very low
- Strata-X is the best solid phase extraction method for extracting metabolites from algal culture conditioned media



Strata-X monomer

Future Work

- Determine chemical features in MS metabolomics spectra unique to microalgae-bacterial consortia co-cultures that did not crash in the presence of rotifers
- Bioassay-guided fractionation/isolation, spectroscopic characterization, and structure elucidation of protective molecules
- Acquisition of full biological profiles for protective metabolites
- Development of a bioassay and testing the bioactivity of bacterial isolate metabolites against chytrids and phagotrophic alga *P. malhamensis*

References and Acknowledgements

1. Sun A et al. (**2011**) Energy, 36, 5169-5179.
2. Hamilton CE and Rossmeissl N. (**2014**) Department of Energy.
3. Smith VH and Crew T. (**2014**) Algal Research, 4, 23-34.
4. Fisher CL et al. (**2019**) 40, 101500.

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