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Natural Products to Protect Algal Biofuel Ponds

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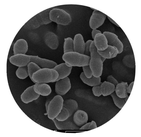
Background

- Algal biofuel production cost can be reduced through elimination of pathogen- and predator-induced "pond crashes"[1,2]
- Current methods to prevent "pond crashes" 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]
- Protective molecules produced by microalgae-bacterial consortia co-cultures can be identified with metabolomics tools

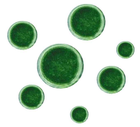
Rotifer Predator

Brachionus plicatilis



Protective Bacterial Consortium

Microalga Prey



Microchloropsis salina

- **Hypothesis:** Bacterial consortia produce chemicals that protect the microalgae against predation from rotifers.
  - **Aim 1**: Determine whether the bioactive molecules are:
    a) Extracellularly released
    b) Intracellular bacterial toxins
  - **Aim 2**: Identify chemicals produced by bacterial consortia that protect against rotifer predation.

Experimental Design

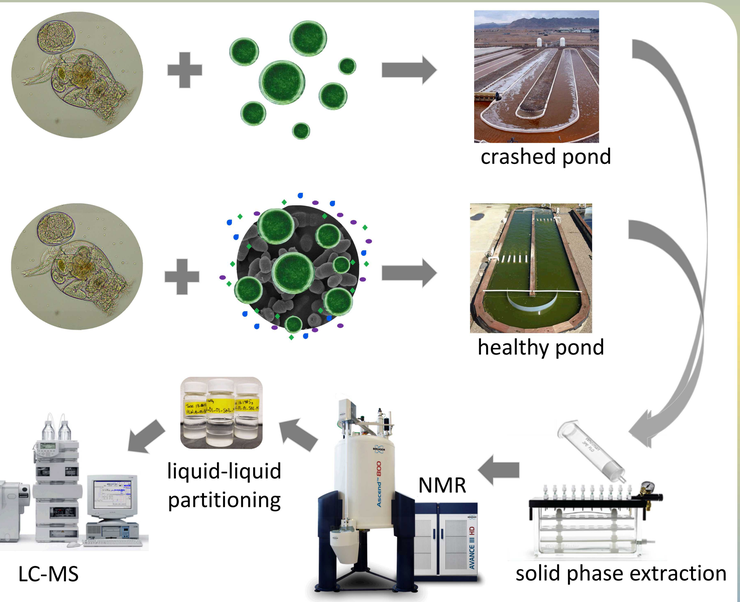


Figure 1: Work flow for identifying anti-predatory small molecules produced by protective bacterial consortia. Rotifers introduced to microalgae-bacteria co-cultures results in a healthy pond. No protective bacteria results in a crashed pond. Metabolites are extracted from algal culture conditioned media and analyzed using spectroscopic methods (NMR and LC-MS) prior to molecule identification.

Results

^{1}H NMR Spectra of Algal Culture Conditioned Media Extracts

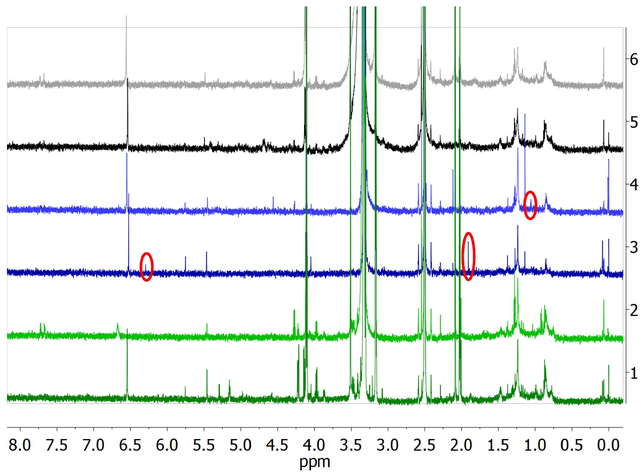


Figure 2: Algal culture conditioned media extract ^{1}H NMR spectra: no bacteria (dark green), no bacteria + rotifers (light green), least protective consortium (navy blue), least protective consortium + rotifers (royal blue), most protective consortium (black), and most protective consortium + rotifers (grey). Although many bacterial consortia were tested, only three treatments with and without rotifers are shown because they are representative of the entire data set. Red circles indicate peaks that are not present in the no bacteria or no bacteria + rotifers samples.

LC-MS Analysis of Algal Culture Conditioned Media Extracts

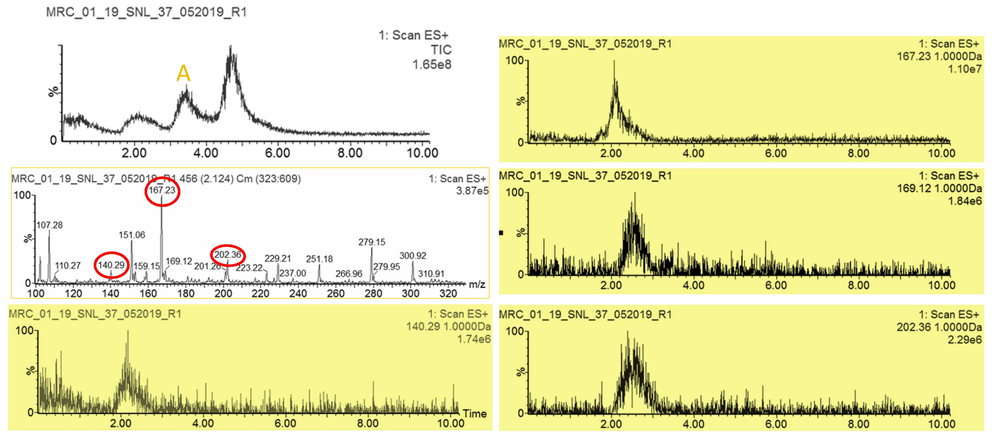


Figure 3: Liquid chromatography (LC) chromatogram for an algal-bacterial (**strongly protective**) culture conditioned media extract. The mass spectrum for peak A (gold outline) shows m/z signals for distinct metabolites (red circles). Extracted chromatograms for each metabolite of interest are shaded yellow.

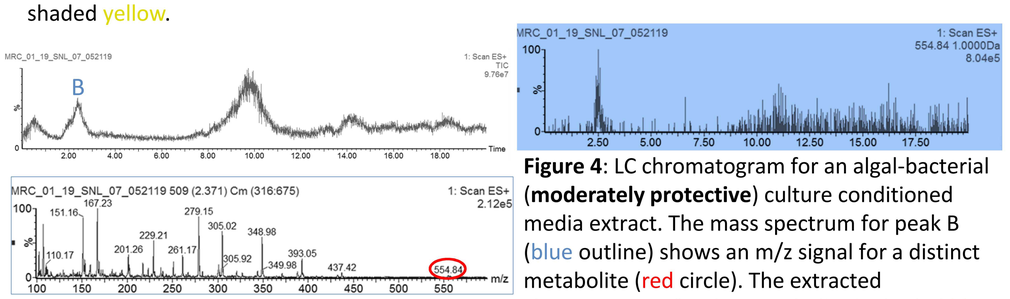


Figure 4: LC chromatogram for an algal-bacterial (**moderately protective**) culture conditioned media extract. The mass spectrum for peak B (blue outline) shows an m/z signal for a distinct metabolite (red circle). The extracted chromatogram for the metabolite is shaded blue.

Conclusions

- Metabolite concentrations are very low
- Peaks present in ^{1}H NMR spectra likely correspond to microalgal or rotifer metabolites
- ^{1}H NMR spectroscopy is not sensitive enough to detect unique chemicals of algal cultures with protective bacterial consortia
- MS chemical profiles of extracts for algal cultures with the most protective bacteria consortium are different from those with a moderately protective consortium.
- Extracted molecules appear to be polar organic metabolites.

Future Work

- Upscaling of algal-bacterial cultures and generation of comparative metabolomics dataset from active culture extracts
- Bioassay-guided fractionation/isolation, spectroscopic characterization, and structure determination of protective molecules
- Acquisition of full biological profiles for identified chemicals of interest
- Development of a bioassay and testing the bioactivity of algal-bacterial culture metabolites against fungal pathogens

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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