



# Application of the Cell Lytic Ability of *Lysobacter* as an Effective Method to Combat Algal Blooms



Amanda Lynn Miller and Dr. Liangcheng Du  
Department of Chemistry, University of Nebraska–Lincoln

## Introduction

- Harmful Algal Blooms (HABs) are algae that plague our marine and freshwater ecosystems when they accumulate in high numbers.
  - HABs can produce many toxins, and their physical shape can cause harmful biomass.
  - Toxins produced by some HABs are harmful to the environment, for example those that cause Red Tides, and when the toxins enter the food chain, the results are catastrophic.
  - The biomass produced by HABs has negative effects on the ecosystems because it leads to oxygen depletion and the migration of native species.
- From 1980-2000, the United States lost over \$1 billion due to damage caused by HABs.
- There are five general categories for typical HABs control mechanisms: mechanical, biological, chemical, genetic, and environmental controls.



<http://oceanservice.noaa.gov/hazards/hab/hab1171.jpg>



[https://ocean.si.edu/sites/default/files/styles/blog\\_public/photos/red%20tide%20alga%20bloom.jpg?itok=0h\\_ODkL](https://ocean.si.edu/sites/default/files/styles/blog_public/photos/red%20tide%20alga%20bloom.jpg?itok=0h_ODkL)

## Materials & Methods

- Experimental co-cultures were made to determine the cell lytic ability of the strains of *Lysobacter* on the algae species.
- OD values were taken using the spectrometer.
- Microscopy images were taken using the light microscope, EVOS, and the Scanning Electron Microscope.
- Mutant Strains of the *Lysobacter* species with deletions of the genes of interest were used to investigate the gene responsible for the cell lytic ability of *Lysobacter* on algae.
- High Performance Liquid Chromatography (HPLC) was used to analyze the amount of HSAF produced by the strains in the growth medium.

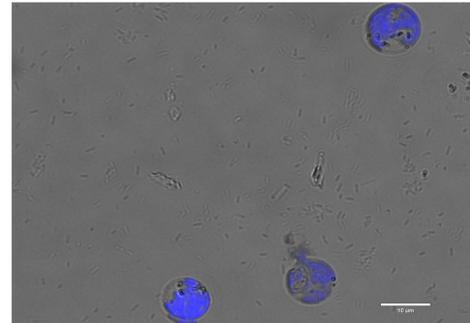


Figure 1: Image obtained from the EVOS microscope of the Co-culture with strain 3655 and algae that shows the algal cell rupturing due to the cell lytic ability of strain 3655 on algae.

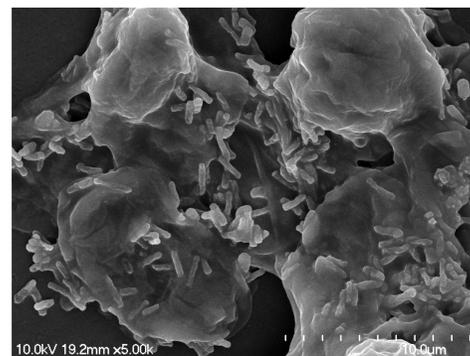


Figure 2: Shows the SEM image of the Co-culture of strain 3655 and algae. This image shows that the *Lysobacter* strain is on the surface of the algal cells.

## Results & Discussion

- All four strains (3655, C3, OH11, and OH13) caused lysis of the algal cells.
- This result was confirmed through co-culturing the strains and the algal species in a 1:1 ratio.
- The EVOS Microscopy images showed that algal cells were ruptured, which is an indicator of lysis occurring.
- EVOS also showed the brightness of the chloroplast of the algal cells and algal cell density both decreased over time.
- SEM images obtained from cultures with strains C3 and 3655 showed the *Lysobacter* species was on both the surface and inside the algal cells, which is another indicator that lysis is occurring in the culture.
- Co-cultures with the mutants of OH11 and OH13 showed that HSAF was a factor responsible for the cell lytic ability in strain OH11, because mutants with the deletion of HSAF biosynthetic genes were unable to effectively decrease algal cell density.
- Co-culturing the C3 mutants and the algae species showed the mutants with the deletion of  $\beta$ -1,3-Glucanase gene A (Glu A) were not capable of decreasing algal cell density.
- HPLC showed the C3 mutants with the deletion of Glu A did not produce HSAF while the wild type did produce HSAF.

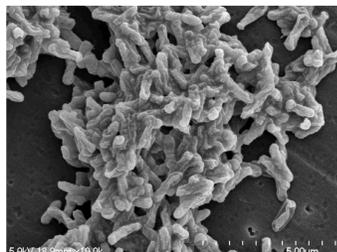
## Background & Present Study

### Prior Research

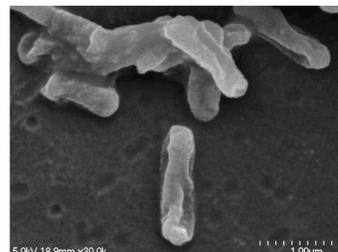
- The tiny bacteria in the genus of *Lysobacter* are known to be capable of causing lysis.
- In theory, the process of lysis could be employed to combat HABs.
- Lysobacter* is a genus of Gram-negative gliding bacteria that are emerging as new bio-control agents.

### Present Study

- The goal of this research was to study the cell lytic property of four different strains of *Lysobacter* species on the algae species *Chlamydomonas reinhardtii*.
- The strains that were used in this study were *Lysobacter enzymogenes* C3 and OH11, *Lysobacter antibioticus* OH13, and *Lysobacter sp* 3655.
- L. enzymogenes* is capable of producing a small product known as Heat-Stable Antifungal Factor (HSAF).
  - HSAF is potentially a factor responsible for the cell lytic ability of *Lysobacter*.



5.0kV 18.9mm x30.0k 1.00um



5.0kV 18.9mm x30.0k 1.00um

## Conclusions

- This study showed *Lysobacter* strains C3, OH11, 3655 and OH13 were all capable of causing lysis in algal cells.
- HSAF is a factor responsible for the cell lytic ability of strain OH11.
- Glu A is related to the cell lytic ability of strain C3, likely through affecting HSAF production, because HPLC showed that HSAF was not produced by the C3 mutants with the deletion of Glu A.

## Future Work

- Obtain microscopy images for the cultures that contain the mutants with the genes of interest.
- Run HPLC for the C3 mutants that do not have the deletion of Glu A.
- Run this trial using a different species of algae.



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Strain	1 ml of OH11 with the deletion of HSAF + 1 ml of TAP	1 ml of C3 with the deletion of Glu A + 1 ml of TAP	1 ml of C3 wild type + 1 ml of TAP	1 ml of Algae + 1 ml of TAP
Day 1				
Day 3				
Day 5				

Figure 3: Shows the experimental Co-cultures of the mutants with deletions of the genes of interest.