A fluorescence microscopy study of ROS production in *Chara australis* (R.Br.)

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Abstract

Our research focuses on the effects of cadmium (Cd) on reactive oxidative species (ROS) production in *Chara australis*. Prior studies [Clabeaux et al. 2011] have shown that *Chara* can absorb cadmium (a pollutant) from aquatic ecosystems, suggesting that *Chara* could be used to clean up polluted systems. However, *Chara* must be able to withstand the toxic metal. *Chara* absorbs cadmium which catalyzes ROS production, which then damages *Chara*. ROS oxidize non-fluorescent dihydrodichlorofluorescein (DCHF) into fluorescent 2',7'-dichlorofluorescein (DCF). By measuring fluorescence in *Chara,* we can determine the concentration of ROS in *Chara* over time. This can help us understand how *Chara* resists Cd toxicity.

Introduction

Phytoremediation "The direct use of green plants and their associated microorganisms to stabilize or reduce contamination in soils, sludges, sediments, surface water, or ground water."[1]

Cadmium

- Toxin that occurs naturally and in polluted systems
- Commonly found in ecosystems near industrial sites
- Highly toxic [2]

Chara australis

- Macroalga that grows primarily in freshwater
- Generates significant biomass in dense mats
- Effective at accumulating various toxins to significant concentrations [3]

Reactive Oxygen Species (ROS)

- Chemically reactive oxygen-containing
- Destructive to biological systems
- Generated by metals such as Cd [2]

Previous Studies

- Other plants: ROS increases after metal exposure [4]

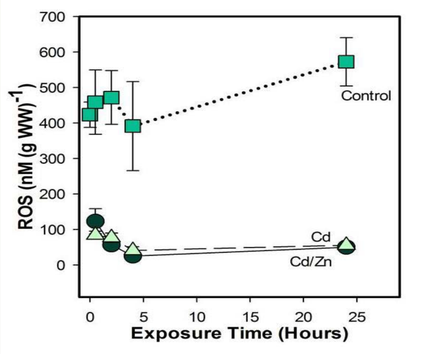

Figure 1. In *Chara* ROS decreases after 0.5 to 24 hr exposure to Cd unpublished work Bisson lab

Hypothesis

Reactive oxygen species increases quickly in response to Cd, but decreases after 30 min.

Methods & Materials

Detecting ROS

We use fluorescence to track ROS production inside *Chara's* cells via the pathway shown in **Figure 2**

1) DCHF-DA enters *Chara's* cells via 2 hour incubation period
2) *Chara* absorbs cadmium which catalyzes the synthesis of ROS
3) ROS oxidize non-fluorescent DCHF-DA into fluorescent DCF

Observing Fluorescence

We used a dissection microscope with fluorescence capability to observe fluorescent DCF inside *Chara's* internodal cell.

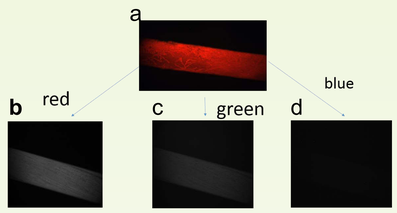

Fig. 2. Fluorescent images. a. True color fluorescence image. Red fluorescence is sue to chlorophyll. b-d. Different wavelengths of the fluorescence separated by filters and presented as gray scale images for analysis (see Fig. 4).

Measuring Fluorescence

We use ImageJ to analyze an area of interest in the images of the fluorescent *Chara* taken over time. (FIJI: http://fiji.sc/Fiji).

Results

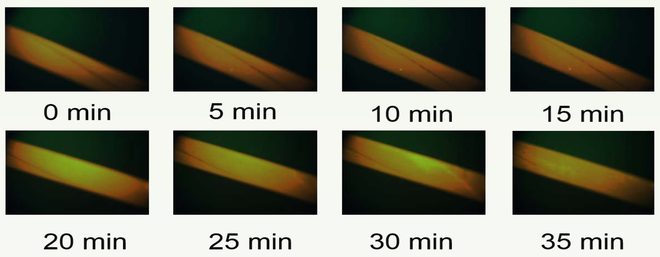

Figure 3. Increase of DCF fluorescence (green) in *Chara* cell after introduction of Cd between 15 and 20 min.

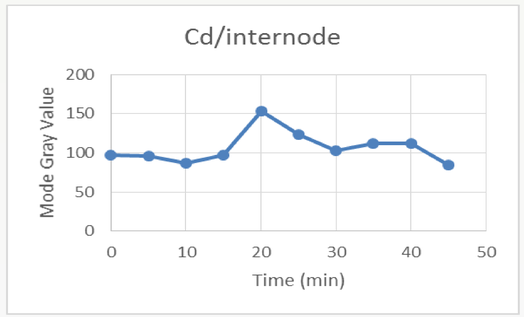

Figure 4. Mean gray values of green fluorescence as seen in micrographs in fig. 4.

Measurements

Treatment	% change in flurorescence (mean ± SE (n))
Control	-4.27 ± 3.14 (4)
Cd	25.1 ± 11.1 (7)

Table 1. Change in fluorescence 4 minute addition of Cd. Means are significant (p=0.0076)

Conclusion

Results show a **statistically significant** difference for change in fluorescence amongst *Chara* plants that have and have not been exposed to Cd. This suggests that ROS production is a factor in regards to Cd's toxicity in *Chara*. We will continue to run shorter term experiments to more accurately measure ROS Production over time (15 min vs 30 min).

Future work

Higher measured fluorescence and more defined areas of observation have led us to start measuring fluorescence in Chara's nodes (**Fig 5.**) as opposed to it's internodes. The dissecting microscope allows us to image such large specimens. However, technical problems has made replication of results difficult.

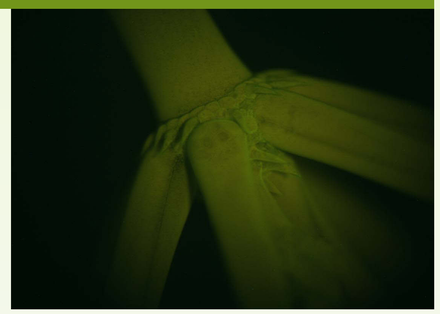

Figure 5. Natural color image of node of *Chara australis.*

If results cannot be replicated we will be using a confocal microscope to measure fluorescence. Although the defined area from which fluorescence is measured will decrease, we may obtain more accurate and quantifiable data on any change in fluorescence.

References

1. *J.c., Paulo, Joo Pratas, Mayank Varun, Rohan Dsouza, and Manoj S. "Phytoremediation of Soils Contaminated with Metals and Metalloids at Mining Areas: Potential of Native Flora." Environmental Risk Assessment of Soil Contamination (2014): n. pag. Web.*

2 *Gallego, Susana M., Liliana B. Pena, Roberto A. Barcia, Claudia E. Azpilicueta, María F. Iannone, Eliana P. Rosales, Myriam S. Zawoznik, María D. Groppa, and María P. Benavides. "Unravelling Cadmium Toxicity and Tolerance in Plants: Insight into Regulatory Mechanisms." Environmental and Experimental Botany 83 (2012): 33-46. Web.*

3. *Clabeaux, Bernadette L., Divina A. G. Navarro, Diana S. Aga, and Mary A. Bisson. "Cd Tolerance and Accumulation in the Aquatic Macrophyte, Chara Australis : Potential Use for Charophytes in Phytoremediation." Environmental Science & Technology Environ. Sci. Technol. (2011): 5332-338. Print.*

4. Collen, Jonas, and Ian R. Davison. "In Vivo Measurement Of Active Oxygen Production In The Brown Alga Fucus Evanescens Using 2',7'-Dichlorohydrofluorescein Diacetate1." Journal of Phycology J Phycol 33.4 (1997): 643-48. Web.

2 *Clabeaux, Bernadette L., Divina A. Navarro, Diana S. Aga, and Mary A. Bisson. "Combined Effects of Cadmium and Zinc on Growth, Tolerance, and Metal Accumulation in Chara Australis and Enhanced Phytoextraction Using EDTA." Ecotoxicology and Environmental Safety (2013): 236-43. Print.*

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