Establishing a Laboratory Culture of *Hypsibius exemplaris* for Use in Molecular Studies

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Introduction

- Tardigrades, also known as water bears, are an invertebrate species capable of surviving harmful environments and harsh treatments. Tardigrades undergo cryptobiosis to form a "tun" state to withstand UV exposure, space, extreme temperatures, and organic solvent treatments.[1]
- Although the physical characteristics of the tun state of tardigrades are well-characterized due to the visible difference under a microscope, little is known about how tuns form on a molecular level.
- To conduct experiments, it is necessary to reproducibly grow tardigrade cultures with similar growth rates.
- Additionally, we are interested in understanding the activation process of tuns in *H. exemplaris*. Preliminary research has suggested an increased level of intracellular calcium in the tuns of *H. exemplaris* via $CaCl_2$ and glucose. Due to this finding, we tested a known inhibitor to intracellular calcium.

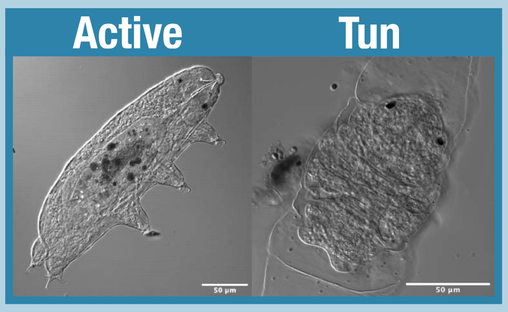

Figure 1. Hydrated and dehydrated tardigrades imaged using confocal microscope.

Abstract

- *Hypsibius exemplaris*, a species of tardigrade, are typically fed Chlorococcum.[2] However, we wanted to test the ability of *H. exemplaris* to grow in *Chlorella vulgaris* algae in a minimally-controlled environment.
- In order to further evaluate the role calcium may have on tun formation, we have explored the effects of phorbol 12-myristate 13-acetate (PMA) on *H. exemplaris* in the presence of glucose. PMA is an activator of protein kinase C, which leads to decreased intracellular calcium when activated.[3] Therefore, we hypothesize that PMA may inhibit the ability of glucose or $CaCl_2$ to induce tuns due to its ability to decrease intracellular calcium via the protein kinase C pathway.

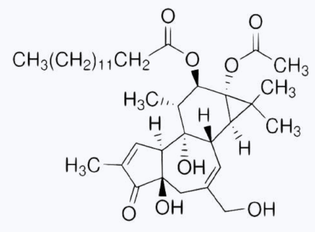

Figure 2. PMA structure.

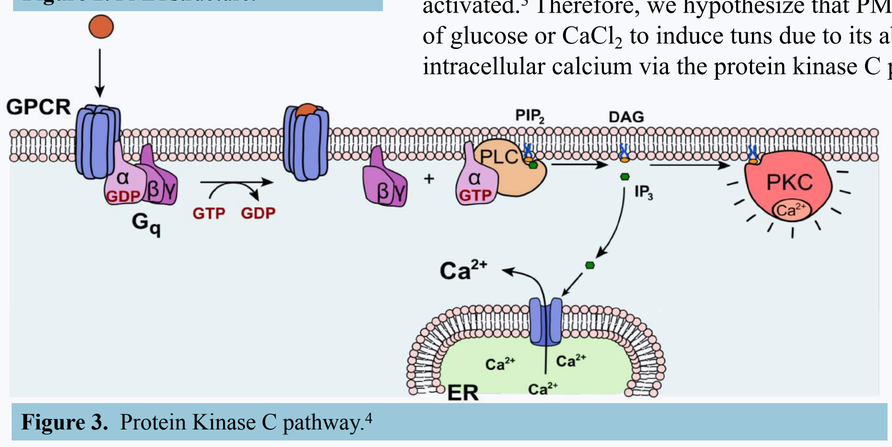

Figure 3. Protein Kinase C pathway.[4]

Methods

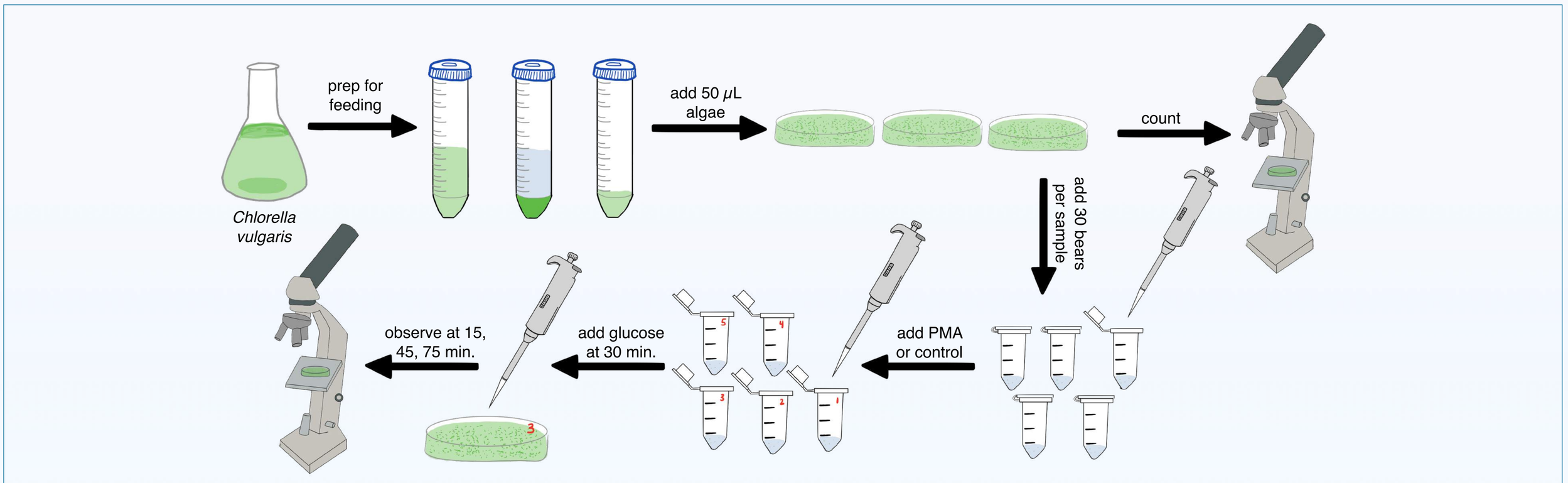

Figure 4. Tardigrades were cultured using *Chlorella vulgaris* as a food source and counted each week to determine growth. Varying PMA and controls were added to each of the five tardigrade samples. Glucose was added to plate 3, an experimental PMA plate, after 30 min. The samples were observed at different time increments.

Results

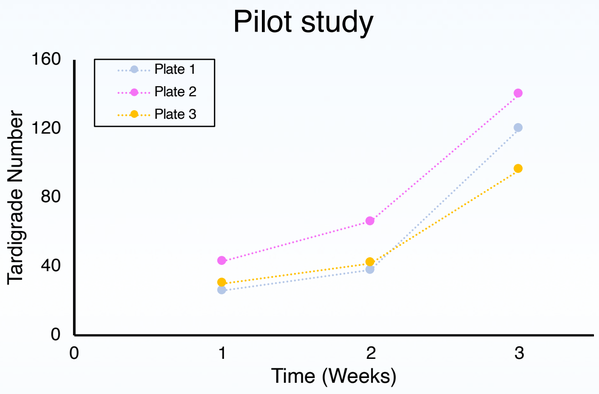

Figure 6. Tardigrade cultures were grown on three separate plates as a pilot study for growth. The number of tardigrades for each plate was documented over a span of 3 weeks. All plates showed a similar growth rate over time and were indicative of healthy colonies capable of reproducing.

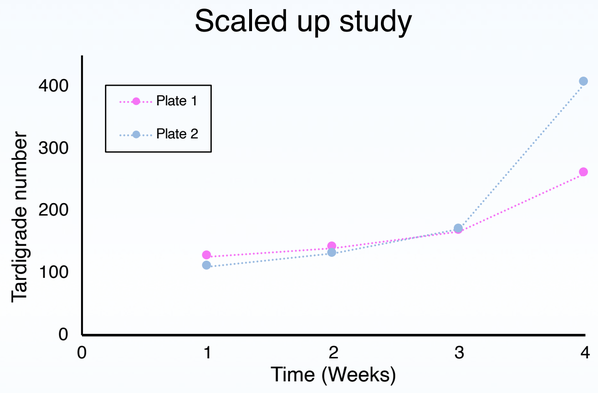

Figure 5. Tardigrade cultures were grown on two separate larger plates to ensure scalability. Tardigrade number was documented over a span of 4 weeks. Plate 2 showed higher growth over this time period, but both plates were indicative of healthy colonies capable of reproducing.

Time	Descriptions	Water Control	DMSO control	PMA + glucose	PMA	PMA
15 min	Movement	Normal	Normal	Slower	Slower	Slower
	Tuns	No	No	No	No	No
	Carcasses	Yes	Yes	Yes	Yes	Yes
45 min	Movement	Normal	Normal	Slower	Slower	Slower
	Tuns	No	No	No	No	No
	Carcasses	Yes	Yes	Yes	Yes	Yes
75 min	Movement	Normal	Normal	None	Slower	Slower
	Tuns	No	No	Yes	No	No
	Carcasses	Yes	Yes	Yes	Yes	Yes

Table 1. Observations after varied time increments of tardigrades under PMA and control conditions. Tube 1: MilliQ H_2O. Tube 2: DMSO. Tube 3: 1 µg/mL PMA, Glucose added after 30 minutes. Tube 4: 1 µg/mL PMA. Tube 5: 1 µg/mL PMA.

Conclusions

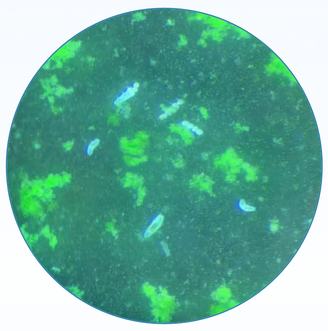

Figure 8. Tardigrade culture with *Chlorella vulgaris* under microscope.

- We were able to observe tardigrades in each stage of their lifecycle (egg, baby, adult, tun, paralyzed, carcass) and did not detect any contamination on the cultured plates.
- We have concluded that the five cultures of *H. exemplaris* were able to survive and successfully reproduce in *C. vulgaris* in an uncontrolled environment (ambient light, room temperature).
- Water bears were not as energetic upon exposure to PMA. This could indicate that the presence of PMA inhibits certain motor pathways in the tardigrade species.
- Water bears exposed to PMA were still able to form tuns upon exposure to glucose, which may indicate that the decrease in intracellular calcium that accompanies PMA exposure does not completely inhibit the ability of water bears to form tuns

Next Steps

- We plan to use the growing cultures for proteomics experiments in order to figure out target protein pathways for further analysis.
- Additionally, we plan to test other inhibitor libraries to determine which protein pathways are essential for tun formation.

Acknowledgements

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- Kolling Lab
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- Office for Undergraduate Research
- University of North Carolina Biology Department
- University of North Carolina Chemistry Department

References:
1. Richaud, M.; Galas, S. Defining the Viability of Tardigrades with a Molecular Sensor Related to Death. *PLOS ONE* **2018**, *13* (10).
2. McNuff, R. Laboratory Culture of Hypsibius Exemplaris. *Cold Spring Harbor Protocols* **2018**, *2018* (11).
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4. File:activation protein kinase c.svg. https://commons.wikimedia.org/wiki/File:Activation_protein_kinase_C.svg (accessed Nov 16, 2021).