Impact of Media Nutrition on Micro-Aggregate Formation of *Batrachochytrium dendrobatidis*

Marina Driver, Dr. Michael San Francisco
Department of Biological Sciences, Honors College

Introduction:

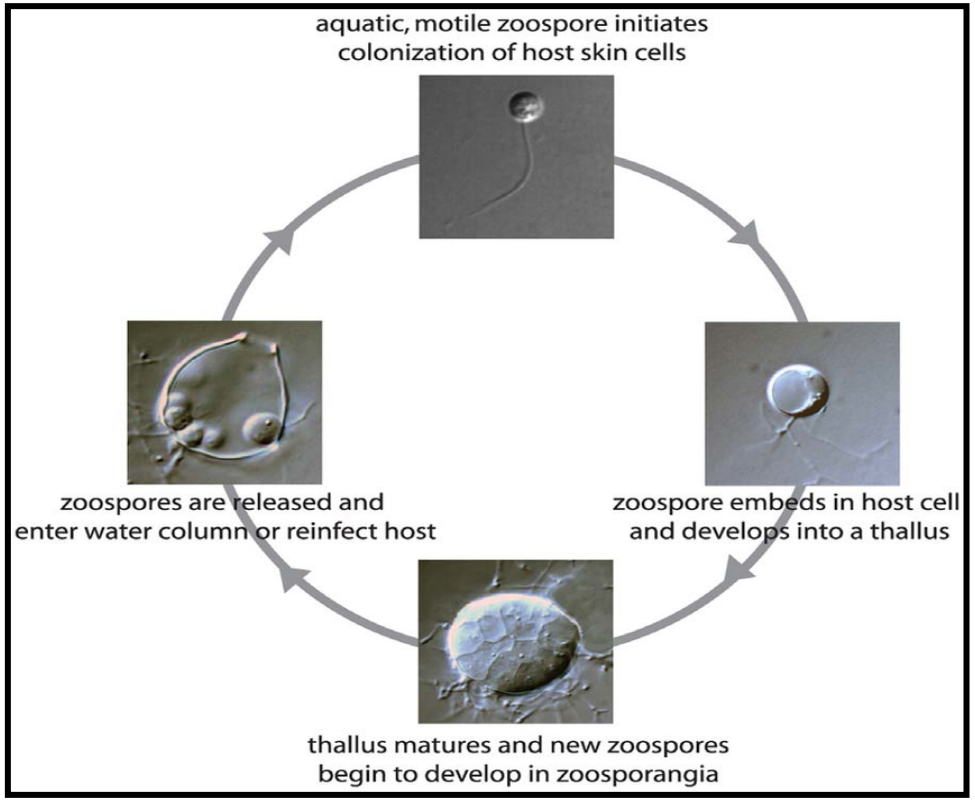
Batrachochytrium dendrobatidis (*Bd*) is a fungus belonging to the Chytridiomycota family, characterized by having two distinct life phases: a wall-less uni-flagellated motile stage and a reproductive chitin-walled zoosporangium stage. *Bd* impacts the host by colonizing the keratinized parts of the frog (the skin of adult frogs and the mouthparts of tadpoles), interfering with normal skin functions leading to hyperkeratosis. Additionally, *Bd* also prevents normal Sodium/Potassium transport, causing a loss of the "righting reflex" and ultimately leading to cardiac arrest. The fungus was initially discovered about 20 years ago by Dr. Joyce Longcore. Her breakthrough into the mystery of the plummeting amphibian populations around the globe is what sparked further research into how *Bd* is able to infect hosts effectively, in addition to surviving in different environments around the world. Our lab's past endeavors focused on the monolayer formation of the fungus in the absence of a host and understanding the gene expression differences between this state and its normal life cycle. Currently, our main goals are to further understand how *Bd* adapts from poor to high nutrient environments and how its growth differs within the pond water. We will also observe sporangial aggregate film formation on plant leaves within poor- and high-nutrient environments.

Abstract:

Batrachochytrium dendrobatidis (Bd) is a global pathogen that is currently the number one threat against the amphibian population worldwide by the Invasive Species Specialist Group (insert citation). The fungus belongs to the Chytridiomycota kingdom which is characterized by having a walless uni-flagellated motile stage in its life cycle and a reproductive stage, a chitin-walled zoosporangium. Our lab is focused on observing how Bd could potentially be able to grow in ponds found in the Lubbock area due to its recent discovery in Clapp Park Lake. Our samples have been taken from three different lakes in the local area to see if Bd may spread to other water sources. Our interests lie in seeing how the fungus grows in these nutrient poor environments in comparison to the nutrient rich environment provided in a lab setting. A serial dilution has been performed on Bd allowing it to slowly progress between environments (1 mL of Bd in 10 mL of nutrient rich broth to a ratio of 1:1000) to enable its adaptation. From here we will study sporangial aggregate film formation on leaves in nutrient-rich (H Broth) and nutrient-poor (pond /lake water) environments from the cultures grown in the procedure above. Based upon our results, further inquiry may be needed to determine why Bd is able to thrive in certain pond environments and not others and how this affects its ability to form sporangial aggregate films on leaves.

Hypothesis:

Growth and micro-aggregate formation of Bd in nutritionally poor media can be enhanced by the presence of a leaf in the diluted medium.



References:

Silva, S., Matz, L., Elmassry, M. M., & Francisco, M. J. (2019, October 31). Characteristics of monolayer formation in vitro by the chytrid Batrachochytrium dendrobatidis. Retrieved from https://www.sciencedirect.com/science/article/pii/S2590207519300097#cebib0010

Rosenblum, Erica & Voyles, Jamie & Poorten, Thomas & Stajich, Jason. (2010). The Deadly Chytrid Fungus: A Story of an Emerging Pathogen. PLoS pathogens. 6. e1000550. 10.1371/journal.ppat.1000550.

Method:

1. Create 100 mL of full-strength H-Broth.
2. Use 25 mL for the control flask without the leaf inside to check for contamination.
3. Perform serial dilutions to obtain full-strength, half-strength, quarter-strength, and eighth-strength H-Broth solutions (25 mL of each).
4. Insert 1 spinach leaf into each flask except for the control and autoclave to sterilize the leaf and solution.
5. Add 1 mL of Bd from a 7 day old culture (grown in 10 mL of full strength H-Broth) to each flask. Ensure that same amount of cells/mL of H-Broth are added to each flask by using a hemocytometer to do a cell count.
6. Add 1 mL of sterile water to each flask to compensate for the added H-Broth in order to maintain proportions.
7. Place flasks inside of the incubator for 1 week to allow for optimal growth conditions of Bd and film formation onto the leaves.
8. Take out flasks and remove leaves to examine them for film growth.

Conclusion:

After gathering the results from this experiment, we will pursue analysis of the protein expressions in both medias to further explore nutritional effects on expression. We plan to test Lubbock ponds that have tested positive for Bd to study how micro-aggregate formation occurs in the environment in comparison to a lab model. From this, we will search for overlapping factors that may contribute to environmental susceptibility to the fungus (pesticides, pollutants, etc.).

Acknowledgements:

I would like to thank the Office of the Provost and Dr. Amanda Starr for their contributions and guidance.

Hypothesis:

Growth and micro-aggregate formation of Batrachochytrium dendrobatidis in nutritionally poor media can be enhanced by the presence of a leaf in the diluted medium.