Algae Culture for tardigrade growth.

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Goal/Purpose

Tardigrades require a moist environment to facilitated gaseous exchange and avoid desiccation (Suzuki, 2003). The main purpose of my project is to be able to find a suitable environment for culturing algae, to use as a food source and culturing medium for these animals. In order to do so, I will test different culture methods, which keeps them, wet and avoid drying. I will culture and test different types of algae and use different protocols to provide nutrients, so that tardigrades can be reared in a laboratory setting in order to provide our Lab with a large amount of tardigrades to facilitate the study of genomics, and also the research on the life cycle and behavior of these animals.

Importance of the Project:

Tardigrades, also known as water bears, are a phylum of microscopic animals (Kinchin, 1994). They can survive for years in a form known as a tun, which resists extreme temperatures and pressures (McNuff, 2007). This makes them a target of research and study, in both their genomic composition as well as the impact in the ecology and the role they play in it. Despite the great interest in these animals, the present status of the research done in this field comprises about 2500 publish papers, most of which are in small, difficult to locate journals. There remains a notable absence of detailed information concerning the life history and embryology of these animals (Suzuki, 2003). In order to improve the information available it is necessary to have large amounts of tadigrades of the same species, in order to perform genomic work. This is why culturing tardigrades becomes essential for the success of research on these animals.
That is the reason why this project is so important; by being able to find a suitable environment we will be able to increase the abundance of experimental animals. If tardigrades are not cultured in the right environment with the adequate nutrients these animal's life expectancy is very short, and also as explained by Suzuki (2003) the restricted feeding decreases the number of eggs per clutch. In order to obtain tardigrades in large numbers, and that way facilitate genomic work, and increase the knowledge of their life cycle and role in the ecosystem, we will need to culture them, and be able to provide them with the most suitable nutrients and growth medium.

Main Proposal:

We will work with two kinds of algae, *Chlorella fisca var. fusc* which was acquired from the University of Texas in Austin, and *Chlorococcum*. Algae will be cultured on Bristol agar (11.25g of $NaNO_3$; 1.125g $CaCl_2$*$2H_2O$; 3.375g $MgSO_4$*$7H_2O$; 3.375g K_2HPO_4; 7.875g KH_2PO_4; 1.125g NaCl, (Suzuki, 2003) in 450mL of distilled water (dH_2O), followed by the addition of 7.5g of agarose) medium. The flask will be filled with $dH_2O$to 500mL, autoclaved in for 20 min, and then let it cool out for 20 min. aprox. We will then pour this mixture into Petri dishes and wait until the mix hardens. We will use two different kinds of nutrients, bold modified basal freshwater nutrient solution (Sigma Aldrich), and the Chalkley's medium, (5 ml of each of the following stock solutions per liter in dH_2O: NaCl, 2 g/100 ml dH_2O; KCl, 0.08 g/100 ml dH_2O; $CaCl_2$, 0.12 g/100 ml dH_2O), (Mcnuff, 2007). *My hypothesis is the following: Null: there is no different between the different cultures of algae, therefore algae is not in optimal conditions to rear tardigrades; alternative: there is a difference in the growth due to optimal conditions in one of the treatments*. In order to find the best treatment and test our hypothesis, we will divide cultures into 4 different groups, 4 Petri dishes with only dH_2O (control group), 4 with bold's medium, 4 with Chalkley's medium, and 4 with both bold's and Chalkley's medium, we will then place a sample of each in a shaded location, leaving the rest of the samples under light. In order to prevent the growth of

bacteria we will sub-culture the algae following the same procedure explained above into new Petri dishes, as explained by Altiero and Rebecchi (2001). Once the algae have been cultured we will place the tardigrades that have been picked by another student in our laboratory from the long-term ecological research sites (LTERS) samples. Tardigrades will be picked under a microscope, after soaking the LTERS samples in dH_2O. We will place the picked tardigrades into a Petri dish containing only dH_2O; these tardigrades will then be separated according to species. We will then place the tardigrades into the cultured algae following 2 different protocols for rearing tardigrades. Once the tardigrades commence to lay eggs we will place the eggs in a different Petri dish with only dH_2O until they hatch. Then those hatched tardigrades will be place in a new Petri dish with the protocol that was used originally. We will separate a control group 1: no food at all, just water, group 2: depending on the species they will be fed algae or nematodes, and the third group will consist of the mold or algae found in the LTERS samples that has been diced and boiled. The discrimination used to pick tardigrades will be based on the samples that have a great amount of tardigrades, the more tardigrades in a sample the more resistant and better to reproduce, which is our desired outcome, that these tardigrades might be able to reproduce in our culture medium.

Anticipated academic outcome:

Publish a research paper in the journal of Soil Ecology, and present a poster or oral presentation at the Soil Ecology Society July 2009. With this project other students will be able to conduct and research on this subject.

Qualification:

I have been doing research on tardigrades for a couple months, and I understand the difficulties of doing molecular work. I am starting the culturing of algae with mixed success. I have also taken and am currently enrolled in a few biology and chemistry classes. More than anything I am very excited about doing this research and look forward to getting this done.

Project timetable:

Culture the algae through October and November 2008, in the meantime collect the tardigrades from the samples brought from different LTERS. Start the culturing of tardigrades at the end of December 2008. The final paper with the discoveries should be done by April 2009.

Work cited:

Altiero, T. Rebecchi, L. (2001) Rearing Tardigrades: results and problems. Zool. Anz. 240: 217-221

Kinchin, I., (1994) The Biology of Tardigrades. Portland Press, London.

McNuff, R. Et al. (2007) The tardigrade *Hypsibius dujardini*, a new model for studying the evolution of development. Develomental Biology. 312: 545 – 559

Suzuki, C. A. (2003) Life History of milnesium tardigradum doyére (tardigrada) under a rearing environment. Zoological Science 20: 49 -57

Fitting with the mission of BYU:

The main reason why I think this project fits with the mission of BYU is due to what President Brigham Young said, “the education is the power to think clearly, the power to act well in the world’s work and the power to appreciate life.” It is through this project that will allow us to understand more about this animals and also developed a greater understanding of those things that are unseen to the natural eye but do affect the environment we live in. the project will also open door for more knowledge and further learning for those that will come after this research project.