Establishing Primary Cell Line from Marine Invertebrate *Botryllus schlosseri*

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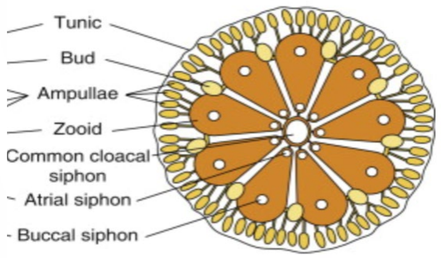
INTRODUCTION

Cell culture lines are essential to scientific research because it allows for cells to regenerate certain cell tissues which is necessary for cell manipulation and can lead to more effective treatments in medicine and vaccine development.

Botryllus schlosseri is a marine tunicate species found typically on barnacles and flat surfaces in marinas. This organism's life cycle is unique since they regenerate their entire colony every 2 weeks. They have stem cells in multiple tissues including their vascular network, this allows them to regenerate clonal replicates of the colony. Studying the cells of this organism would allow for advanced research possibilities, these cells could be used for various applications, in stem cell production, cell aging evolution as well as regeneration mechanisms.

To establish a cell culture line, we first aimed to achieve cell adhesion and a state of cell proliferation of cells from this organism in a culture dish. We utilized different types of cell content from the colony and looked for a period of cell proliferation before cell death occurs when seeded in a culture dish.

This experiment explored five different cell contents that were micro dissected from the colony- individual zooids, ampullae which is the vasculature system of the organism, ampullae cell suspension and whole zooid system with the tunic still attached as well as whole system cell suspension.



METHODS AND MATERIALS

- All colonies were washed with 70% ethanol and then rinsed with artificial sea water- penicillin streptomycin, amphotericin-B (ASW-PSA) rinse before dissection. Artificial seawater and gentamycin (ASW-G) soaks were utilized on some colonies for increased sterilization
- Cell contents that were microdissected away from the colony: cluster of ampullae, individual zooids, ampullae cell suspension and a whole colony system (ampullae + zooid) with tunic still attached
    - Cell suspension: cell content was rinsed with ASW-PSA and smushed through a cell strainer to dissociate the cells from tissue and tunic context, centrifuged and then the pellet is seeded
- Samples were seeded on monolayer cell culture dishes with 500 µl of Tunicate Culture Media (TCM)
- All seeded trials were checked every 24-48 hours and new media was added if needed

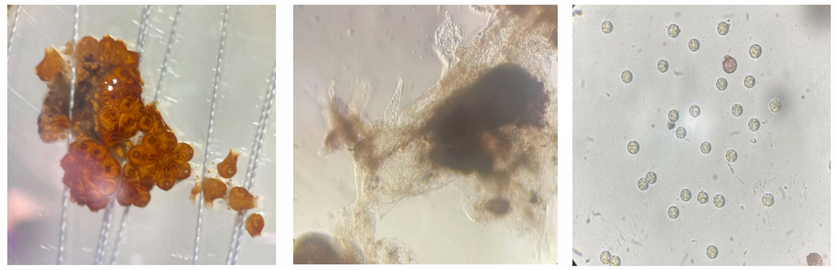


Figure 1: Whole B. schlosseri colony with 4 individual zooids *(left)* dissected away from the system, ready to be seeded. After 48 hours, cell adhesion started *(middle)* but contamination was discovered. After trial 2, contamination and growth of Thraustochytrid organism *(right)* was present after being seeded prior to an ASW-G soak

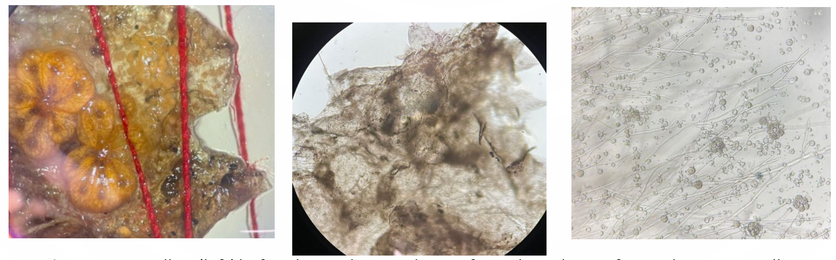


Figure 2: ampullae *(left)* before being dissected away from the colony. After 24 hours, ampullae cells started to adhere *(middle)* to culture dish, after 48 hours, fungal growth (*right)* and contamination was observed under microscope

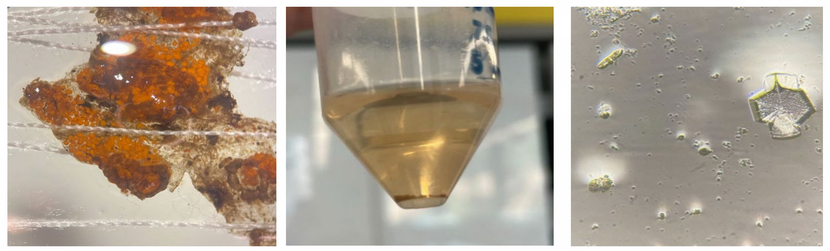


Figure 3: Ampullae (*left)* still attached to the colony. Pellet and supernatant *(middle)* of ampullae cell suspension after being centrifuged. Results from under microscope *(right*), 48 hours after being seeded, fungal growth observed

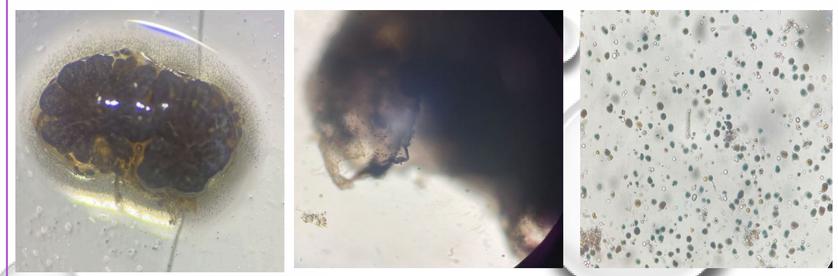


Figure 4: Two whole systems connected to each other *(left)* dissected away from colony. Cell adhesion *(middle)* started in the culture dish 24 hours after being seeded. Fungal growth *(right)* and contamination observed

RESULTS

- Zooid- two ethanol washes and a one-hour ASW-G soak before dissection of the colony was preformed. 24 hours after seeding there were no signs of contamination or fungal growth present. After 48 hours, mold and fungi growth as well as Thraustochytrid organism growth was observed
- Ampullae- cells started to adhere to the culture dish after 24 hours. 48 hours later cell death occurred. Trial two, the ampullae were soaked in ASW-G for one hour to possibly aid in controlling contamination, 24 hours later the ampullae started to adhere, 48 hours later contamination and fungal growth was observed
- Ampullae cell suspension- 48 hours prior to being seeded, fungal and crystal growth was observed under microscope. First time thraustochytrid organism was observed
- Whole system- cell adhesion started 24 hours prior to being seeded, after 48 hours the cell culture dish appeared to have signs of bacteria growth and contamination

We started to establish some protocols from the *B. schlosseri* species for isolating and culturing cells, but 48 hours after each cell content trial, immense contamination and bacterial growth from the wild colonies were observed which created a major obstacle for this research. The bacteria growth and contamination hinders the advancement of the next steps of this experiment.

Once the contamination and bacteria issue is resolved, these studies should be continued to explore if any of these cell content culture approaches would yield any adhesion or proliferation

Research studies on creating a cell line out of this organism has been ongoing for years and unfortunately there are no successful cell lines available for this species yet. It is important for science to continue this research because studying more about the cells of this organism and having a cell line created would allow for various possibilities and applications in science and medicine.

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