Effect of Amino Acid Culture Mediums on Botryllus Schlosseri

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ABSTRACT

- Immortalized cell lines are a group of cells that have been mutated and changed, enabling them to proliferate infinitely. One organism that researchers have been trying to establish a immortalized cell line for is *Botryllus schlosseri*, a marine vertebrate called a colonial tunicate.
- ***B. schlosseri*** unique traits: Extraordinary regenerative abilities, nonrandom senescence
- The aim of this research was to assess whether amino acid mediums could positively infleunce the cell growth and proliferation of *B. schlosseri* cells.
- After performing cell counts, we found that amino acid-based mediums, namely Dulbecco's Modified Eagle Medium (DMEM) and Medium-199, do not have a positive impact on the growth and proliferation of *B. schlosseri* cells, as no live cells were observed throughout the entire experiment.
- Despite containing various antibiotics, such as streptomycin and penicillin, an overwhelming amount of contaminants and debris were observed.

INTRODUCTION

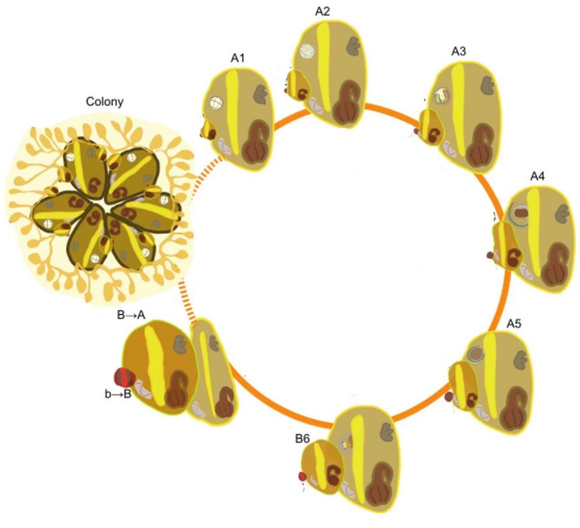
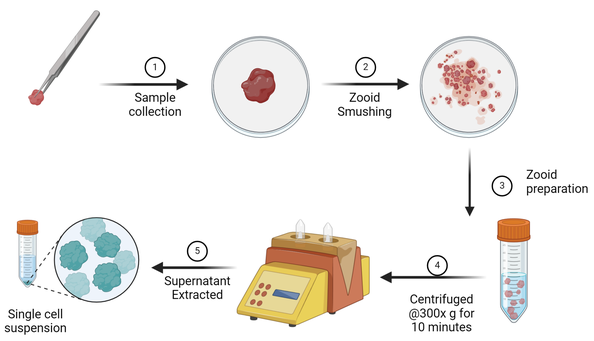


Figure 1: **Common Life Cycle of *B.Sclosseri.*** **(**Adapted from Kowarsky et al. 2021)

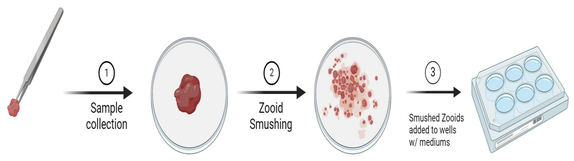
METHODS

Seeding Methods:

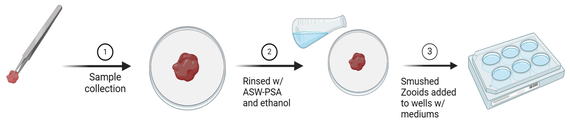
- **Method 1: Single-Cell Suspension**



- **Method 2: Direct Smush Without Centrifuging**



- **Method 3: Direct Zooid Addition**



Culture Media Chosen:

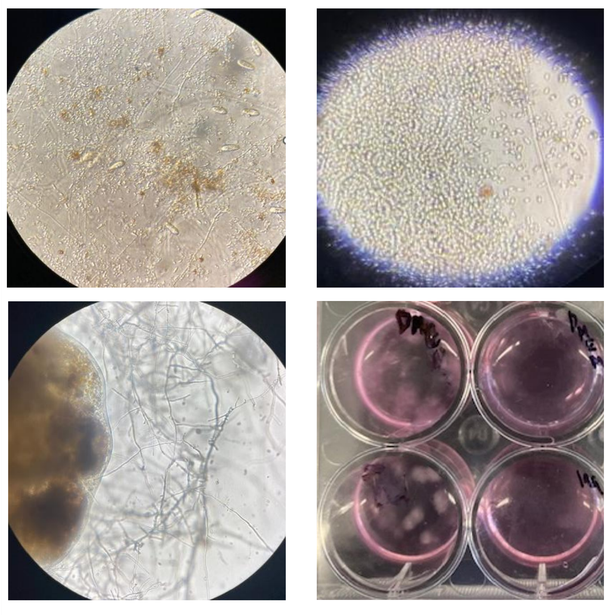
- **M199:**
  - Supplemented with artificial sea water containing penicillin, streptomycin, and amphotericin-B (ASW-PSA) to reduce contamination.
  - sodium pyruvate to further decrease contaminants during seeding.
- **DMEM:**
  - Supplemented with artificial sea water, fetal bovine serum, L-glutamine, ASW-PSA, and sodium pyruvate.
- **TCM:**
  - Used as a baseline medium to compare the effects of the amino acid-rich mediums DMEM and M199.

RESULTS

Trial #	MEM	TCM	199
1-2(CS)	0	0	N/A
3-4(DS)	0	0	0
5-6(DZ)	0	0	0

Table 1: Results of 6 trials to test the effectiveness of different amino acid mediums on cell proliferation and growth of *B. Schlosseri* cell material. Cell count that came from methods: Cell suspension (CS), Direct smushing (DS), and direct zooid (DZ) in three different media.

A) **B)**



C) **D)**

Figure 2: Examples of Contaminants (A-D) that appeared in all three of the mediums, taken at 10x magnitude. Many of the contaminants that were continuously found throughout the mediums were the same. Figure 2C offers a closer look at the white fungus that was continuously growing in the mediums, which is also seen in Figure 2D.

CONCLUSIONS

After conducting 6 one-week trials using the culture mediums selected for this study, with different methods of seeding the zooids and cell material:

- Observed no signs of cell proliferation or growth in any of the wells across all trials
- The amino acid-based mediums used may not support cell division or proliferation in *Botryllus schlosseri* cells.
- Identified contamination of cell culture as the possible primary obstacle and is likely a contributor to inhibited cell proliferation and growth in medium.

Future Studies

Contamination of cell culture was the biggest obstacle throughout this study, as it most likely was hindering cell proliferation and growth in all wells. It will be extremely important in the future to take steps to prevent this type and amount of contamination in culture, which could be done through research and testing of different antibiotics and antimycotics on common contaminants found alongside the cultures.

Citations