Establishing of Immortal Cell Lines from *Botryllus schlosseri*

Ayasha Phuyal, Mindy Yates, Dr. Saumya Sankaran, Dr. Alison Gardell

Abstract

Botryllus schlosseri are marine colonial tunicates with exceptional regenerative abilities. Deriving an immortal cell line from *B. schlosseri* cells holds significant promise as a resource for studying the cellular physiology of regeneration and growth capabilities. However, no cell lines from this organism have been established to date. Here, we attempted to grow cells from wild colonies of B. schlosseri in culture by exposing them to genotoxic stressors. Whole organisms were seeded in cell culture dishes, then exposed to nickel chloride, UV radiation, and TPA (12-O tetradecanoylphorbol-13-acetate). Two trials were run, and these experiments aimed to induce DNA breakage, however in both trials of the experiments, data retrieval was hindered by contamination issues. Once the contamination issues are overcome, proceeding with this project would contributes to a deeper understanding of regenerative mechanisms and establish an immortal cell line, presenting significant potential for scientific advancements.

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Introduction

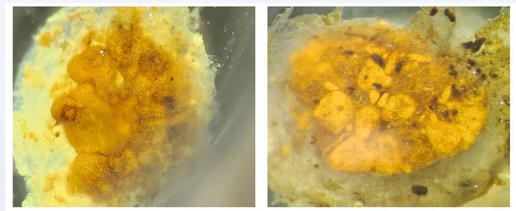
Botryllus schlosseri, a member of the Chordata family, exhibits a fascinating life cycle within its colonies. These colonies consist of interconnected zooids, each possessing the potential for regenerative processes. Within a single zooid, the presence of primary and secondary buds contributes to the intricate colony formation. As the adult zooid undergoes coordinated apoptosis, the primary bud takes over, emphasizing the organism's capacity for continuous expansion.

These zooids harbor essential stem cells crucial for the regeneration of the organism. Isolating these stem cells from *Botryllus schlosseri* could enhance study of regenerative biology. However, immortal cell lines in *Botryllus* have not been successful yet. Our aim is to extract these cell and try to maintain and proliferate these cells in an in-vitro conditions to generate a cell line.

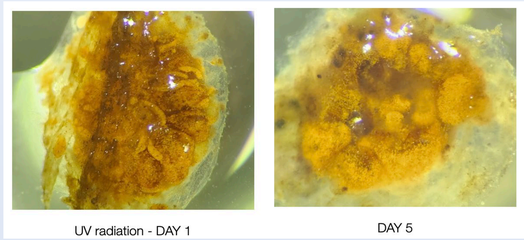
Our aim is to extract cells and try to maintain and proliferate these cells in i*n-vitro* conditions to generate a cell line by utilizing genotoxic agents—Nickel chloride, 12-O-tetra-decanoylphorbol-13-acetate (TPA), and UV radiation—as potential catalysts for inducing mutations. This strategic selection aims to mimic conditions conducive to abnormal cell growth, a strategy inspired by successful attempts in other organisms to establish immortalized cell lines. These specific genotoxic agents were successful in causing mutation resulting in abnormal growth in other organisms, which has helped establish cell lines from other organisms. They cause changes in cells, making them grow differently, which is essential for strong cell lines in a lab. Their accessibility adds practicality to our experiments, making them a vital component of our approach.

Methods

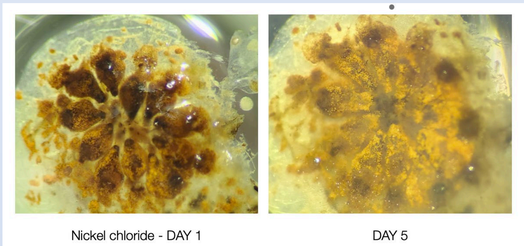
- Healthy *Botryllus Schlosseri* colonies were extracted from Tacoma Marina, transferred to glass slides in ASW, and acclimated in University of Washington-Tacoma's artificial seawater system.
- A healthy system was selected for dissection, sterilized with 70% ethanol, rinsed in ASW (Artificial seawater) and PSA (penicillin, streptomycin and amphotericin)
- Dissection was conducted under a microscope, dirt or damaged tunics were removed, zooids were exposed, and systems were placed in a cell strainer with ASW+PSA.
- The strainer was rinsed with 30% ethanol and ASW+PSA, and systems were transferred to individual, sterilized wells with 200µl ASW+PSA, then incubated at 18°C for 48 hours.
- Six colonies from *Botryllus Schlosseri* were received, labeled, and treated with ASW+PSA in wells for 2 days in the incubator.
- Exposures (nickel chloride, UV radiation, combinations, and controls) were introduced after 48 hours.
- Two control colonies were maintained with ASW+PSA to prevent dehydration, with an additional 500µl added after 48 hours.



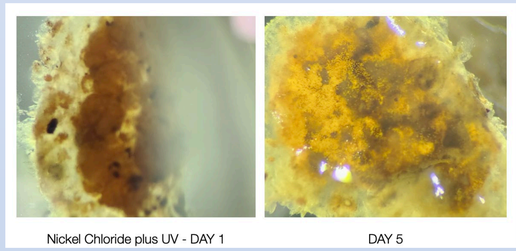
This system was submerged in 500µl of ASW+PSA,5 days post dissection.



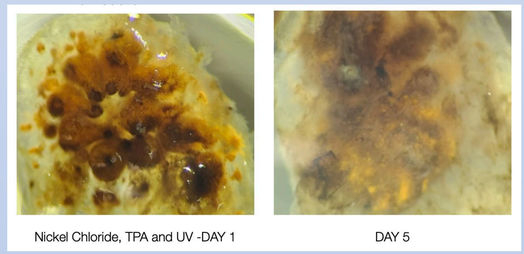
The system was immersed in 200 µl of ASW+PSA, exposed to radiation doses of 6.22 KJ/m2 for 157 seconds two days after dissection, and subsequently incubated at 18°C for three days.



The system underwent a two-hour exposure to Nickel chloride at a concentration of 0.01g/l. Following this, the medium was switched to 500 µl of ASW+PSA, and the system was incubated at 18°C for the next three days.



The system underwent exposure to radiation doses of 6.22 KJ/m2 for 157 seconds, followed by a two-hour exposure to Nickel chloride at a concentration of 0.01g/l. The medium was switched to 500 µl of ASW+PSA, and the system was then incubated at 18°C for three days.



The system underwent exposure to radiation doses of 6.22 KJ/m2 for 157 seconds, followed by a two-hour exposure to Nickel chloride at a concentration of 0.01g/l with another addition of 2 mmol of TPA(dissolved in DMSO) two days after dissection. The medium was switched to 500 µl of ASW+PSA, and the system was then incubated at 18°C for three days.

The dissection focused on a chosen colony, selecting the healthiest systems. Following dissection, 10 systems underwent sterilization and were placed in plates with 200µl of ASW+PSA for adhesion over the next two days in an incubator at 18°C. Row A figures depict systems in ASW+PSA on day 2 post-dissection, while row B shows the systems after exposure and incubation on day 5.

Results

- There were no signs of proliferation seen in most of the setup, but the samples were adhered to the plate.
- ASW+PSA: The system remained healthy with no visible contaminants, but the tunicate appeared slightly less transparent than on day 1.
- NICKEL CHLORIDE: The system was viable with no contaminants; however, the tunicate was less transparent, and zooids appeared more swollen. Vascular circulation was detected on day 5.
- UV RADIATION: The system remained healthy with viable vascular circulation on day 5, and no early contaminants were observed.
- NICKEL CHLORIDE AND UV RADIATION: This system showed signs of distress; zooids' contents seemed exposed, no vascular circulation on day 5, and early contaminants were observed.
- NICKEL CHLORIDE, UV RADIATION, AND TPA: The system exhibited decreased health, with the tunicate becoming opaquer and zooids more swollen. No vascular circulation was observed on day 5, and a unique dot-like formation appeared.
- After 2 More Days in the incubator resulted in contamination, resembling fungal-like growth, covered the organisms, hindering analysis. Foggy and cloudy growth was observed, preventing meaningful results.

Conclusion and follow Up steps

Due to a consistent issue of contamination seen throughout the experimental setup, we were unable to test our hypothesis. As except for the observed contamination, our results did not reveal any significant morphological findings. To definitively assess DNA breakage success or failure, additional experiments are necessary.

Unfortunately, constraints in time and funding hindered our ability to complete this phase. The next verification step involves utilizing DNA from our exposed systems and subjecting them to a "comet assay" (Olive and Banáth, 2006). Which is a crucial experiment segment that is pivotal for determining DNA breakage, leaving the conclusion of our experiment uncertain.

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