Inducing Mutations in *Botryllus schlosseri* for Immortalization in Cell Culture

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Abstract

Botryllus schlosseri is a marine colonial tunicate with an incredible regenerative stem cell functionality. The organism follows a strict life cycle that allows it to be replaced by a new genetically identical organism every seven days. The organism can regenerate a whole colony just from small pieces of the organism, which makes it a perfect model organism for future studies of stem cells, regenerative biology and apoptosis research. Establishing cell lines from this organism has been challenging because, when these cells are planted from colonies into cell culture, they cease to proliferate. To try and establish cell lines from this species, whole organisms were treated with a known genotoxic agent which should induce mutations in hopes for allowing some type of one cell to proliferate in cell culture. Organisms were dissected out of their colonial tunic and placed in a tissue culture media with ENU (N-ethyl-N-nitrosourea) at high and low dose. The outgrowth of cells from these organisms was monitored after 24, 48, and 72 hours. Due to high contamination of the trials ran, ENU could not be tested for it effect on cell proliferation.

More On Botryllus schlosseri

- *B. schlosseri* is a very invasive species and can be found all over the world, this mostly has to do with there transporting from bottom of ships to other harbors.
- *B. schlosseri* feed on phytoplankton and are consumed by crabs, starfish, snails, and sea urchins.
- *B. schlosseri* can be found in many different color morphs from orange, black, brown, and even blue.
- *B. schlosseri* life cycle at 18-20 ℃ is about 7 days.
- *B. schlosseri* can reproduce both asexually by they budding process, and sexually through egg and sperm. The organism is a hermaphroditic so one organism can release both egg and sperm.
- It has been shown that even just an ampulla in the tunicate can regenerate whole systems from what stem cells are in the ampulla.

Life Cycle of Botryllus schlosseri

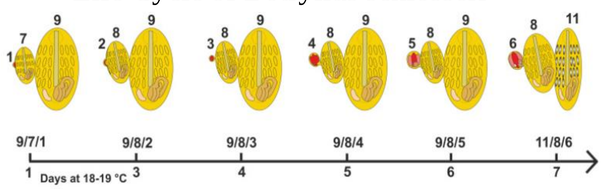

Figure (1) This diagram show the life cycle of Botryllus and shows the parent bud, primary bud, then the secondary bud.

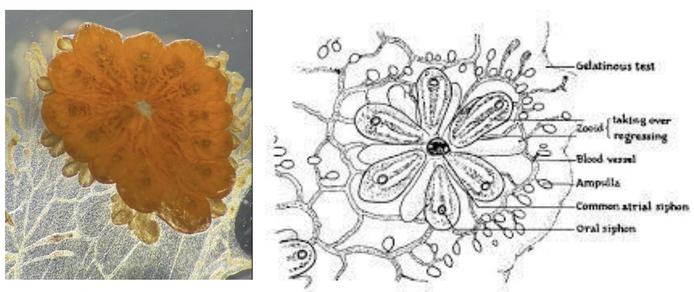

Figure (2) these figures show a cartoon version of Botryllus anatomy along side a picture of the animal taken in lab to show some of these key features.

Methods

In order to induce mutation in *B. schlosseri* zooids were dissected out the their tunicate systems. These colonies were taken from the wild and maintained in the University of Washington seawater system, tied to glass slides, instead of there substates in the wild. Through a dissection microscope *B. schlosseri* were staged and if between $B_2 - C_2$ zooids were dissected to be seeded in a plate. Colonies were rinsed with 70% ethanol and then with artificial sea water also containing Penicillin, Streptomycin, and Amphotericin-B. (ASW+PSA) before dissection occurred. Then using insulin needles zooids were dissected from their tunicate. Once about 3-4 zooids were collected for each well they were rinsed with a 30% ethanol and then again ASW +PSA. Then they were transferred to a cell culture hood to be placed in dishes. The zooids were placed in 200 microliters of tunicate culture media to let them adhere to the plate for 24hrs. Then once zooids were attached then media was replaced and topped off too 700 microliters. The cells were checked on every 24, 48, and 72 hours with an inverted microscope. If zooids showed low/ no contamination and were attached to the dish then they were exposed to the ENU treatment. The exposure included a control where only DMSO was added. One was exposed at 20 µg of ENU in media for an hour, and the last was exposed to 5 µg of ENU in media for 24 hours. After allotted time of expose media was replaced and cell outgrowth was checked.

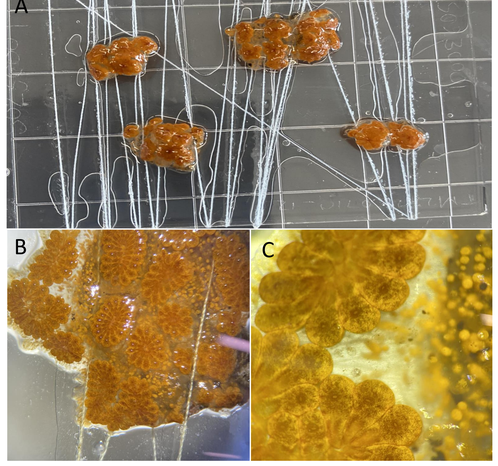

Figure (3) This photo above shows The animal that was taken from the wild and tied down to a slide instead of the substrate it was found on. Then Shows increasing magnification under a dissection microscope. The pictures show whole zooids and ampullae that are in the tunicate.

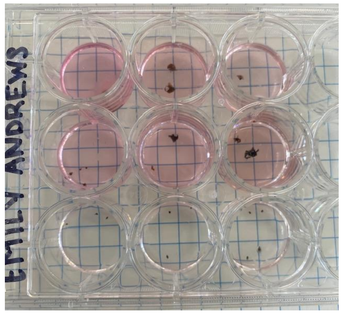

Figure (4) This picture shows the wells the zooids were placed in, along with how they looked after incubation

Results

Seeding Zooids Trials and ENU Exposure

Trials	Materials Seeded	Days Incubated (18°C)	Result
Week 5: Trial (1)	2 Ampullae 4 Zooids	5 Days	Material was black and dead, no sign of cell outgrowth. Not much media left in well
Week 6: Trial (2)	4 Ampullae 5 Zooids	2 Days	Material was black and dead, no sign of cell outgrowth. Not much media left in well
Week 7: Trial (3)	5 Zooids	10 Days	Organisms were alive some zooids attached, yet well showed signs of contamination
Week 8: Trial (4)	11 Zooids	7 Days	Organisms were not attached and showed signs of contamination
Week 8: Trial (5)	10 Zooids	5 Days	Organisms attached and showed very small signs of contamination, was treated with ENU

- Ampullae was not used again after Trial 2 because of lack of attachment to well and seemed to die outside tunicate.
- After Trials 1 and 2 it was obvious that the media needed to be changed and topped off daily to give cell material optimal conditions to attach and show signs of life.
- Since these organisms were taken from the wild they all showed many signs of contamination.
- Trial 5 even though experienced the ENU treatment did not seem to live and showed signs of contamination under the microscope so I could not measure how ENU affected cell proliferation.

Conclusion/ Future Work

For future work with this species, I would want to work with lab grown organisms to reduce the risk of contamination. If we could lower the contamination then we would have more time to monitor outgrowth and then expose with ENU to see if it can mutate the cells. Given all trials were contaminated we could not see how ENU effects outgrowth of zooids. Next, if cells were proliferating I would have a way to measure DNA breakage from the ENU, through qPCR assay or DNA-based assay for damage.

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References