

L. birnbaumii Identification and Testing

Preface: In 2020, a particular fungal strain lesser known to the dart frog hobby began to surface frequently in vivariums and related substrate materials such as isopod enclosures. While other species of the genus *Leucocoprinus* have been known to the dart frog hobby for a long time and generally referred to as “Flower Pot Fungus”, *L. birnbaumii* is much more vigorous/aggressive and quickly overtook many vivariums and isopod colonies. While harmless to humans (unless ingested) and not seeming to harm plants or frogs (other than heavy spore loads), this fungus became a great nuisance and was found to be toxic to many species of isopods which would try to predate upon it, resulting in the loss of countless colonies (some quite expensive). Due to its propensity to spread rapidly through vivariums and isopod bins, many hobbyists rushed to find the source of contamination. In a joint effort between Houston Frogs (Chase Jennings) and FrogDaddy (Alex Menke), the source of the infestation was identified as the New Zealand Fern Fiber (Figures 1-4), which had gained popularity in the hobby due to the shortage of Guatemalan Tree Fern Fiber. Several newly delivered, infested bags were opened by Houston Frogs, photographed, and compared with materials received by FrogDaddy. Several other dart frog related businesses were contacted, and it was concluded that the fern fiber was the source of the outbreak.

After speaking with the supplier/importer of the New Zealand Fern Fiber, Houston Frogs recommended heat treatment of the materials and cleaning of the processing and storage facilities for these materials.

Recent Shipments in August of 2021 of New Zealand Fern Fiber have been heat-treated and tested for live spores and mycelium of *L. birnbaumii* before importation. An independent lab has already declared this material to be free of *L. birnbaumii*; however, random bags of product were also sent to the Houston Frog’s laboratory in Houston, Texas and have been tested to ensure the material was free of *L. birnbaumii* contamination after importation.

Abstract/Objective: The objective of this experiment is to show that there is no living mycelium or spores of *Leucocoprinus Birnbaumii* evident in the New Zealand Fern Fiber Substrate.

Methodology: Samples were taken of two species of *Leucocoprinus* mycelium visible in frog vivariums here at Houston Frogs (one of which was identified as *L. birnbaumii* from its mycelial structure and fruiting bodies) and placed in Petri dishes with water agar to isolate and observe growth as a control sample against which mycelial growth from the New Zealand Fern Fiber samples would be compared (Figure 5).

Test tubes were used to gather ~10 grams of substrate from each of 4 bags of New Zealand Tree Fern Fiber substrate and numbered 1-4 (Figure 6). The test tubes were hydrated with distilled water, and liquid samples placed on Petri dishes meant for spore germination. The liquid was also be placed on a slide (Figure 7) and viewed under a microscope in attempt to find ungerminated live spores of any kind. If found, they would be compared to the spores of *L. birnbaumii*. If there was no match, an attempt would be made to identify the spores.

32oz containers were used to gather ~12oz(volume) of substrate from each bag and numbered 1-4 (Figure 8). The 32oz containers were misted as needed with distilled water in an attempt to encourage germination of live spores, which can be visually observed.

Findings: Ungerminated spores were observed in samples 2 and 4 at 400x magnification (Figures 9-10). The spores in sample 2 were ellipsoid and mostly clear, like *L. birnbaumii*, however many fungi have ellipsoid clear spores. *L. birnbaumii* cannot be ruled out for sample 2, and liquid from sample 2 was placed on two Petri dishes with recipes intended for spore germination.

Spores in sample 4 were also ellipsoid, but had a green tint, indicating a different species (unknown, Figure 14). Liquid from sample 4 was placed on Petri dishes with recipe for spore germination. After allowing the Petri dishes to colonize, there were both bacteria and fungi present in the dishes (Figures 11-14). All of the fungi in each dish had visible surface sporulation in colors from green to black. The four 32oz samples had no visible mycelium growth after two weeks.

Conclusion: The samples of New Zealand Fern Fiber substrate from 4 bags were tested for bacteria and fungi, both of which were found as expected. Neither living spores nor mycelium of the species *Leucocoprinus birnbaumii* were found to be present in any of the samples, and a mold species of no significant concern was found to be present and prolific enough to likely fend off most other species of fungi. Our laboratory concurs with the results from previous laboratory testing before importation that no living spores or mycelium of *Leucocoprinus birnbaumii* is present in the current heat-treated New Zealand Fern Fiber products.



Figure 1: *L. birnbaumii* visible in newly opened bags of New Zealand Tree Fern Fiber, opened in 2020 by Houston Frogs.



Figures 2-3: *L. birnbaumii* mycelium observed in newly opened bags of New Zealand Tree Fern Fiber, opened in 2020 by Houston Frogs.



Figure 4: *L. birnbaumii* mycelium observed in plant pot containing New Zealand Tree Fern Fiber at FrogDaddy in 2020.

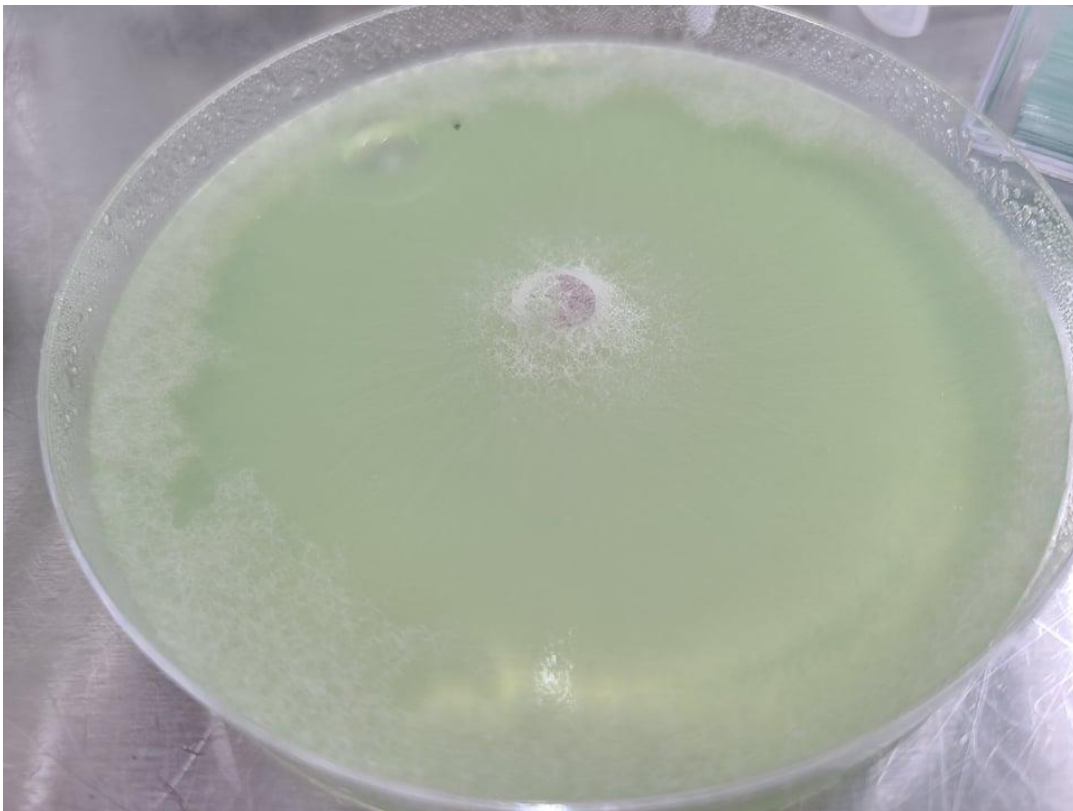


Figure 5: Cultured *L. birnbaumii* to be used for a comparison sample.

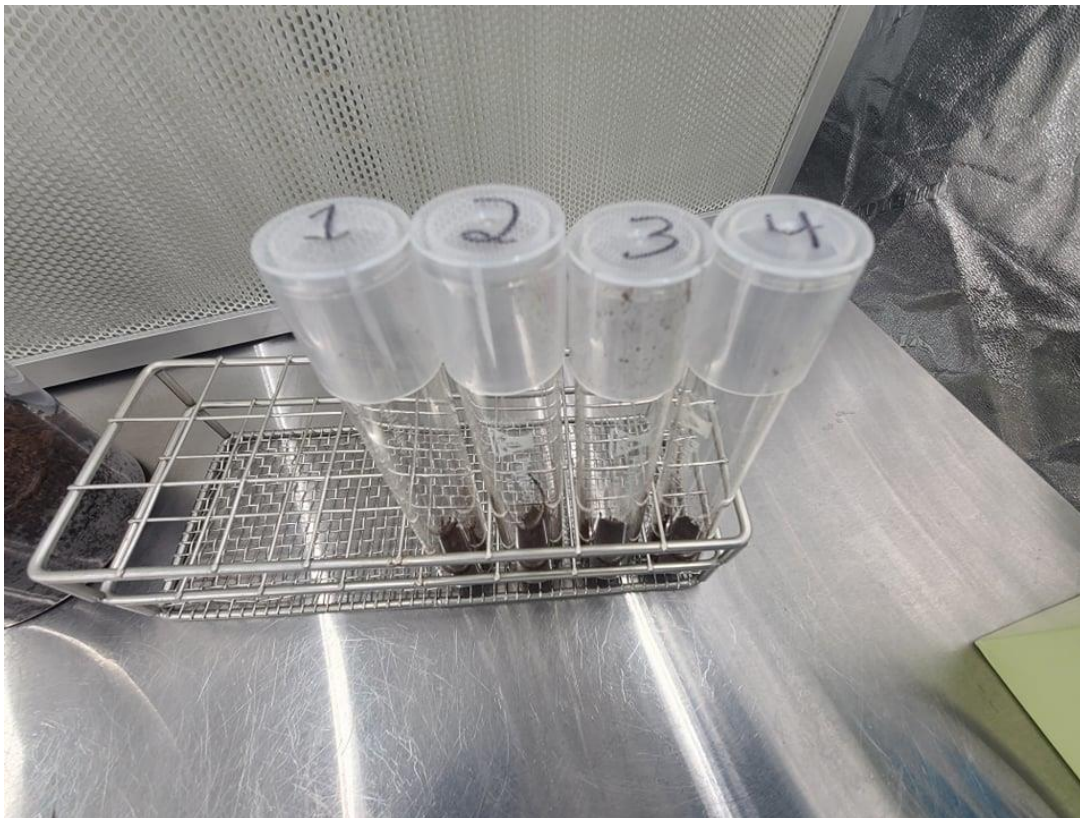


Figure 6: Hydrated New Zealand Fern Fiber Samples to be used for inoculation of sample plates and for slide preparation.

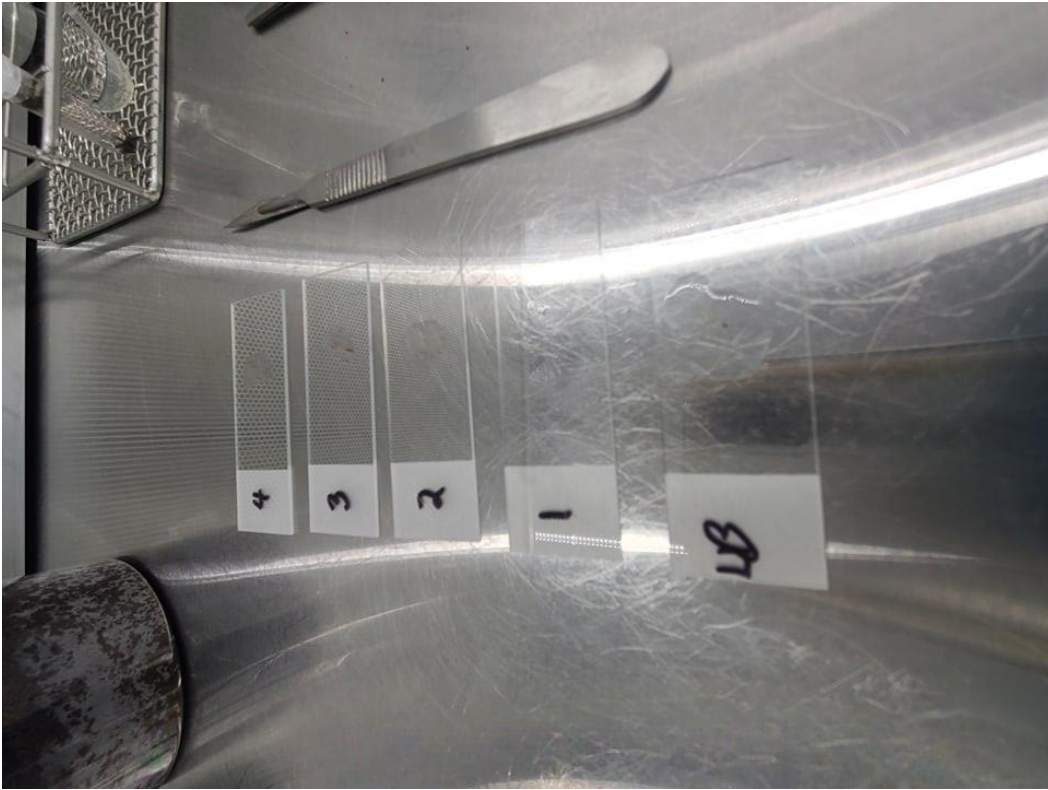
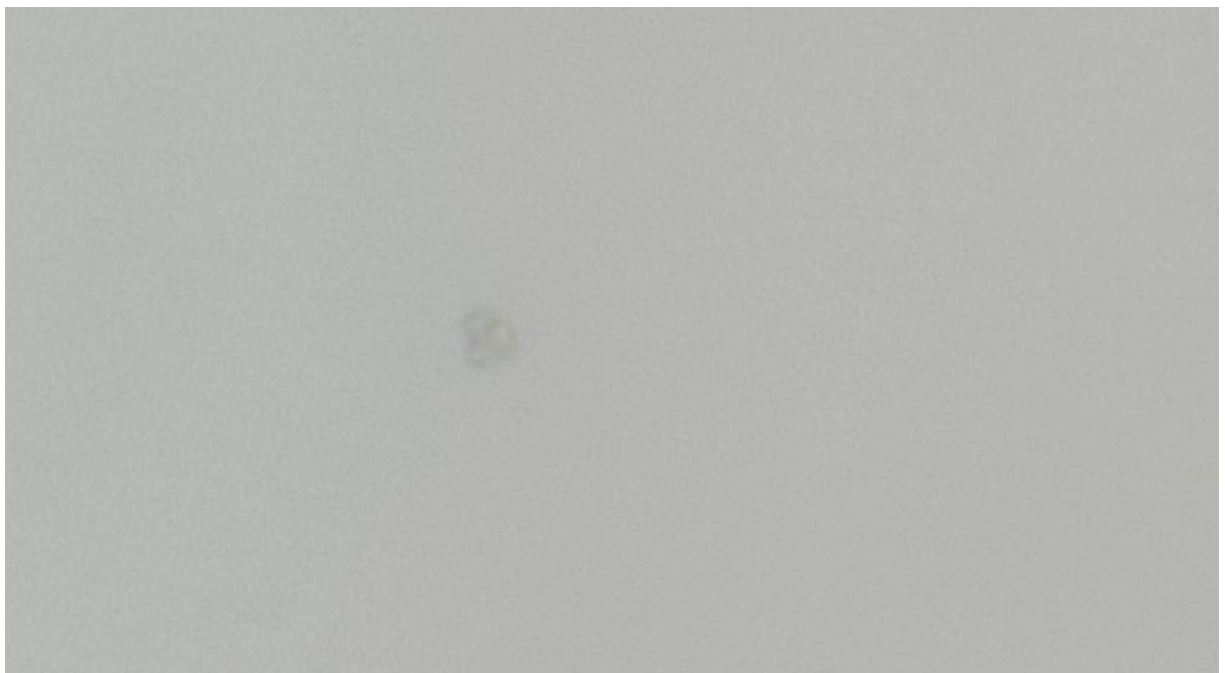
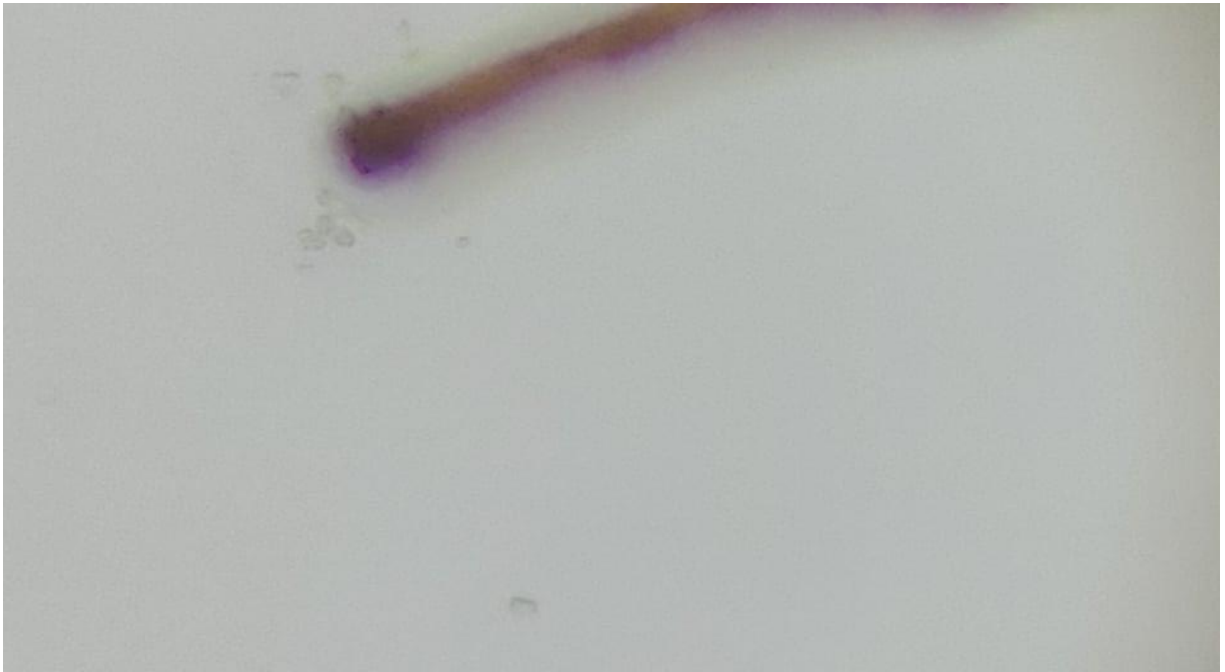


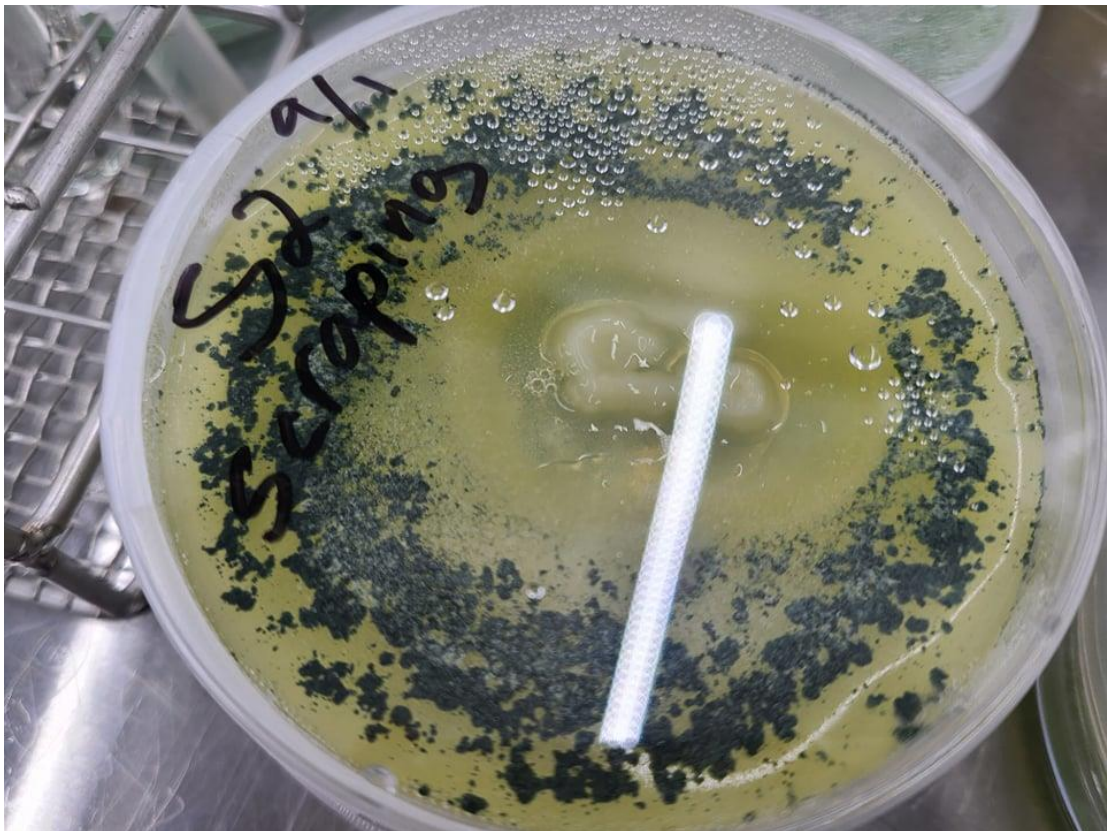
Figure 7: Microscope slides prepared to search for *L. Birnbaumii* spores at 400x magnification with Lugal's Reagent.



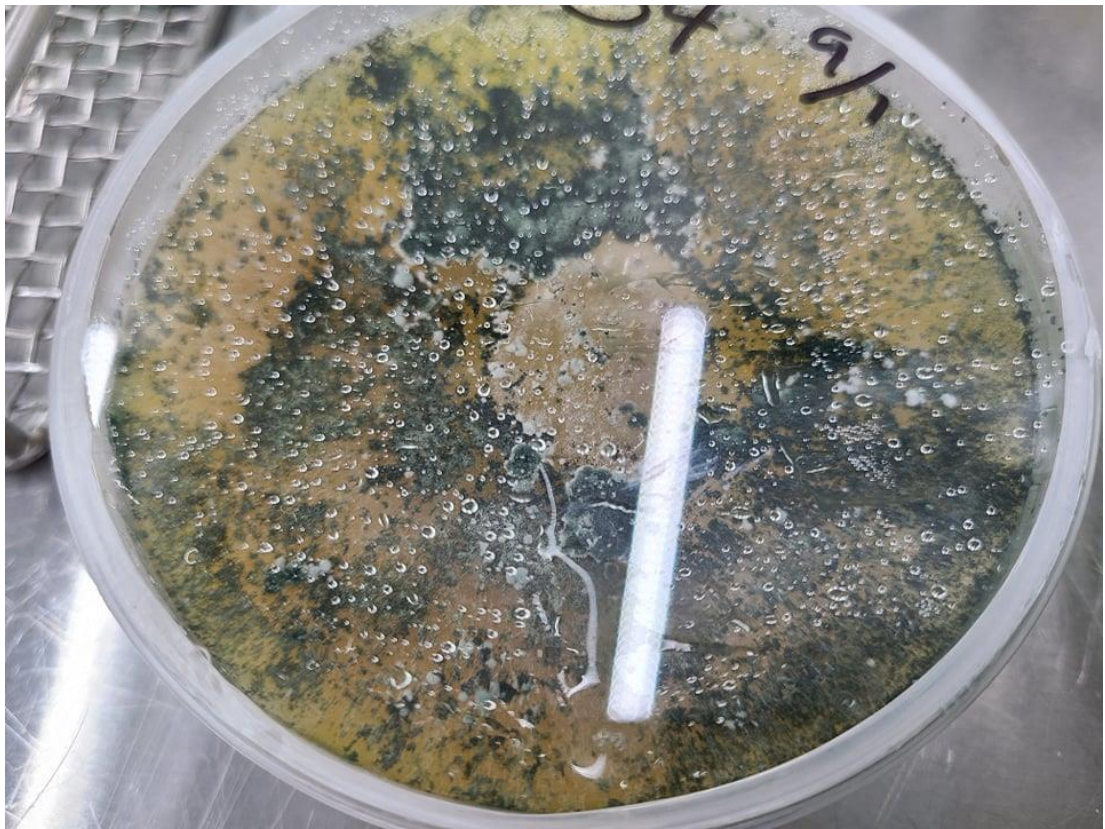
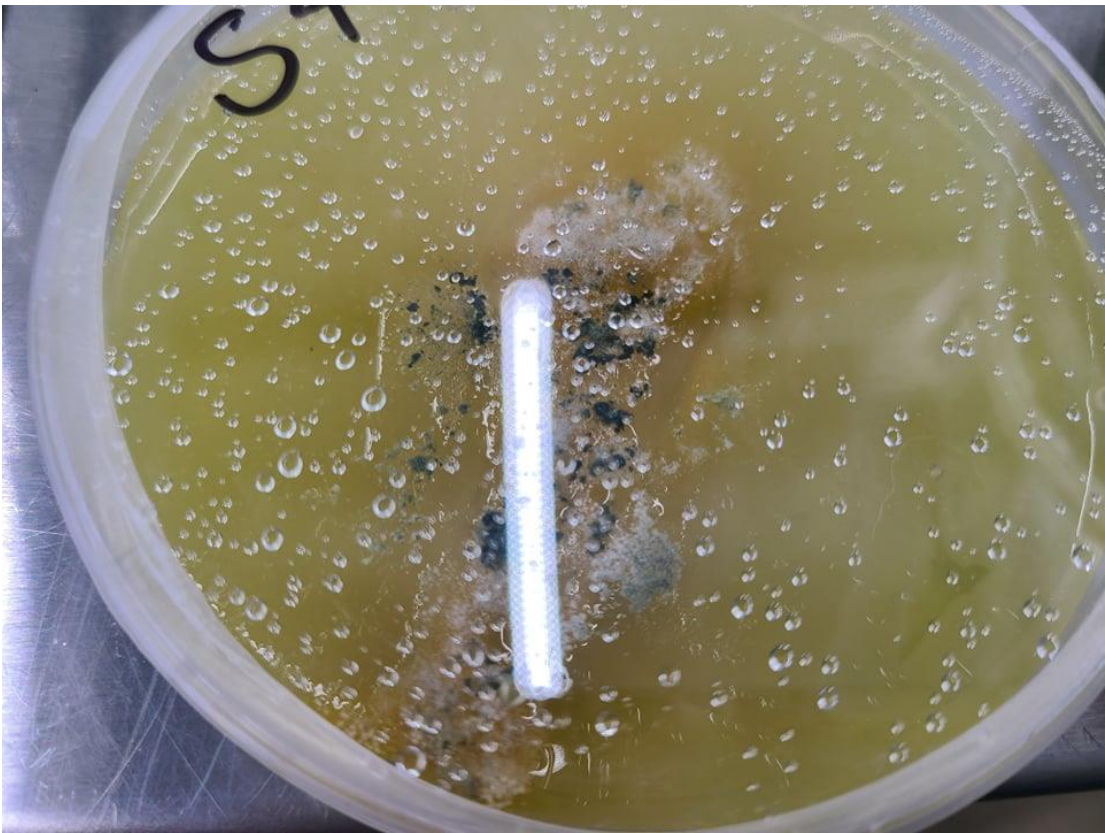
Figure 8: Samples from each bag of New Zealand Tree Fern Fiber hydrated in 32oz containers with vented lids to simulate a humid vivarium environment. Samples were observed for two weeks for visible growth of *L. birnbaumii*.



Figures 9-10: Ungerminated spores observed in samples 2 (top) and 4 (bottom) at 400x magnification



Figures 11-12: Agar plates from sample #2 originally containing unknown ungerminated spores. Spores have germinated showing the presence of multiple species of mold and bacteria but the absence of *L. birnbaumii*.



Figures 13-14: Agar plates from sample #4 originally containing unknown ungerminated spores. Spores have germinated showing the presence of multiple species of mold and bacteria but the absence of *L. birnbaumii*.