The Role of Environmental Factors in Formation of Hyphal Aggregation in *Fomitopsis betulina*

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Abstract

Fomitosis betulina is a brown rot fungus used in laboratory research. The species has a unique mycelial morphology in solid state culture, forming raised hyphal aggregations. To determine the impact of environmental factors, *F. betulina* was grown in extreme environmental conditions, including High Light, High Temperature, and High CO^2 concentration. Hyphal aggregation counts were compared, and found to be significantly different in some but not all of the treatments. This suggests that environmental factors play a role in the formation of hyphal aggregations.

Introduction

Fomitopsis betulina is a wood degrading fungi commonly found in boreal forests, where it only grows on birch trees. Being a brown rot fungus, it selectively degrades cellulose and hemicellulose, leaving behind the lignin (Kaffenberger and Schilling 2015). However, when grown on solid state media in a laboratory setting, it demonstrates an unusual mycelial morphology. Rather than an even layer of fluffy mycelium radiating out from the center of the plate, *F. betulina* typically grows in thin, spindly strands pressed flat against the surface of the mycelium, with a network of finer hyphae spread between then, nearly imperceivable to the naked eye. This sparse mycelium is interrupted periodically by small, dense masses of hyphae rising above the surface of the culture. These masses, henceforth referred to as hyphal aggregations, occur at random across much of the surface of an individual culture, and vary in size, number, and distribution between plates. Little has been documented about this unusual morphology, and both their cause and purpose are unknown.

In order to assess the environmental factors impacting the presence of hyphal aggregations, I cultured *F. betulina* under three extreme environmental conditions; High Light, 35° C, and High CO^2 concentration. Hyphal quality and number of hyphal aggregations were measured.

Materials and Methods

To determine factors contributing to hyphal aggregation formation, I exposed *Fomitopsis betulina* grown in solid state culture to varied environmental conditions. Initial samples were taken from ATF strain isolates of *F. betulina* and grown on PDA, then twenty eight plugs were transferred to individual PDA plates. Seven randomized plates, the Control plates, were wrapped with a single 10.16 cm by 2.54 cm strip of parafilm and grown at 27° C in a dark incubator. Seven randomized plates, those in the High CO^2 treatment, were wrapped with two 10.16 cm by 2.54 cm straps of parafilm, and grown in the same incubator (Banerjee et al. 2019). Seven randomized plates, those in the 35° C treatment, were wrapped with a single 10.16 cm by 2.54 cm strip of parafilm, and grown at 35° C in a dark incubator. The final seven plates, those in the High Light treatment, were wrapped with a single 10.16 cm by 2.54 cm strip of parafilm, and grown in a makeshift incubator at a starting temperature of 27° C, outfitted with a humidifier to maintain humidity, and a home-use grow light (ZXMEAN three head Full Spectrum LED Grow Light).

After two weeks, plates were removed from the incubators and stored at 4° C for 3 weeks. Each plate was photographed, and the hyphal aggregations were counted. Samples from each plate were examined under light microscopy. In the Control and High CO^2 treatments, one sample from the ordinary mycelium and one sample from a hyphal aggregation were used. In the High Light treatment, one sample from the ordinary mycelium and one sample from a hyphal aggregation were used, with a third sample taken from discolored patches for those plates that showed discoloration. One sample per plate was taken from the high temperature treatment, as they had not grown any hyphal aggregations, and very limited ordinary hyphae. From each sample, five randomly selected hyphae were measured for width, and samples were photographed. Hyphal aggregation counts were analyzed by treatment, with distinct hyphal aggregations and uncountable clusters being analyzed separately. The data was not normally distributed, so the Kruskal-Wallis Test was used. The Dunn Test was then used as a post-hoc test.

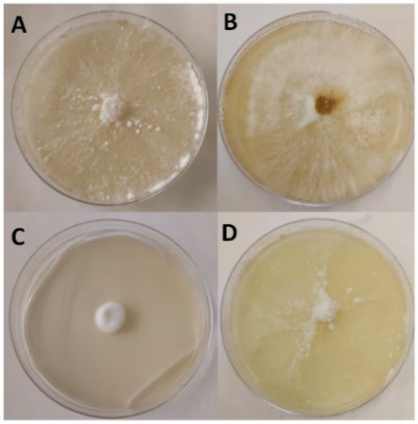

Figure 1: Photographs of plates from each treatment. High CO^2 (**A**), High Light (**B**), 35° Celsius (**C**) and Control treatments (**D**). Note the location of the hyphal aggregate clusters around the edge in the High CO^2 treatment, the growth rings, fluffy hyphae, and discoloration on the High Light treatment, and the limited growth on the 35° Celsius treatment. All photographs were taken following the three week refrigeration period.

Results

The mycelium from each treatment were visually distinct on a macroscopic scale. Those plates in the high light group grew fluffy, raised hyphae over much of their surface, with distinct growth rings (Figure 1). Plates in the high temperature treatment failed to colonize the plate, growing only a small amount of fresh mycelium on the agar plug in the middle of the plate (Figure 1). In the High CO^2 treatment, the hyphal aggregations seemed to be more concentrated around the outer edge of the mycelium than they were in the control.

On a microscopic scale, the individual hyphae themselves did not measurably vary in width between any treatment. Hyphae appeared more densely packed within the hyphal aggregations but could not be measured. Portions of the High Light treatment were discolored (Figure 2); in these zones, the hyphae were a slightly darker shade, and hyphae around the edges had the appearance of beginning to break apart. These discolored patches were found on five out of seven plates in the treatment.

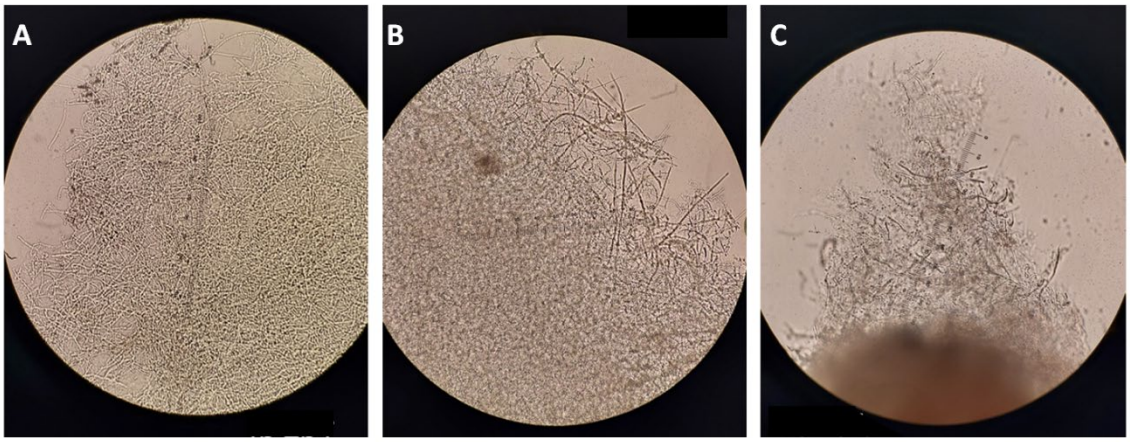

Figure 2: Visual difference between hyphal types. All samples are taken from the high light treatment. Note the increased hyphal density in the hyphal aggregation (**B**) compared to the ordinary hyphae (**A**), and the brown coloration of the discolored sample (**C**). Hyphal samples photographed under 10X magnification.

Treatments varied in hyphal aggregation counts for both individual aggregation and clusters (Figure 3), and the Kruskal-Wallis Test indicated that the treatments were statistically different for both the individual hyphal aggregations (p-value < 0.001) and the clustered hyphal aggregations (p-value < 0.001). The Dunn Test results were more varied. For the individual hyphal aggregation, only the comparisons of the High CO^2 treatment to the 35° C treatment (p-value < 0.001) and the 35° C treatment to the Control treatment (p-value < 0.001) were statistically significant. For the clustered hyphal aggregations, the comparisons of the High CO^2 treatment to the High Light treatment (p-value = 0.028), High CO^2 treatment to the 35° C treatment (p-value < 0.001), and the 35° C treatment to the Control treatment (p-value < 0.001) were statistically significant. While all other comparisons appear different (Figure 1), the Dunn Test contradicts that.

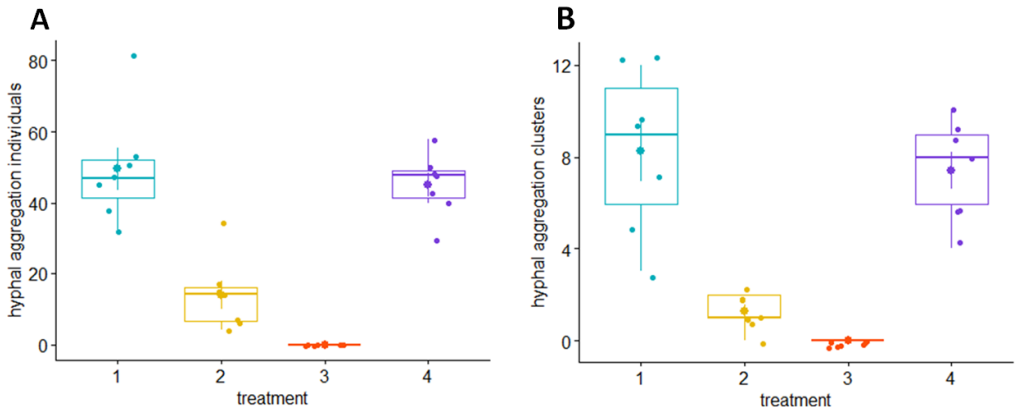

Figure 3: hyphal aggregations varied by treatment. The High CO^2 treatment (1) and the Control treatment (4) have similar hyphal aggregation counts for both individual aggregations (**A**) and aggregation clusters (**B**), while the High Light treatment (2) is lower for both measures. The High Temperature treatment (3) had no aggregations of either type.

Discussion

The three treatments each had their own distinct macroscopic mycelial morphologies. Of note were the growth rings in the high light treatment. Similar growth rings have been noted in other fungal species, which may be related to daily light and dark exposure (Hein 1930). However, these cultures were grown under light around the clock, and were not exposed to darkness during their two week growth period. Also in the high light treatment, the discoloration on five of the plates seemed to correspond with placement under the growth light. This discoloration likely indicates damage to or death of the mycelium, given the seeming breakdown of the hyphae in the edges disturbed by being removed from the plate. Non-discolored hyphae did not disintegrate around the edges in that way, but rather broke off cleanly. In the high temperature treatment, 35° C initially appeared to exceed the heat tolerance of *F. betulina*, which did not reach the fresh PDA of the plate, barely covering the sides of the original agar plug. In spite of that, once moved to the refrigerator, the culture did begin growing again, and while it did not spread across the plate, it increased in density in the areas it was already growing (Figure 1). This indicates that, while 35° C is too hot for *F. betulina* to thrive, it is not too hot for it to survive, at least for a short time. The high CO^2 treatment was the most visually similar to the Control, other than the distribution of the clusters of hyphal aggregations. Given this species' niche as a wood decomposition fungus, it spends the majority of its life cycle inside of wood; therefore, the higher CO^2 or decreased gas exchange may mimic its natural environment, and be less stressful for the fungus than the other treatments.

While the Kruskal-Wallis test showed significance, the Dunn test indicated that few of the individual comparisons were significant for either type of hyphal aggregation. While several of the comparisons with 35° C were significant, the only one that was not a comparison with a mean of zero (Figure 3) was the comparison between the clustered hyphal aggregation counts in the High Light treatment and the High CO^2 treatment. This seems to indicate that light exposure, CO^2 concentration, and temperature can all impact hyphal morphology in certain ways, but more research is needed to better ascertain in what way they do that.

The high light treatment had a few complicating factors. It was set up in a cabinet and the humidity was maintained with a humidifier filled daily, as there were no available incubators that could also hold a grow light. Due to the combination of a humidifier and grow light running 24/7 for two weeks, the temperature exceeded the desired 27° C towards the end of the first week and remained closer to 30° for the remainder of the growth period as attempts to cool the space failed. Additionally, while I attempted to evenly distribute the plates under the light source, it is likely that some plates were receiving more direct light than others, possibly resulting in the discoloration noted.

This study demonstrates that environmental processes play a role in determining mycelial morphology of *Fomitopsis betulina* but does not resolve the question of how. More research is needed to determine what the role of hyphal aggregations are in the growth of *F. betulina*, as well as how each environmental factor controls their growth. A study with larger sample sizes may yield more information in this regard.

Reflection

Flexibility and creativity in research have been the most valuable skills I have learned from my UROP experience. Going into this project, I did not predict that my objective of measuring hyphal morphology with thin slicing agar would prove ineffective under light microscopy, and so when it became clear I was not going to be able to acquire usable data in that way, I had to learn to think outside the box to find a way to accomplish my goal with different methods. Others with more experience than I have assured me that this adaptability is an important skill to have when working in research of all levels. Aside from that, I now more than ever understand the need for community in research; every step of this project, as small as it was, I had the help and input of those with more experience than me, even for things I had not predicted that I would need help with (I do not think anyone predicted that setting an incubator would be a multi-person operation, but it was). It would certainly not have been possible to do this without the help and support of everyone in the lab, and I am grateful to have a community I can reach out to for guidance.

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