Mycelium Propagation in Various Oil Mediums and Applications in Asphalt Decomposition

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Introduction

Fungi are often overlooked when examining the dynamics of an ecosystem, though such organisms are an essential component in the decomposition of both organic and inorganic matter. Many mushroom spores grow mats of thin filaments below the soil known as *mycelium*, which can then aggregate to form a recognizable mushroom body.[1] *Mycoremediation* is the relatively new field of utilizing mushrooms to remove environmental pollutants. Common oyster mushrooms native to Van Cortlandt Park, *Pleurotus ostreatus*, are categorized as "white rot fungi" for its ability to break down brown fiber in wood.[1] White rot fungi excrete lingin peroxidases, maganese peroxidases, and laccases, which target hydrogen-carbon bonds.[1]

The fungi was placed on agar plates containing two oils in the hopes that the results of the experiment would be applicable in implementing the fungi in asphalt mycoremediation. Asphalt is a petroleum-based material of various composition depending on the specific manufacturer. However, asphalt usually contains various resins, aliphatic hydrocarbon compounds (chains),[3] and polycyclic aromatic hydrocarbon compounds (rings, known as *PAHs*).[3] Various motor oils typically contain resins, benzene-based substances, and petroleum-based hydrocarbons which consist of carbon, hydrogen, and polyalphaolefins.[4] Canola oil is an oil rich in saturated fatty acids–lipids consisting of a carboxyl group attached to a hydrocarbon chain.[5]

Purpose

The purpose of this experiment was to test the mycoremediation capabilities of white rot fungi on canola and motor oils for the potential application of this fungi on decomposing asphalt in Van Cortlandt Park.

It was hypothesized that the presence of hydrocarbon-containing oils would increase the growth of the white rot fungi by providing it a source of food.

Methodology

Pleurotus ostreatus mycelium were grown on two different agar mixtures in addition to different types of oil.

1. Each plate was prepared with an agar mixture with just water added or a minimal mixture of peptone, yeast, monopotassium phosphate, and magnesium sulfate.
2. 10µ of canola or motor oil were spread across select plates.
3. A tube of approximately 1.25 cm in diameter was used to cut a portion of a previously inoculated agar plate and transferred to the prepared plates.
4. Five plates for each of the six distinct plate types were inoculated for a total of 30 plates.
5. Pictures were taken of each plate on a regular basis and analyzed for surface area using Fiji™ software.

Image 1. Motor oil on agar plate Day 0

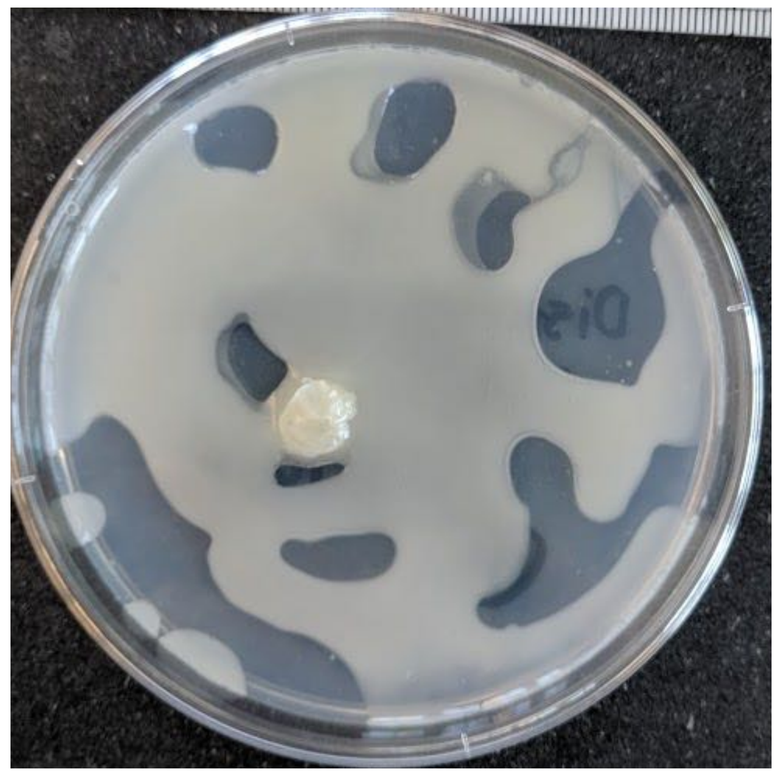

Image 2. Motor oil on agar plate Day 5

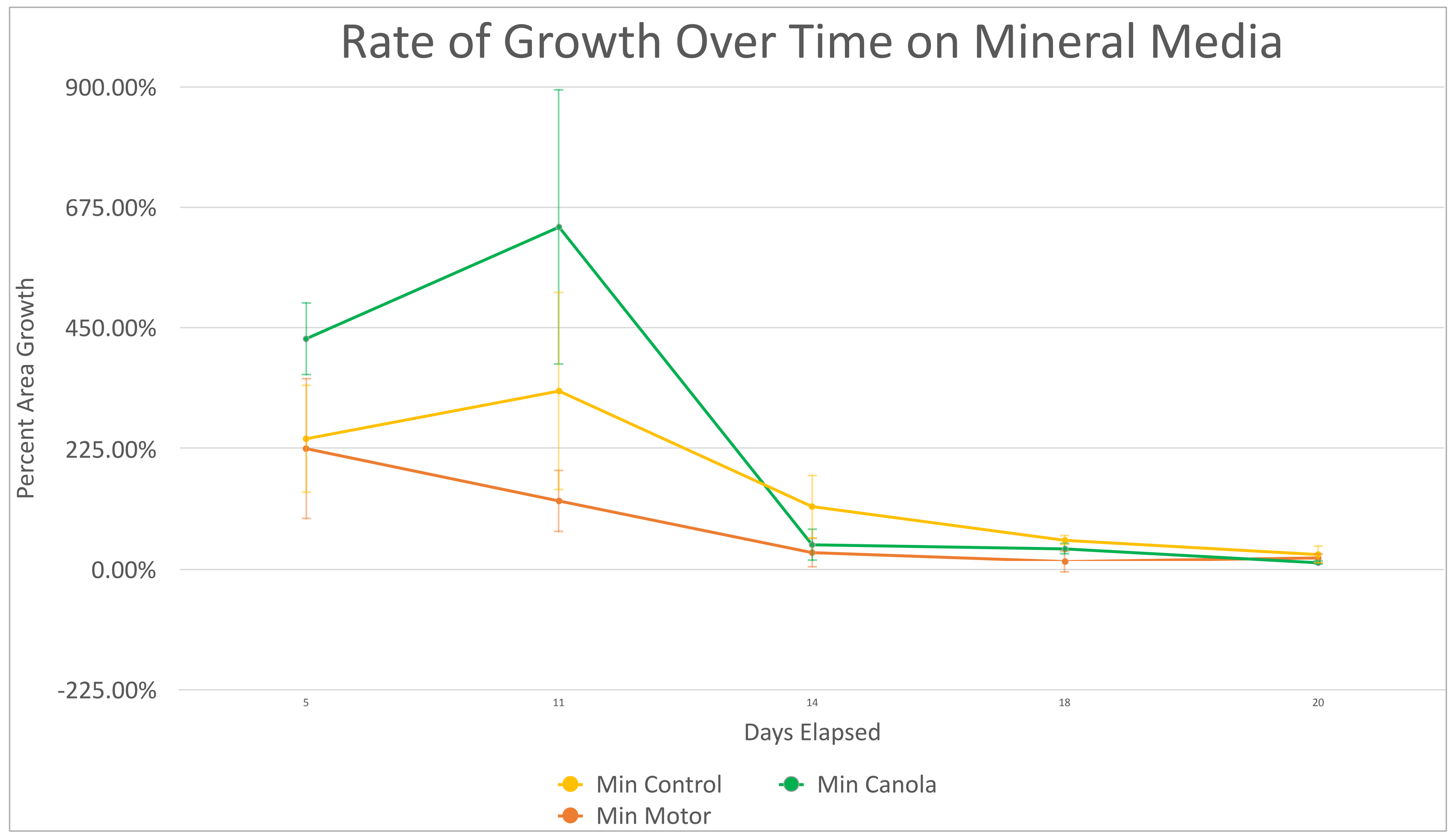

Figure 1. The percentage increase in surface area of Pleurotus ostreatus grown on a mineral and agar plate in various oil mediums. Error bars are assigned different colors to make differentiation easier. Mycelium grown in canola oil shows the sharpest initial increase in surface area but subsequently drops at around Day 14. It should be noted that as each day progressed, the size of the plate itself became a limiting factor in mycelium growth and propagation

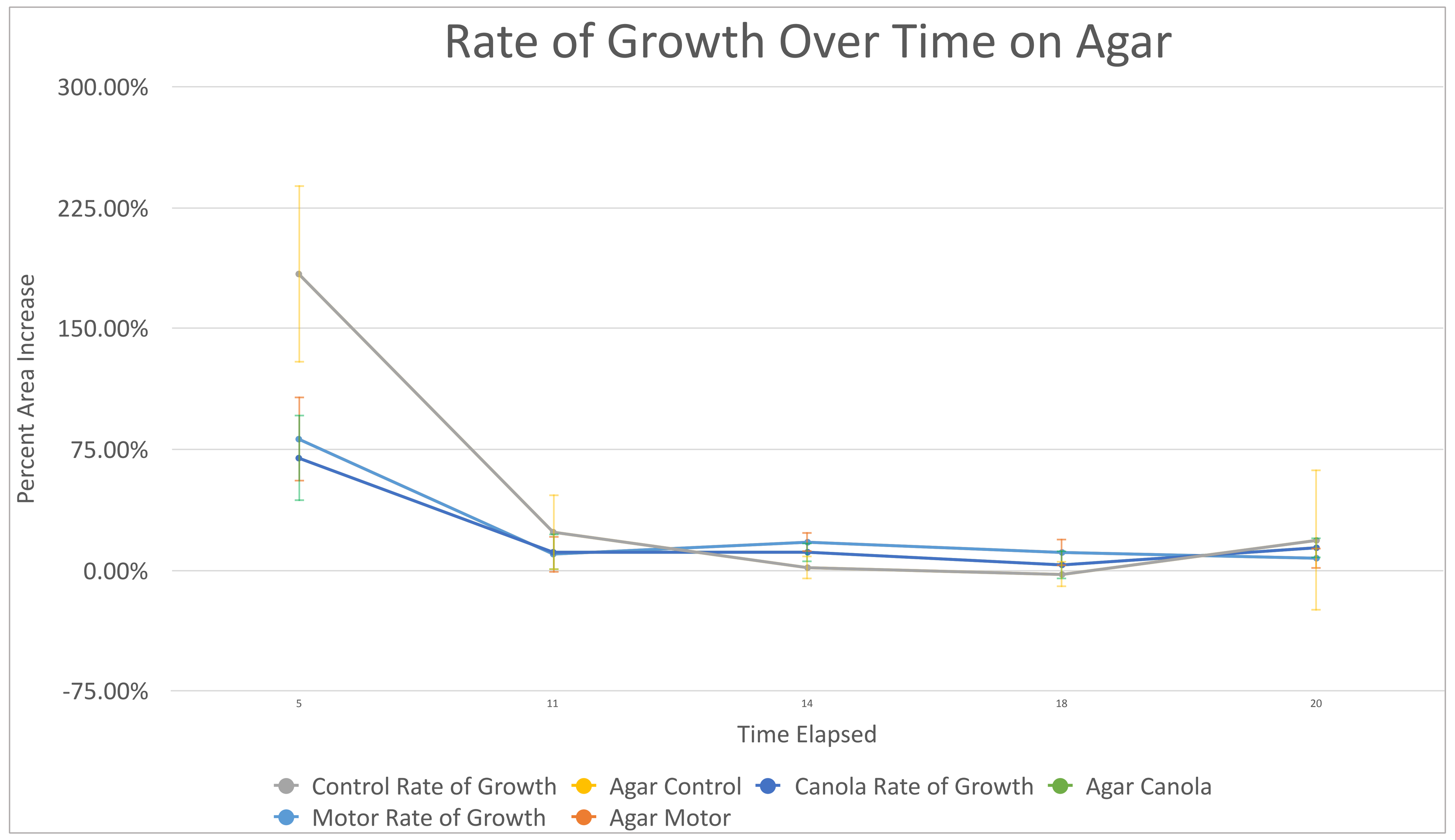

Figure 2. The percentage increase in surface area of Pleurotus ostreatus grown on a simple agar plate in various oil mediums. Error bars are assigned different colors to make differentiation easier. The control group showed the sharpest increase in surface area from Day 5 to 11, though mycelium growth on all plates plateaued (and even declined) as time passed, as shown by the 0% growth on all plate types from Day 14 to 20.

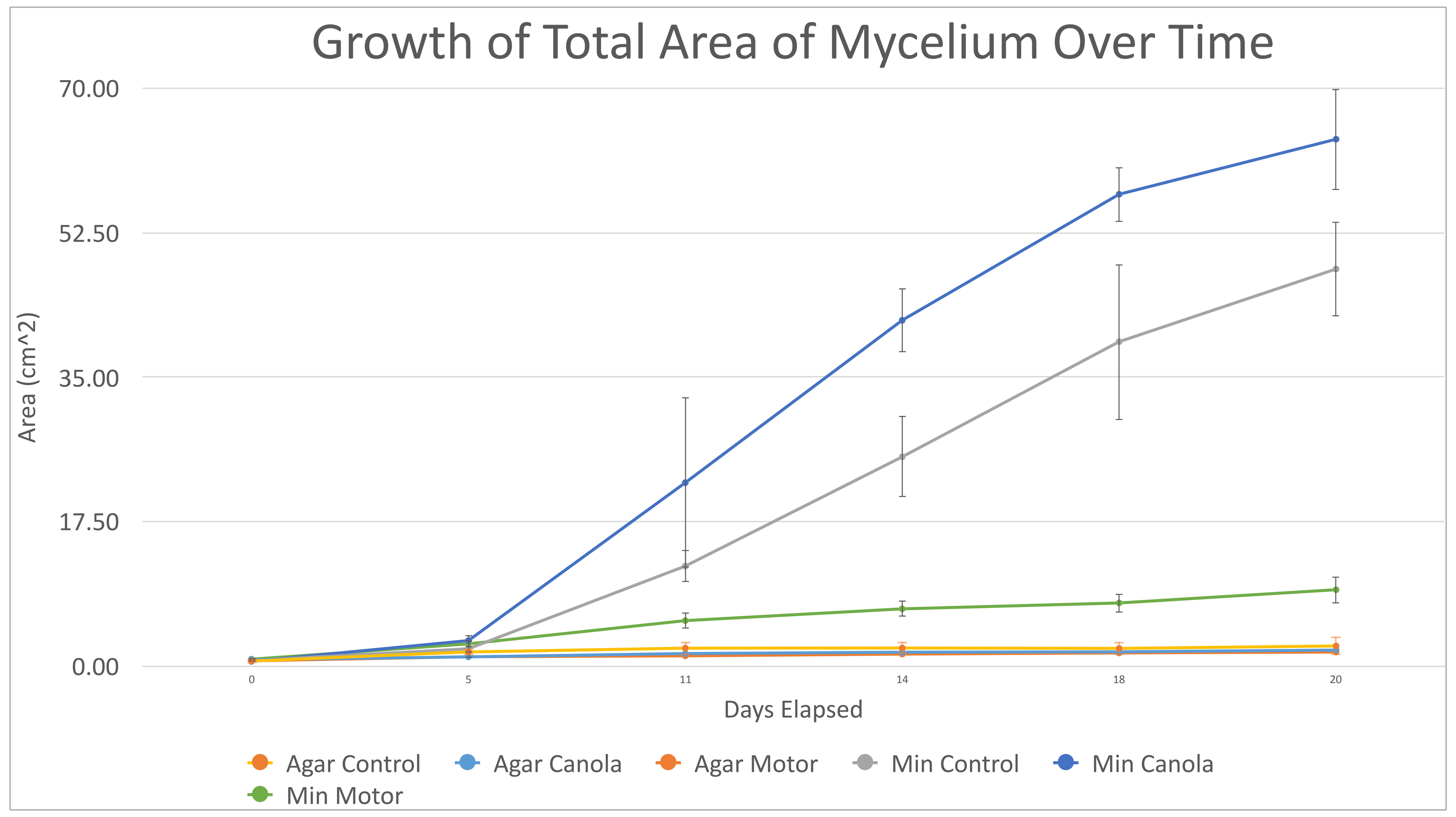

Figure 3. Total surface area covered by mycelium on each plate type. It is evident that Pleurotus ostreatus grew best on the mineral mixture of agar, regardless of oil, though of all the mineral + agar plates, mycelium growing in canola oil covered a significantly larger area from each other plate type. It is important to note that the starting size for the mycelium sample on each plate, while an effort was made to standardize it as much as possible varied a fair amount between .4 and .9 cm^2 (presenting a problematic source of uncertainty). However, this variance was random and it is still useful to see the difference between mycelium grown in canola oil and the control group.

Conclusions and Observations

- Of the six different types of plates analyzed in this experiment, results show that oyster mushroom mycelium growing on an agar + mineral mixture and canola oil had the fastest rate of growth, significantly different from both control groups.
- This difference reveals that *Pleurotus ostreatus* propagates best in canola oil when in an environment with other life-essential minerals.
- Mycelium growth was greatly affected by the mineral mixture in the agar plates compared to the mixture of water and agar as each of the three oil conditions grew best on a mineral plate.
- As seen in Image 4, the pattern *Pleurotus ostreatus* mycelium growth very closely follows the oil pattern on the agar plate more clearly seen in Image 3. The irregular growth pattern should be compared to the radial symmetry of Image 2 to determine the effect of the presence of canola oil to mycelium propagation.

Discussion

The data presented supports the idea that the growth rate and therefore the metabolism of *Pleurotus ostreatus* is affected by the presence of compounds containing hydrocarbon bonds such as those found in canola oil. These results support the ongoing research that suggests that *Pleurotus ostreatus,* along with other fungal species, has the ability to metabolize compounds found in asphalt to aid in its decomposition as a form of mycoremediation. A t-test was also performed on the data used in Figure 3 to see whether the differences between the three mineral agar plates were statistically significant. The t-tests showed that the canola oil and the control plates were different with a value of 0.0092 and that the control and motor were different with a value of 0.00011. This proves that the propagation on the mineral plates was best for canola and worst for motor. As seen in Figures 1 and 3, mycelium growing in the presence of motor oil showed the slowest rate of growth. Images 1 and 2 suggest that the presence of *Pleurotus ostreatus* changed the color of the motor oil on the plate. Furthermore, an additional control plate with just motor oil sitting on agar shows that the oil does not change color in the absence of this mycelium.

In future experiments, different methods of analysis should be considered, such as cell count, to further the common understanding of fungal metabolism and its applications. The results shown suggest that the next step in pursuing such research is to observe *Pleurotus ostreatus* growth in the presence of asphalt to determine its possible applications in mycoremediation.

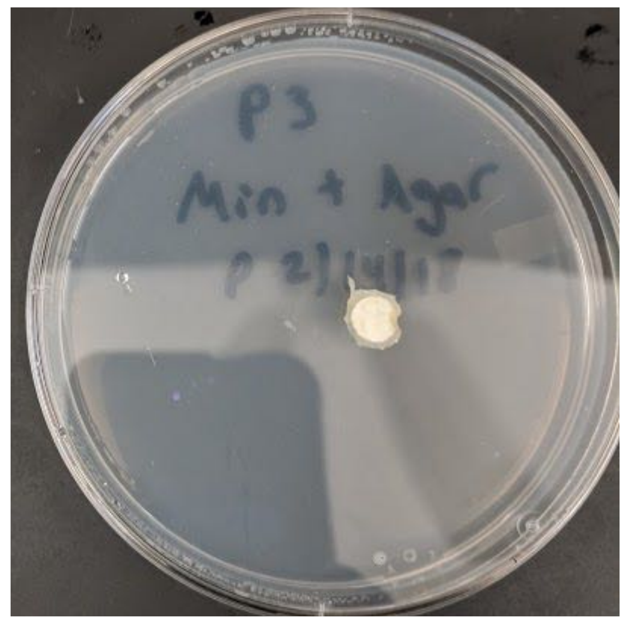

Image 3. Control on mineral plate Day 0

Image 4. Control on mineral plate Day 11

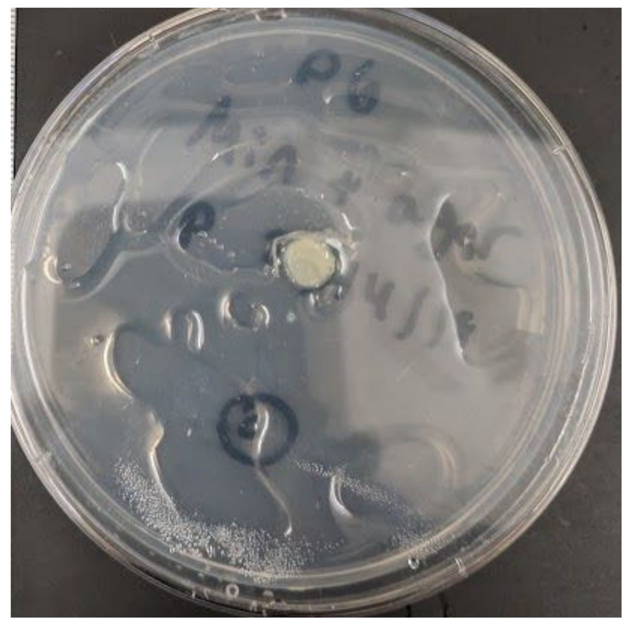

Image 5. Canola on agar plate Day 0

Image 6. Canola on agar plate Day 11

References

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