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How to prepare *P. Polycephalum* for future microplastic studies

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INTRODUCTION

In today's society, microplastic pollution has been steadily increasing throughout the world and can be found in various sources, such as in food, the oceans, and wastewater treatment plants. Due to its ever-increasing prevalence, it is a potential threat for both human health and the environment. Yet there is a surprisingly low amount of research that has been done, regarding microplastic pollution, in other environmental areas, such as in forests. Slime molds are one of many natural decomposers of organic matter and can be found all over the world. Using the slime mold species *Physarum Polycephalum* we hereby want to test the environmental influences of microplastics on its growth and oscillating properties.

OBJECTIVES

- study the right cultivation conditions and to cultivate *P. Polycephalum* for subsequent experiments
- create a permanent state of *P. Polycephalum* (sclerotium)
- investigate the oscillation behaviour of *P. Polycephalum*
- preparation of fluorescence loaded microbeads – with different polymers
- observe if *P. Polycephalum* ingests the previously prepared microbeads
- observe if *P. Polycephalum* degrades the microbeads in any kind of way

METHODS

Cultivation of *P. polycephalum* – *under sterile conditions*

- sclerotium of *P. Polycephalum* was placed on agar in petri dish
- some drops of sterile water and oat flakes were placed on and next to sclerotium
- *P. Polycephalum* was stored in a dark incubator at 25 °C
- piece of plasmodium was cut out with a sterile scalpel or an overgrown oat flake was placed upside down on a new petri dish
- Petri dish was sealed with parafilm, which had small holes to ensure air exchange

Production and testing microbeads

- Nile red was mixed with water and added to Poly(D,L-lactide-co-glycolide) solution
- emulsion was homogenized with a sonotrode
- homogenized solution was added in polyvinyl alcohol and mixed with a homogenizer
- solution was stirred overnight and dichloromethane evaporated
- after centrifugation, microbeads were washed
- finished microbeads were injected with a syringe into a plasmodium and examined by fluorescence microscopy

Oscillation behavior of *P. Polycephalum*

- change of flow direction of *P. Polycephalum* was examined by microscopy
- time till *P. Polycephalum* changed its flow direction was measured with a timer

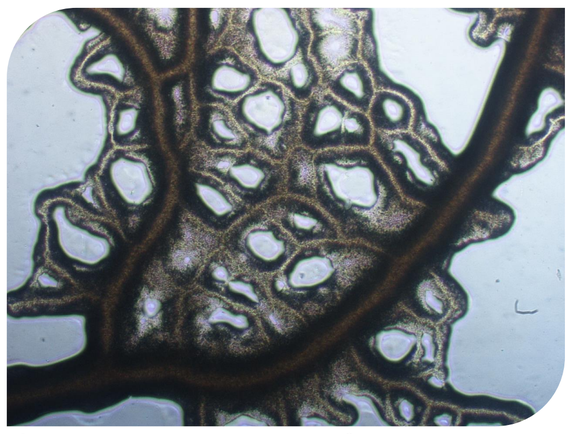

Fig. 1: Microscopic image of *P. Polycephalum; 100x magnification*

Fig. 2: *P. Polycephalum* connecting oat flakes

RESULTS

During the project, the cultivation conditions were established and the occuring contamination of the initial slime mold was eliminated by changing the supplier. The fastest and most reliable growth was achieved at a temperature of 25 °C and a humidity over 80 %. For future experiments a permanent state of the *P. Polycephalum*, called sclerotium, was cultivated and stored. *P. Polycephalum* exhibits oscillation, which was captured by microscopy. Nile red loaded PLGA-particles were therefore synthesized as a representative for microplastic polution and injected into the *P. Polycephalum*. The average particle size of 1-10 µm was examined by microscopy. After heating *P. Polycephalum* at 37 °C for an hour, no visible flow was detected.

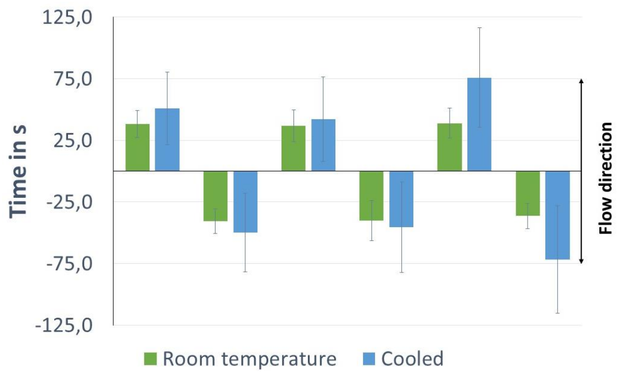

Fig. 3: Temperature dependet oscillation

DISCUSSION

By changing various cultivation conditions, we finally found out, that the purchased sclerotia from the company were already contaminated. Herein we proofed that our techniques didn't have any impact on the growth of mould inside our petri dishes. Additionally, we found out that it is not necessary to cultivate *P. Polycephalum* under such sterile conditions we first did. The change of temperature however showed some differences to the one cultured at cultivation conditions. This results in a slower growth of *P. Polycephalum*. Further research objectives could be to investigate the influence of microplastics, such as PS or PLGA, of the slime molds oscillation properties and the impact of different amounts of environment pollutants on *P. Polycephalum*.

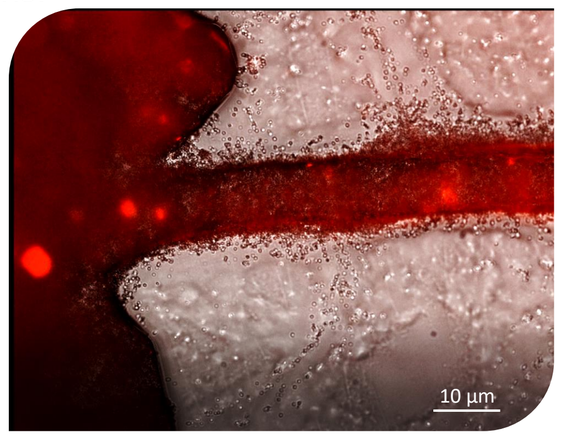

Fig. 4: Injected fluorescence labeld PLGA microbeads in *P. Polycephalum*