

Background

EPA Superfund sites classify locations with hazardous levels of containments, posing potential health risks to humans and the environment^[1]. PAHs are a group of chemicals often found at Superfund sites due to their mutagenic and carcinogenic properties^[2,3].

There are numerous strategies implemented to remediate PAH contamination, many being ecologically intrusive^[4]. A sustainable and cost-effective alternative is mycoremediation. However, minimal success in PAH degradation has been seen due to planktonic microbes not properly establishing within the novel environment^[5].

This project will utilize known PAH degrading microbes isolated from a PAH contaminated Superfund site^[6]. Though this project, we aim to develop methods to encapsulate fungal genera and quantify growth and viability. This research will work to promote the survival and delivery of PAH degrading microbes back into the environment^[7].

Current Work

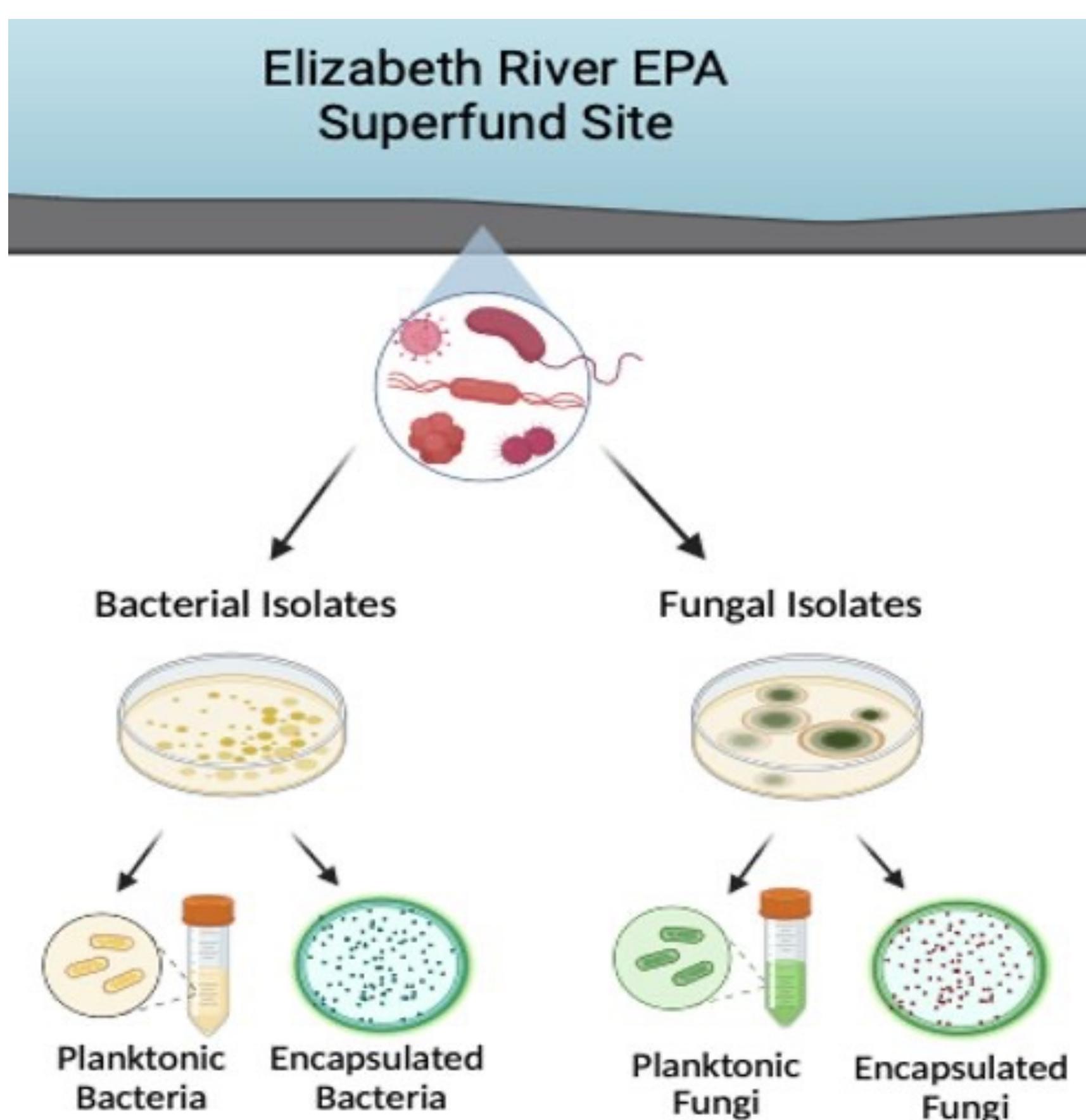


Figure 1: Schematic describing monoculture process from Elizabeth River

14 PAH degrading Fungal Strains were isolated from the Elizabeth River Superfund Site. Culture plates and slant stocks have been created to revitalize old stocks. Grown on MEA at 30C

Will use three genera to investigate the differences in encapsulation of sporulating versus yeast-like fungi

- *Auerobasidum*
- *Penicillium*
- *Trichoderma*

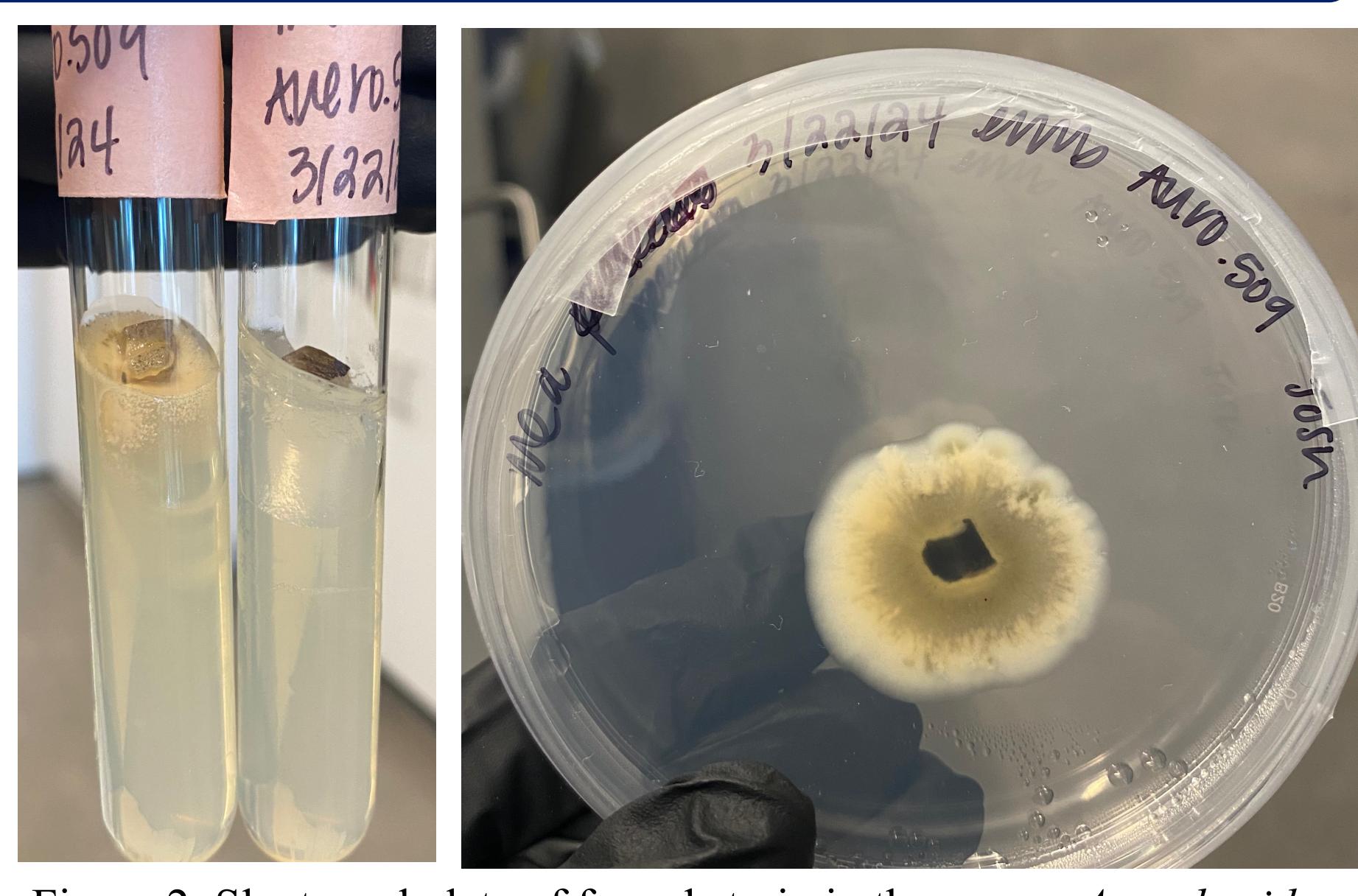


Figure 2: Slants and plate of fungal strain in the genera *Auerobasidum*

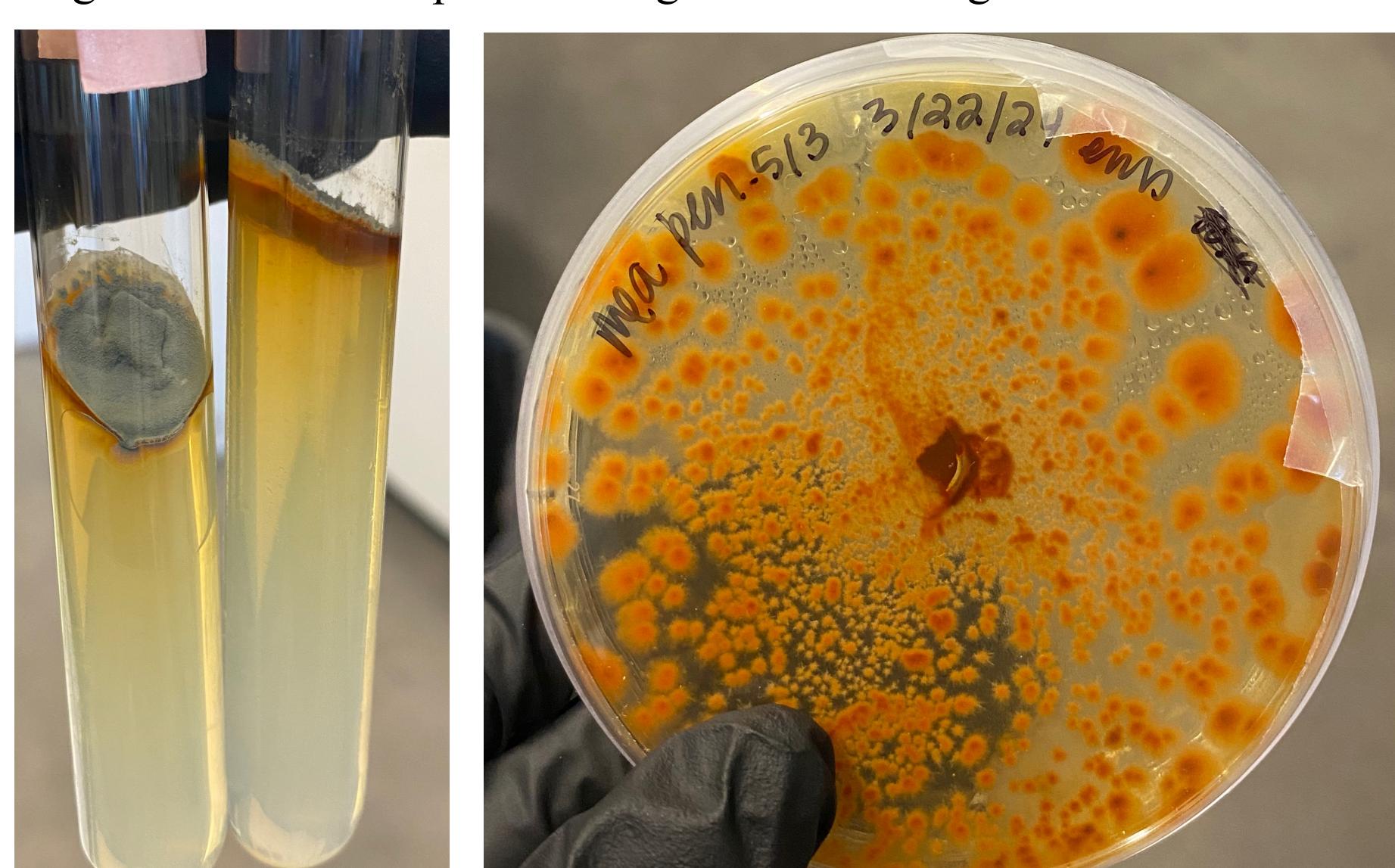


Figure 3: Slants and plate of fungal strain in the genera *Penicillium*

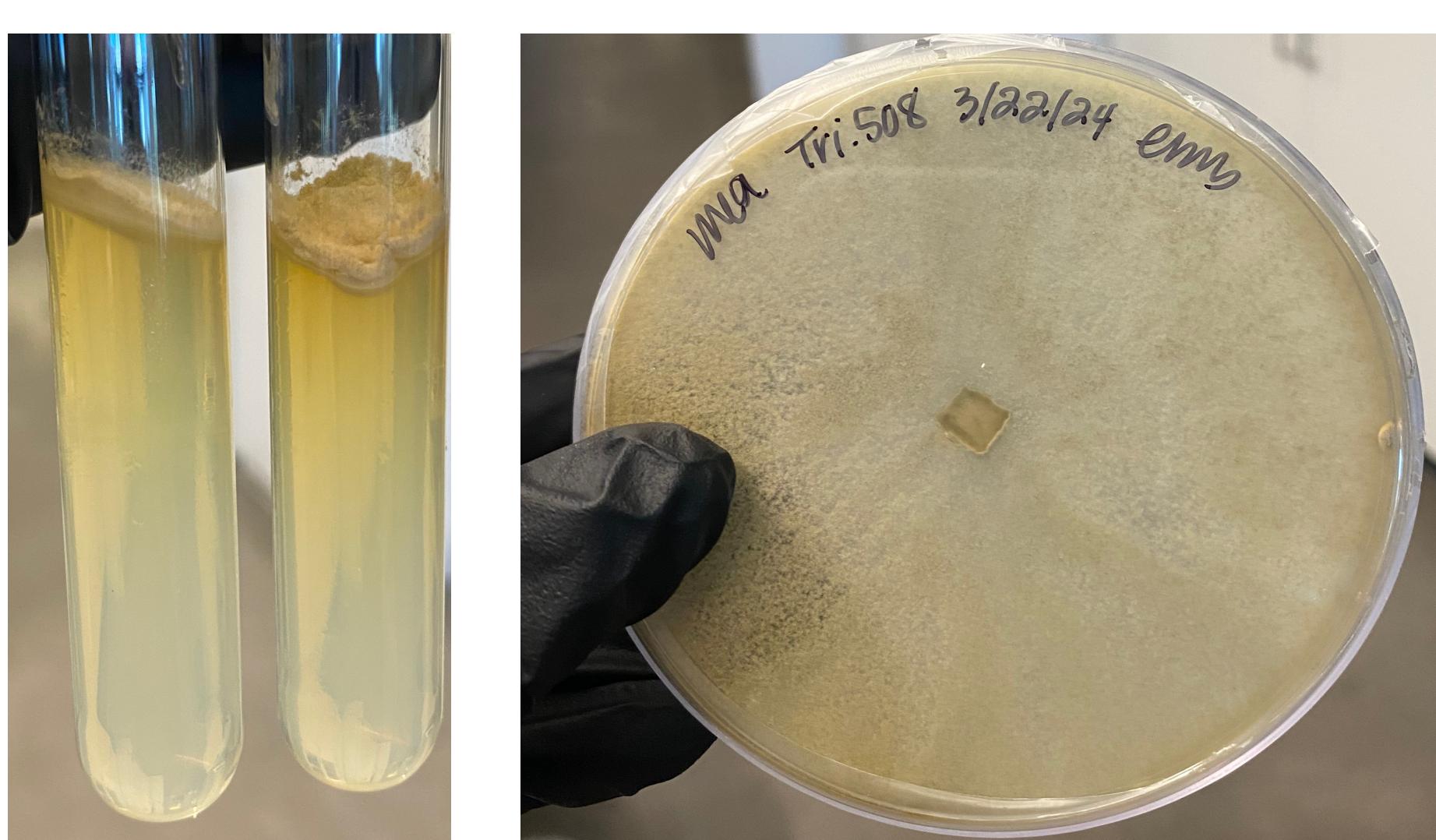


Figure 4: Slants and plate of fungal strain in the genera *Trichoderma*

Next Steps

Previously Developed Methods

Monocultures

Fungal and bacterial isolates were isolated from Sediments of the Elizabeth River. Monocultures were then grown in LB media at 30 C as both planktonic and encapsulated cultures

Bacterial Encapsulation

Capsules were generated via extrusion using a 1% sodium alginate solution and 10% bacterial culture through a 30G needle into 0.1M calcium chloride bath^[8]



Figure 5: Syringe pump setup for producing microcapsules via extrusion

Measuring Growth

OD600 was used to measure bacterial growth of the media surrounding the capsule to quantify the release of bacteria. Capsules were dissolved in sodium citrate and measured using OD600 to quantify the total bacterial concentration^[9]

Confocal Microscopy

Distribution of bacteria within alginate microcapsules was determined via the constitutive fluorescence of previously constructed fluorescence bacterial strains as observed via a confocal microscope at 10x, 20x, and 40x.

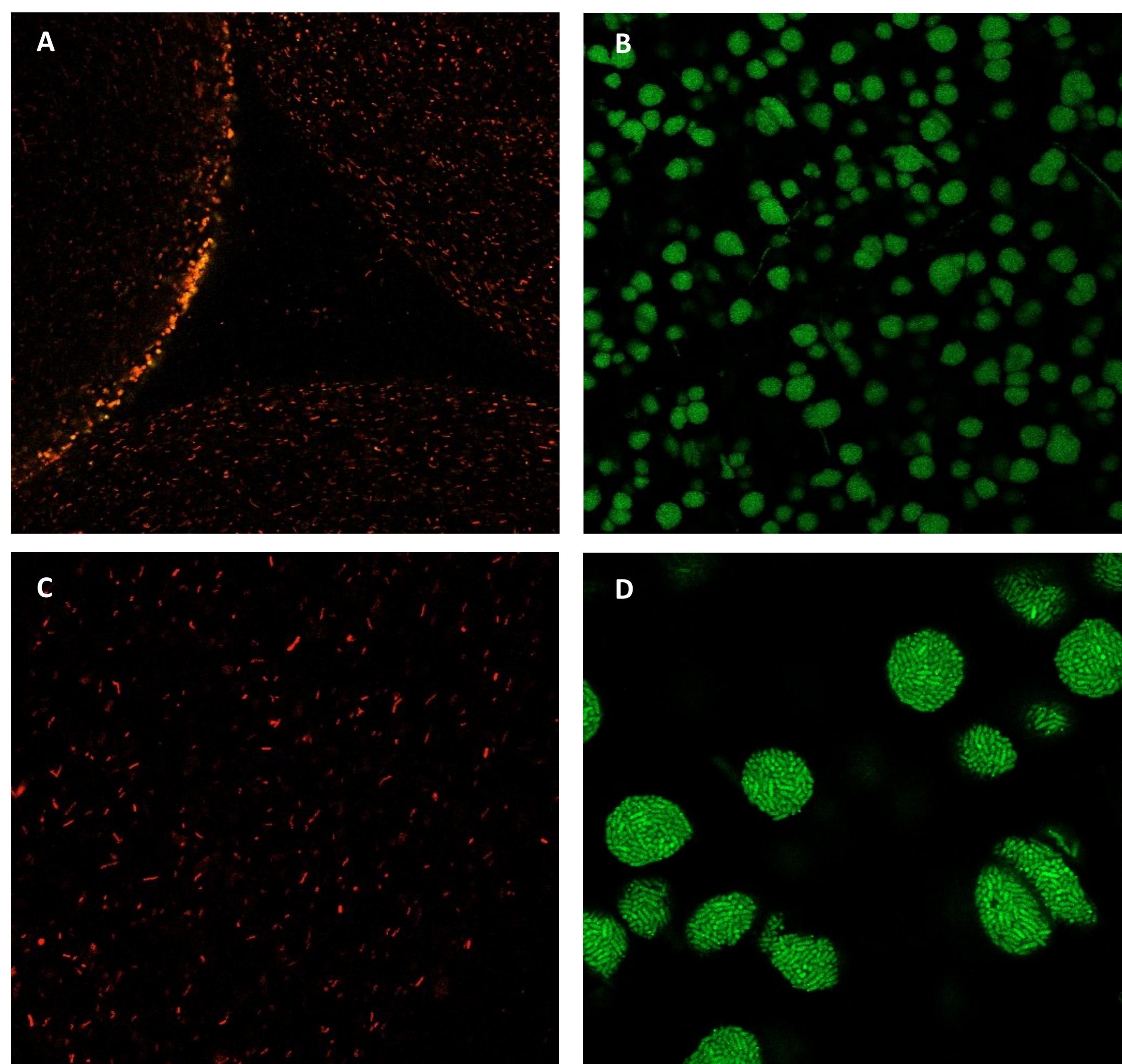


Figure 6 (A-D): Pictures showing the distribution of bacteria on the surface of sodium alginate microcapsules. Figure via Amelia Foley

Fungal Encapsulation

Using a 1% sodium alginate gel, microcapsules will be generated via extrusion to determine if the bacterial encapsulation protocol can be adapted to fungi. The gel will be inoculated with a spore suspension and growth in ME media at 30C. Yeast-like strains will follow the bacterial protocol

Future Directions

- Evaluate if these bacterial adapted microcapsules also improve viability of fungi compared to planktonic cultures
- Investigate the viability and fitness of microencapsulated fungi over longer time scales
- Compare the encapsulation success between filamentous and yeast-like fungi strains
- Encapsulate a consortia of bacteria and fungi
- Test the encapsulated microbes under different environmental conditions (i.e. pH and ionic strength) to determine if capsules can persist in *in-situ* like conditions

References and Acknowledgements

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