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Title: Investigating Bacterial-Fungal Interactions Using Fungal Highway Columns in Diverse Environments and Substrates

Landing Page Title (not for video use): Utilizing Fungal Highway Columns for Studying Bacterial-Fungal Interactions

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

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Current Protocol Length

Number of Steps: 15
Number of Shots: 34

Introduction

NOTE for VO producer: Please record the interview statements.

NOTE: The statements and the questions have been edited to remove any personal pronouns.

REQUIRED:

1.1. This study focuses on bacterial-fungal interactions to understand how these microbes can influence each other's behaviors. This work investigates bacteria that utilize fungal hyphae for transport. Gaining insight into these interactions enables the study of complex microbiomes in environments like soil **[1]**.

1.1.1. *2.1.2.*

What technologies are currently used to advance research in this field?

1.2. Examinations of bacterial transport along hyphae have traditionally been done on modified Petri dishes, but recent advances in 3D printing technology have allowed for the design and manufacturing of devices that can better mimic native conditions for these microbes to interact **[1]**.

1.2.1. *2.7.4*

How will these findings advance research in this field?

1.3. These fungal highway columns have been successfully used in soil, rhizospheres, and herbivore dung across environments on two continents. These devices may also serve as effective teaching tools or be applied by other research groups to expand collective knowledge on bacterial-fungal interactions **[1]**.

1.3.1. *2.10.1*

Protocol

2. Construction and Utilization of Fungal Highway Columns

Demonstrator: Julia Kelliher

2.1. To begin, pour sterilized agar-based media into 90-millimeter Petri dishes **[1-TXT]** until the agar is close to the top of the sides of the Petri dishes **[2].**

2.1.1. WIDE: Talent pours sterilized agar media into 90 mm Petri dishes. **TXT: Media: Sodium Carboxymethyl Cellulose/ Malt Extract Agar/ Potato Dextrose Agar/ R2A Agar**

2.1.2. CU: Shot of the agar almost at the top of the Petri dish.

2.2. Next, place the column end of a fungal highway column on one of the solidified media plates **[1].** Lift the column from the media in a twisted motion so that the agar stays within the column end and creates a media plug **[2].**

2.2.1. Talent places the column end of a fungal highway column on one solidified media plate.

2.2.2. Shot of the column being lifted in a twisted motion and the media plug is being created.

2.3. Add the small cap over the end with the media plug to maintain a sterile microenvironment for the target media **[1]**. Flip the column over **[2]** and create a media plug for the other end **[3].**

2.3.1. Talent adds the small cap to the end of the column.

2.3.2. Shot of the column being flipped.

2.3.3. Shot of a media plug being created on the other end of the column.

2.4. Using sterilized scissors, cut a circular piece of an autoclaved nylon mesh with a 2-centimeter diameter **[1]**. Lay the mesh over the exposed end of the column **[2].**

2.4.1. Talent cut a 2 cm circular piece of nylon mesh.

2.4.2. Shot of the mesh being placed over the exposed end of the column.

2.5. Twist the threaded ring onto the bait media end of the column **[1]** while securing the mesh within the threads **[2].** Now, place a large cap on the bottom of the column over the mesh and the other end of the threaded ring **[3-TXT].**

2.5.1. Talent twists the threaded ring onto the bait media end of the column.

2.5.2. Shot of the mesh being tied within the threads.

2.5.3. Talent places a large cap on the bottom of the column. **TXT: Keep the large cap on while storing or transporting the column**

2.6. Set up a laboratory soil microcosm **[1]**. Remove the large cap from the bottom of the column to expose the threaded ring and mesh **[2]**. Add the column to the soil microcosm such that the entire bottom section is within the soil **[3-TXT].**
  2.6.1. Shot of a set up laboratory microcosm.
  2.6.2. Talent removes the large cap from the bottom of the column.
  2.6.3. Talent inserts the column into the microcosm. **TXT: If necessary, make a depression in the substrate before column insertion**

2.7. Remove the column after it has been in contact with the substrate for the desired amount of time **[1].** Carefully shake off any excess substrate **[2]** and replace the large cap back onto the bottom of the column below the mesh **[3]**. Place the column in a 50-milliliter tube for transport **[4].**
  2.7.1. Shot of the column being pulled out of the soil microcosm.
  2.7.2. Talent shakes the column.
  2.7.3. Talent places the large cap onto the column, below the mesh.
  2.7.4. Shot of the column being placed in a 50 ml tube.

2.8. Transfer the column to a sterile environment such as a biological safety cabinet **[1].** Remove the bait media large cap, the threaded ring, and the mesh **[2].**
  2.8.1. Talent places the column in a biological safety cabinet.
  2.8.2. Shot of the bait media large cap, the threaded ring and the mesh being removed.

2.9. Then remove the small cap from the column end containing the target medium **[1]**. Use sterilized forceps to extract the plug **[2-TXT]**.
  2.9.1. Shot of the small cap being removed from the end containing the target medium.
  2.9.2. Shot of the plug being removed with sterilized forceps. **TXT: Alternatively, flip the column to let the target plug fall out**

2.10. Transfer the target medium plug directly onto the center of a 90-millimeter Petri dish containing agar medium **[1].**
  2.10.1. Shot of the target medium plug being placed on the center of a 90 mm Petri dish with agar medium.

2.11. To extract DNA *(D-N-A)*, first freeze the entire agar plugs or the selected pieces from the columns in 1.5-milliliter centrifuge tubes at minus 20 degrees Celsius **[1].** Alternatively, submerge the plug pieces in a preservative contained in a 1.5-milliliter centrifuge tube prior to extraction **[2].**
  2.11.1. Shot of frozen agar plug in 1.5 mL centrifuge tubes.
  2.11.2. Talent transfers plug pieces into a 1.5 mL centrifuge tube containing a preservative solution.

2.12. Carve out a 1-centimeter piece of agar containing microbial growth from the cultured target or bait media **[1]**.
  2.12.1. Talent cuts out a 1 cm piece of agar from plates containing cultures from the target or bait media.

2.13. For extractions of isolated bacterial colonies, use a sterile inoculation loop to swipe a colony from the plate **[1].** Swirl the loop directly in the commercially available DNA extraction buffer **[2]**.
  2.13.1. Talent swipes a sterile inoculation loop over a plate containing isolated bacterial colonies.
  2.13.2. Shot of the loop being transferred directly into a labelled tube with DNA extraction buffer.

2.14. For extractions of isolated fungi, use a mortar and pestle to grind the agar pieces separately in liquid nitrogen **[1].** Transfer the ground samples into extraction tubes **[2]**.
  2.14.1. Talent grinds the sample agar pieces in liquid nitrogen with a mortar and pestle.
  2.14.2. Shot of the ground samples being added into extraction tubes.

2.15. Using a commercial DNA extraction kit optimized for bacteria and fungi, extract the DNA **[1].** Then use a fluorometer to quantify the resulting DNA **[2].**
  2.15.1. Talent holds a tube with extracted DNA.
  2.15.2. Talent transfers the DNA sample into a fluorometer.

Results

3. Results

3.1. Microbial growth was visible on both the bait and target media plugs after the fungal highway columns were removed from the substrate and disassembled **[1].** Bacteria and fungi were isolated from the target and bait media *via* subculturing techniques **[2].** The microbes present on the media plugs were taxonomically identified **[3].**

3.1.1. LAB MEDIA: Figure 4 A

3.1.2. LAB MEDIA: Figure 4 B

3.1.3. LAB MEDIA: Figure 4 C and D

3.2. No recovery of colonized microbes was found when the columns were added to extremely low humidity environments **[1].** In some cases, microbes simply did not grow from the target media plug **[2],** or an overgrowth of fungus was seen through the column top **[3].**

3.2.1. LAB MEDIA: Figure 5 A

3.2.2. LAB MEDIA: Figure 5 B

3.2.3. LAB MEDIA: Figure 5 C

Pronunciation Guides:

1. Sodium Carboxymethyl Cellulose

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/sodium%20carboxymethyl%20cellulose
- **IPA**: /ˌsoʊdiəm ˌkɑːrbɒksiˌmɛθəl ˈsɛljəˌloʊs/
- **Phonetic Spelling**: SOH-dee-uhm kar-BOX-ee-meth-uhl SELL-yuh-lohs

2. Malt Extract

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/malt%20extract
- **IPA**: /mɔːlt ˈɛkstrækt/
- **Phonetic Spelling**: mawlt EK-strakt

3. Agar

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/agar
- **IPA**: /ˈeɪˌgɑːr/

- **Phonetic Spelling**: AY-gar

4. Petri Dish

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/petri%20dish
- **IPA**: /ˈpiːtri ˌdɪʃ/
- **Phonetic Spelling**: PEE-tree dish

5. Microcosm

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/microcosm
- **IPA**: /ˈmaɪkrəˌkɒzəm/
- **Phonetic Spelling**: MY-kruh-kah-zuhm

6. Centrifuge

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/centrifuge
- **IPA**: /ˈsɛntrəˌfjuːdʒ/
- **Phonetic Spelling**: SEN-truh-fyooj

7. Fluorometer

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/fluorometer
- **IPA**: /flʊəˈrɒmɪtər/
- **Phonetic Spelling**: floo-RAH-muh-ter