EXPERIMENT 13

Screening Fungi for Antibacterial Activity

INTRODUCTION

As bacteria and fungal pathogens become increasingly resistant to current drugs, there is a growing demand for novel antibacterial and antifungal therapeutic compounds. Natural compounds especially from fungi are considered a crucial source for new compounds with bioactive properties. Bioactive compounds from fungi have always been a vital component in microbiology research. Since the discovery of penicillin by Fleming in the 1920s, we have understood the importance of fungi-derived beneficial drugs.
Fungi are especially of interest for **bioactive compounds** since they are abundant in the environment, have high diversity, and are rich in secondary metabolites. To this day numerous fungal bioactive compounds have been extracted from marine fungi with cytotoxic, anticancer, antiviral, antibacterial, and antifungal properties. Common species of fungi that produce potent secondary metabolites include *Aspergillus, Penicillium, Fusarium,* and *Acrimonies.*

To discover and produce new antibiotics, scientists often employ the process of screening. **Screening** involves using selective procedures that allow the detection of microorganisms that produce the desired metabolite(s). Screening is often interchangeable with the methods of **bioprospecting**, which involves the search for microorganisms (in this case) from which valuable therapeutic drugs may be obtained for commercial purposes. Screening of microorganisms is often tedious since a large number of microorganisms have to be screened in order to find a potentially vital one. Nonetheless, it is still the most important step in the discovery of new drugs in order to eliminate the unwanted strains and characterize new compounds.

In this experiment, you will be screening various strains of fungi for the ability to produce antibacterial compounds. In order to verify a fungus for its antibacterial properties, we will use the agar diffusion method as we have seen previously in screening various antibiotics in Experiment 5. The agar diffusion method differs however because first the fungus of question is grown onto a selective medium.

When enough growth is established, a small piece of agar containing the inoculated fungi is cut out and placed onto an agar plate with a lawn of the bacterium in question. If the fungus is producing any antibiotic compounds, the area around the agar piece will have a **zone of inhibition** and inhibit the growth of bacteria. If the fungi produces a zone of inhibition, the bacteria is said to be susceptible to the compounds present in the fungi.

Materials:

1. **(4) Four fungal strains or source of fungi**
2. **(4) Sabouraud agar plate**
3. **Flammable metal forceps**
4. **Loop**
5. **Glass Pasteur Pipette**
6. **(4) Four Nutrient Agar plate per group (WEEK 2)**
7. **Bacteria samples (WEEK 2)**

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Procedures for WEEK 1: (Optional)

Part I. Isolation of Fungi

1. Obtain 4 Sabouraud agar plates.
2. **Label** each Sabouraud plate with its respective fungal source.
3. Using a sterile loop, take a sample of the mold by rubbing the loop sufficiently onto the fungus to remove enough material.
4. With the loop, streak for isolation onto one of the Sabouraud agar plates (Practice Figure 9).
5. Repeat steps 3 and 4 for the remaining three fungal sources.
6. Incubate the plates at 30°C for 48 hours.

Procedures for WEEK 2:

Part I. Fungi Antibacterial Assay

1. Obtain your four Sabouraud agar plates with the inoculated fungi and your large sterile nutrient agar plates.

2. **Label** each of your nutrient agar plates with its respective Fungi and bacteria.

3. Inoculate all of the nutrient agar plates with the bacterium provided and spread it and create a bacterial “lawn” as in the Practice Figure 10.

4. With a sterile glass Pasteur pipette, begin by gently forcing the rounded edge of the pipette into one of the Sabouraud Agar plates. Make sure to place pipette over a single colony of your fungi.

5. With a sterile forceps, pick up the circular agar piece and place this small piece onto the center of the nutrient agar plate.

6. Gently press the circular agar piece with the fungal growth firmly onto the agar without damaging specimen.

7. Repeat steps 5 and 6 for the other nutrient agar plates with the other fungal strains.

8. Incubate these plates at room temperature for 2-3 days.

9. Next lab you will observe and measure (mm) the zone of inhibition (if any) produced on the nutrient agar plates.

Part I. Antibacterial Activity

Directions: Record the size of the zones of inhibition (if any) in mm produced by any fungal strains corresponding to the correct bacteria.
(5 points)

Fungi	**Bacteria 1**	**Bacteria 2**
Aspergillus		
Penicillium		
Fusarium		
Unknown		
	From Group 1	From Group 2

Part II. Questions

Directions: Answer the following questions in full sentences. Use online searches to assist in answering any questions. **(5 points)**

1. Was a bacterium more susceptible to a certain fungi? Why do you suppose so?

2. How would you determine if the antimicrobial agent produced by the fungus is bacteriostatic or bactericidal?

3. What would be the next step after discovery of a large zone of inhibition by a specific fungus? **(2 points)**

4. Are there any medical or commercial applications of your fungi? If so, what are they?