

Identifying Antibiotic Producing Microorganisms from Soil

[a lesson plan for a classroom activity]

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Activity Appropriate for a classroom demonstration or hands-on activity

Abstract

Many soil microorganisms produce anti-bacterial or anti-fungal chemicals, compounds which are termed antibiotics. It is relatively simple to identify antibiotic producing microorganisms from isolated soil bacteria or fungi by testing them against standard microbial strains on Petri dishes containing a nutrient medium. In this activity antibiotic microorganisms will be identified by their ability to produce compounds which inhibit the growth of a common bacterium, *Micrococcus*.

National Science Education Standards Addressed: B & D

Keywords

Antibiotics, microorganisms, fungi, bacteria, soil

Intended Audience: grades 5-8 or 9-12

ACTIVITY

Learning Objectives

This activity is intended to demonstrate that common microorganisms isolated from soils are sources of antibiotics. A simple growth method can demonstrate how the effect of diffusible compounds from different microorganisms can be used to observe the inhibition of growth of a common bacterium associated with humans. This same activity scaled up many times is how the pharmaceutical industry discovers new antibiotics for human use.

Preparation Time

Preparation time will vary depending on whether you choose to prepare and sterilize materials yourself or to purchase pre-sterilized materials. Preparation of the nutrient media in Petri plates will require several hours and the use of an autoclave or pressure canner. If pre-sterilized media and supplies are purchased, preparation time should be less than one hour.

Learning Time

This activity will typically require _ to 1 hour in each of three classroom sessions, several days apart.

Background

Soil microorganisms routinely produce antibiotics compounds through a chemical process called secondary metabolism. It is believed that antibiotic production by soil microorganisms provides them the opportunity to compete with other microorganisms. In this activity students will screen a selection of microbial isolates from soil and test them for antibiotic activity against a microorganism commonly occurring on humans.

Materials

1. Disposable inoculating loops, 10 μ L – 5 to 10 per student or team [metal inoculating loops may also be used, but must be flamed to sterilize just prior to use, as well as after]
2. Petri dishes containing Mueller-Hinton agar – 2 or 3 per student or team
3. A microbial culture of *Micrococcus luteus* [ATCC 381] – living cultures in Petri dishes so that students may inoculate their Petri dishes
4. Microbial cultures of bacteria or fungi isolated from a natural soil – 5 to 8 different living cultures so that students may inoculate their Petri dishes

Procedure – Instructor Version

1. Prepare 150 mL of Mueller-Hinton agar for each student or team (e.g., for 10 students or teams you will need 1.5 L of the medium). To autoclave the agar-based media you generally must not fill the containers (flasks or bottles) more than half to two-thirds full. Cover the flasks or bottles with loose-fitting caps or a square of aluminum foil wrapped over the top.
2. Sterilize the agar-based media by autoclaving for 15 minutes at 121 °C [a home pressure canner may be used for this step provided that you use the 15 p.s.i. setting on the pressure regulator]. Allow the pressure in the autoclave or pressure canner to decrease slowly to prevent the contents from the flasks and tubes from boiling over.
3. After autoclaving, allow the agar-based media to cool slightly. Aseptically dispense 25-30 mL (or until each plate is about half full) of this warm media into each sterile Petri dish, taking care not to create bubbles as you pour. Allow to cool and solidify for 3 to 4 hours or overnight. If plates are not to be used within a day or so, place in plastic bags and store in a refrigerator. Plates may be stored without problems for several weeks to a month if poured aseptically.
4. Inoculate 4 or 5 plates of the Mueller-Hinton agar with the *Micrococcus luteus* bacterial culture and incubate at 28-37 °C for 24-48 hours. The inoculation of the plates may be done as typical quadrant streaking, or streaking as lawns over the entire surface of the plate. Alternatively, these starter cultures of *Micrococcus* may be generated in liquid cultures (Mueller-Hinton medium without agar) in test tubes. The preparation of these should be coordinated with the class schedule.
5. At the beginning of the class, be prepared to demonstrate aspects of sterile technique, which must be followed to prevent common microbial contamination from hands or non-sterile equipment. The first rule of sterile technique is not to touch with the hands any item which is intended to remain sterile, or allow the sterile item to touch any other object which is itself not sterile. This can sometimes be a difficult concept to teach students, who are frequently encouraged to touch and feel objects in science classes. Secondly, for laboratory equipment which must be held, there will be some part of it which cannot be touched to maintain sterile technique. For example, a sterile inoculating loop may be held by the handle but not be touched or be allowed to touch anything at the loop end. Because this is initially a foreign concept to grasp, you might have students practice prior to beginning the activity and correct any problems that are observed.

Procedure – Student Version [the text below can be copied and made into a handout, if desired]

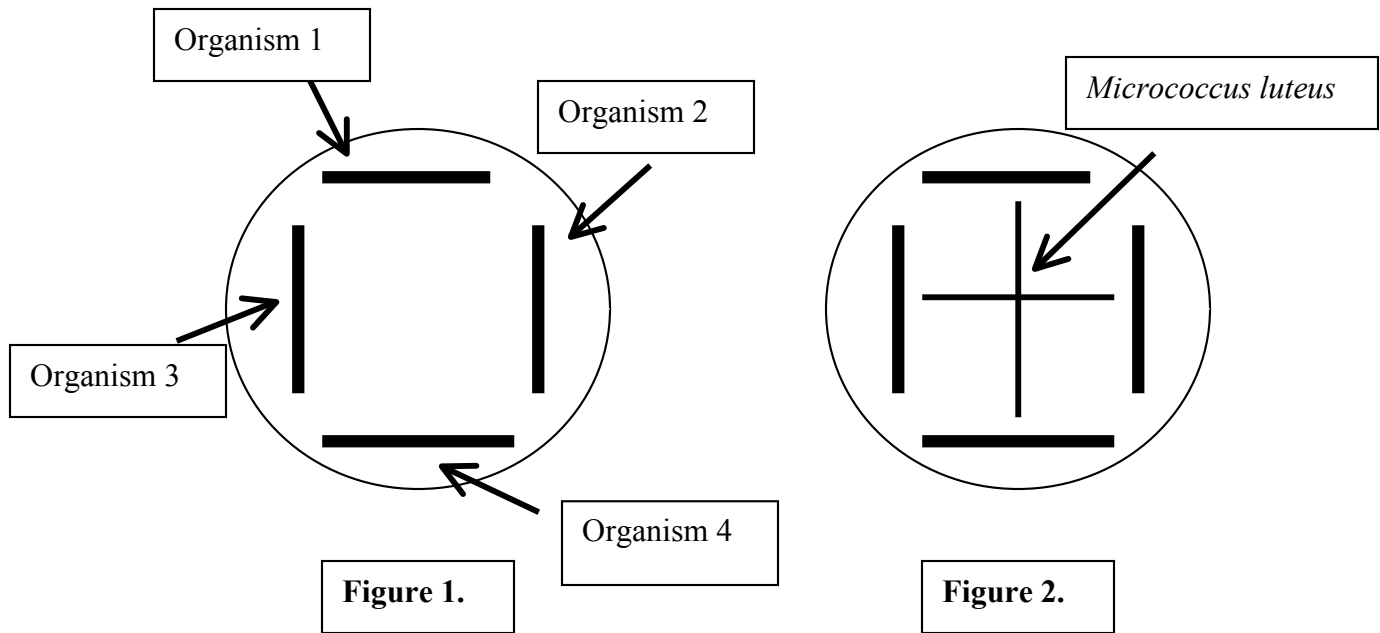
In this laboratory activity, you will be testing for the production of antibiotic compounds by common soil microorganisms. These isolated microorganisms might have been isolated by you in a previous activity, or supplied by your instructor. Soil microorganisms are the primary source of

most of the antibiotics used in human and animal therapy, and they were discovered in much the same way that you will be testing your microorganisms in this activity.

Petri dishes containing a microbial growth medium, Mueller-Hinton agar, will be available to you. Using a sterile transfer loop, rub the loop across the colony of a microorganism you wish to test. Then rub the loop across one edge of the surface of the growth medium in one of the Petri dishes. Next using a new sterile transfer loop, transfer some of the material from a different microorganism colony to the opposite edge of the surface of the medium in the same plate. Repeat another two times with additional microorganisms so that you have made a pattern on the surface of the medium in the Petri dish like that shown in Figure 1. Be careful not to overlap any of the lines in the pattern on this inoculated Petri dish. If you are testing more than four microbial isolates, repeat the procedure again using another Petri dish containing growth medium.

Invert the Petri dish(es) and place inside a zipper-lock storage bag. Incubate the bag at a temperature between 25-30 °C for 3-4 days.

With a sterile loop, transfer a small amount of the microbial material from a colony of *Micrococcus luteus* into the center of the inoculated and incubated Petri dish(es) in such a way that you make a cross perpendicular to the existing growth pattern observed as shown in Figure 2. Again, be careful not to overlap any of the lines in the pattern on the medium.



Invert the Petri dish(es) and place inside a zipper-lock storage bag. Incubate the bag at a temperature between 25-30 °C for an additional 2-3 days.

After the incubation period is complete observe the plate for growth of the microorganisms. Make notes about the quantity of growth of the *Micrococcus luteus* and its proximity to the soil isolates. Make notes also on the color, texture and quantity of growth of the soil isolates themselves. Are there any pigments being produced by the soil isolates which appears to discolor the growth medium? Does it appear that the pigments are inhibiting the growth of the *Micrococcus*?

Safety Issues

In most areas of the United States soils do not contain appreciable numbers of pathogenic (disease-producing) microorganisms and therefore you should be able to conduct this activity with uncharacterized soil isolates without major concern for exposure to pathogens. However, in some areas of the United States, for example, the mid-west and the arid southwest, the soils contain some significant fungal or viral pathogens and care should be taken to minimize direct exposure to the soil, soil dilutions or isolated microbial cultures.

Micrococcus luteus is a gram-positive bacterium commonly associated with humans. The strain recommended for use in this activity is not pathogenic. However, students should be advised to follow good hygienic practices when working with any type of microorganism and wash their hands frequently during and after undertaking this activity.

The wearing of safety glasses, lab coats and gloves while conducting this activity may be appropriate.

Assessment

Students of this age group should be able to follow directions and complete the activity without significant supervision. Bioactivity of microorganisms against a gram-positive microorganism like *Micrococcus* is relatively common, especially if some of the isolates tested are fungi or actinomycetes. Therefore students should be able to identify a number of soil microorganisms producing some type of antibiotic. The students should be able to write a summary report indicating what tasks they performed and what the results were.

SUPPLEMENTARY INFORMATION

Possible Modifications

If a set of positive control microorganisms is desired to demonstrate the production of antibiotics, the American Type Culture Collection (www.atcc.org) can provide a number of fungi or actinomycetes which are known to produce common antibiotics (tetracycline, streptomycin, etc.). Living cultures of these can be used to supplement the strains isolated from natural soils.

References and Resources

1. Textbooks
2. Internet websites
 - a. Cubist Pharmaceuticals, Inc. (www.cubist.com): Cubist conducts isolation of microorganisms for antibiotic discovery and information on microorganisms and their activities can be found here.

- b. American Society for Microbiology (www.asm.org): a general resource for microbiology information
- c. Society for Industrial Microbiology (www.simhq.org): check out the kid's zone and the Resources and Links for additional information
- d. VWR Corporation (www.vwr.com): a supplier of laboratory supplies and sterile items
- e. Fisher Scientific (www.fishersci.com): a supplier of laboratory supplies and sterile items

Appendices

1. Recipe for Mueller-Hinton agar (this is typically purchased as a commercially prepared dry powder, but can be prepared from its primary components)

Beef Extract	2	g/L
Acid Casein hydrolysate	17.5	g/L
Starch	1.5	g/L
Agar	17.0	g/L

pH 7.3