

Antibiotic Producing Bacteria and Fungi in Soil Samples

Summary: Students use prior microbiological skills to create a lawn, or carpet, of *S. epidermidis* on Petri dishes. They then sprinkle the plate lightly with soil they have gathered from home. Antibiotic-producing microbes can be found by the zones of inhibition, areas where the *S. epidermidis* cannot grow, surrounding their colonies.

Grade Levels: 9-12

Prior Knowledge: Students should have completed Labs 1 and 2 in this series, or an equivalent set of labs. Students should have an understanding of what antibiotics are, how they work and what type of organism they are effective against.

Materials: In all labs in this series, the agar tryptic soy agar (TSA) is used. This is only one of several that are suitable. Others, such as nutrient agar or LB agar will also work. All students should practice the skills found in this lab, though material can be shared between pairs or groups of three students. More than three to a group means too much down time for students and the lab will not be finished in a reasonable amount of time. All of the following quantities are for pairs of students, unless otherwise noted.

45 TSA plates (3 TSA plates/group)

15 sterile swabs

15 fine tipped marking pen

15 bent glass rods

15 dropper bottles of 70% isopropanol (rubbing alcohol)

15 Bunsen burners

soap and matches

1 each per lab table: nutrient broth culture of *S. epidermidis*.

biohazard bag, parafilm and scissors (one set for the class)

Teacher Instructions:

- Students groups need to have met before this lab takes place to discuss where to get their soil samples. They should be directed to considered places where there would be a lot of competition, from a microbe's point of view. Students only need about a spoonful of each type of soil brought to school in a sealed zip-lock plastic bag. If working in pairs, each student should bring in one soil sample. You may wish to provide the zip-lock bags, if you feel the students need them.
- You may wish to refer students to Part 2 of Lab #1. They will be substituting a cotton swab for the loop, but they will be using the bent glass rod to create a lawn of bacteria. You may wish to modify the lab and simply use the cotton swab to

spread the bacteria. Complete coverage of bacteria over the entire plate is essential, though.

- This lab was written for using 60mm Petri dishes. If you prefer 100 mm dishes, you may wish to modify step 2 of the procedure by having the students divide the Petri dish into quadrants on the bottom of the dish. Number the quadrants 1 through 4 and use three soil samples instead of two. Reduce the amount of soil to 10-20 particles per quadrant and skip step 5. Students will have to be very careful when putting the Parafilm on the dish so the soil particles don't roll into a neighboring quadrant.
- Always have students dispose of their Petri dishes in the biohazard bag.

Correlations to State and National Standards:

- **Colorado State Standard 3:** Life Science-- Students know and understand the characteristics and structure of living things, the processes of life, and how living things interact with each other and their environment.
- **Colorado State Standard 5:** Life Science-- Students know and understand interrelationships among science, technology, and human activity and how they can affect the world.
- **Colorado State Standard 6:** Life Science--Students understand that science involves a particular way of knowing and understand common connections among scientific disciplines.
- **National Content Standard C (Life Science):** As a result of their activities in grades 9-12, all students should develop understanding of the cell; the molecular basis of heredity; biological evolution; interdependence of organisms, matter, energy, and organization in living systems; and behavior of organisms.

Materials Price List/Ordering Information:

Carolina 1-800-334-5551 www.carolina.com

Item	Ordering Number	Price
Bacterial Spreader	BA-21-5820	\$2.70 each
Inoculation Loop	BA-21-5826	\$1.80 each
Staphylococcus epidermidis	BA-15-5556	\$7.50/tube
TSA plates (100 x 15 mm)	BA-82-2022	\$15.25/pack of 10 \$13.25/10+ packs
TSA media tubes	BA-82-7322	\$13.75/pack of 10 \$12.60/10+ packs
TSA Dehydrated media	BA-78-8420	\$17.95/100 g
Biohazard Bags 12 x 24 in.	BA-64-7051	\$36/100 bags
Parafilm 2 in. x 250 ft.	BA-71-3044	\$17.95 each
Sterile Cotton Tip applicators	BA-70-3033	\$19/box of 200

Teacher Note: Isopropyl (rubbing) alcohol can be purchased at any grocery store; Q-tips (sterile in the box, or can be sterilized in a test tube, stoppered by nonabsorbent cotton) could be exchanged for the Cotton Tip applicators; glass pipettes or stirring rods can be melted into a hook shape by a Bunsen burner. 60 x 15 mm Standard petri dishes (500/case) can be purchased through Life Science Products (1-800-245-5774) for \$48.70.

Correlation to Confronting the Microbe Menace:

Cross reference information given on bacteria and antibiotics found on the **DVD 2000 and Beyond Confronting the Microbe Menace** with lab 5.

General Information on Bacteria	T5C5	07:45
Size: Analogy one, ping pong ball	T5C5	07:51
Analogy two, ruler	T5C5	08:11
Gram Stain Identification	T5C6	08:55
Chart: Bacteria Are Everywhere and Numerous	T5C7	09:45
Slide: Normal Flora of the Mouth Bacteria Gram Strained	T5C9	12:11
Video: Bacteria E.Coli, show actual reproduction	T5C10	13:25
Chart: Some Bacterial cause Disease (Sometimes)	T5C14	16:52
Chart: Examples of Bacterial Diseases	T5C16	19:30
Chart: Infectious Agents Are Easily Spread	T5C18	21:50
Combat Infectious Diseases		
Chart: How to combat infectious Disease	T5C20	24:21
Chart: Antibiotics	T5C21	25:18
Chart: Antibiotic Mechanisms	T5C23	27:02
Picture E. Coli on a plate Disk Diffusion Kirby-Bauer	T5C24	27:25
Video E. Coli being lysis	T5C25	27:54
Chart: Antibiotics	T5C26	28:39
Picture: E. Coli on plate that is antibiotic resistant.	T5C27	29:30
Chart: Antibiotic Resistance	T5C38	29:38
Demonstration of Super Bug (Antibiotic Resistant)	T5C29	31:45
Chart: Antibiotic Resistance (Super Bug)	T5C29	32:09
Video: Conjugation	T5C31	34:52
Chart: Shelf Life of New Antibiotic	T5C32	35:29

Lab #5: Antibiotic Producing Bacteria and Fungi

Introduction:

The term “antibiotic” literally means “against life”. In our every day usage, however, we use the word to describe a set of chemicals that inhibit or kill bacteria. The British scientist Alexander Fleming is credited with being the first to notice that another organism could inhibit bacterial growth in 1928. He noticed that growth of the bacterium *Staphylococcus aureus* was inhibited by a mold (fungus) that had contaminated his plate. The mold was later identified as *Penicillium notatum* and the antibiotic, isolated a short time later, was named penicillin.

The value of penicillin was immediately recognized, but it wasn't until 1940 before the first clinical trials of penicillin were tried on humans. The reason for the long delay was because of the difficulty in producing large enough quantities of pure penicillin. While research was progressing World War Two reached Britain and the entire research project was given the highest priority and moved to the United States for safety. This was before the time of genetic engineering; so one of the aspects of the research project was to find a mutant strain of *Penicillium* that would produce massive amounts of penicillin. *Penicillium* mold was collected wherever it was found and cultured to examine its antibiotic producing potential. Finally, a high-producing strain was found growing on a cantaloupe in a market in Peoria, Illinois. This strain finally enabled large-scale production of the antibiotic penicillin.

Its impact was immediately appreciated. Largely because of penicillin, World War Two was the first war in history where more soldiers died from wounds than from disease. Penicillin was described as a wonder drug and it was widely believed that infectious disease would never again be a dominant issue for mankind. Those who understood the mechanisms and potential of evolution should have known better. Since then, we have seen numerous antibiotics discovered or developed and we have also seen bacteria become resistant to many of them. Several diseases, once thought to be controlled are re-emerging as potent public health hazards because of antibiotic resistance and a lax attitude toward the potential of infectious diseases. It has gotten to the point where many scientists and medical personnel fear we are teetering on the brink of disaster and infectious disease may once again inhibit our ability to enjoy life.

Problem: Describe the purpose in doing this lab and then identify the experimental and dependent variables.

Hypothesis: State your expected outcome for this lab and then describe how and why the experimental variable will affect the dependent variable.

Materials:

Broth culture of <i>S. epidermidis</i>		3 plates of TSA
Sterile cotton swab		fine tipped marking pen
Soap	Parafilm	Bunsen burner
matches	70% isopropanol	bent glass rod

Safety:

1. A microbiology lab is potentially a very dangerous place. For this reason it is extremely important that you follow all safety guidelines and always practice sterile technique when handling microbes, unless instructed otherwise.
2. There should be no books or papers at your workstation except this lab packet.
3. Never have any food or drink at your workstation.
4. Always thoroughly wash your hands with disinfectant soap or alcohol before leaving your workstation.
5. Never open a Petri dish after you have inoculated it and allowed it to incubate overnight.
6. Always dispose of used material in the biohazard bag, unless instructed otherwise.

Procedure:

1. Clean your work area as you did in Lab #1.
2. Swirl the contents of the broth culture of *S. epidermidis* until it is equally murky throughout. Use the sterile cotton swab and glass rod to create a lawn, or carpet,

of *S. epidermidis* on all of the plates of TSA as you did in Part 2 of Lab #1.

(Don't forget to hold the cap in your pinky and to flame the mouth of the test tube before and after dipping the sterile swab into it).

3. Label the bottom of the petri dish with your name, the date and the location where the soil sample was collected. Label the third Petri dish "control". The control will contain only a lawn of *S. epidermis* (i.e., **NO soil!**).
4. Sprinkle about 20-30 particles of soil on the surface of the agar (less is better than more). Wrap the edges of the dish with Parafilm.
5. Gently shake the Petri dish to have the soil particles touch a variety of locations on the agar. If the lid has moisture on it, be careful not to shake so much that the soil sticks to the lid. Before finishing, agitate the plate back and forth horizontally to get the soil particles as evenly distributed as possible on the surface of the agar.
6. Place the plate in the incubator at 30°C lid-side up so that the soil doesn't fall off of the agar.
7. After 24 hours in the incubator, check for the presence of antibiotic activity. This is done by looking for a clear area, called a zone of inhibition, surrounding a colony. Remember to never open the dish for a better view. To determine the affectivity of an antibiotic, measure the distance, in millimeters, from the edge of a colony secreting an antibiotic to the edge of the zone of inhibition.

Results:

Source of Soil	Colony Morphology	# of Colonies with Similar Morphology	Zone of Inhibition (mm)

Analysis/Conclusion:

Use class data to answer the following questions.

1. What was the source of the soil with the greatest diversity of organisms producing antibiotics?
2. What was the source of the soil with the highest population of organisms producing antibiotics?
3. Use class data to evaluate your hypothesis.

Answers to analysis/conclusion questions:

1. What was the source of the soil with the greatest diversity of organisms producing antibiotics?

Answers will vary depending on class data.

2. What was the source of the soil with the highest population of organisms producing antibiotics?

Answers will vary depending on class data.

3. Use class data to evaluate your hypothesis.

Answers will vary depending on class data.

4. Why do organisms produce antibiotics?

Organisms produce antibiotics to compete for a particular niche and to survive. For example, Ascomycetes (Penicillium) produces penicillin. Penicillin kills bacteria by interfering with the enzyme that links sugar chains in the cell wall. Bacteria growing in penicillin develop holes in their cell walls. As a result, when water enters a cell by osmosis, the bacterium ruptures and dies. Note that the penicillin is effective only against bacteria cell walls due to the peptidoglycan (protein-sugar complex) that is a major component of the cell wall. Plant and fungi have different cell wall structures.

5. What was the role of the control plate in this experiment?

The control plate should contain only S. epidermis growth on it. If there are any zones of inhibition, then an antibiotic is being

produced by the test subject and not any organisms in the soil. The control plate also gives one a plate to compare against.

6. Offer suggestions why numbers one and two gave the results they did. Be sure to support your answers with rationale.

Answers will vary depending on results.

7. Why are antibiotics affective only against bacteria?

Antibiotics are affective only against bacteria because they attack the unique peptidoglycan cell wall or smaller ribosomal unit of the bacteria. Viruses and eukaryotic cells either lack a cell wall or have a different cell wall structure and composition. Additionally, eukaryotic cells have larger ribosomal units, which aren't affected by antibiotics in the same manner as bacterial ribosomes are.

8. Do you think that any of these antibiotics inhibit bacterial growth as well as commercially available antibiotics? Explain.

Answers will vary depending on results; however, commercially prepared antibiotics usually are more concentrated than those produced by living organisms.

