“BUGS" THAT PRODUCE DRUGS TO KILL "BUGS”
Microbes Produce Antibiotics

Science in the Real World
Microbes In Action

“BUGS" THAT PRODUCE DRUGS TO KILL "BUGS” is a curriculum unit developed as part of the *Science In The Real World: Microbes In Action Program*. The curriculum units were developed with support from the National Science Foundation, The Coordinating Board for Higher Education, Sigma Chemical Company, Pfizer Foundation and the Foundation for Microbiology.

Don Cohn & Elmer Kellmann
Developer of Curriculum Unit

Teresa Thiel, Ph. D.
University of Missouri- St. Louis
Program Director & Microbiologist

Victoria L. May, M.A.T.
Science Education Resource Center
Co- Director & Curriculum Specialist

Mark R. Kalk, M.S.
Science Education Resource Center
Lab Supervisor & Technical Specialist

Sandra Alters, Ph. D.
Brian Alters, Ph. D.
Program Evaluators

Kimber Mallet
Illustrator

Judith O’ Brien, Ph. D.
Ralston Purina
Industrial Consultant

Bruce C. Hemming, Ph. D.
Sigma Chemical Company
Industrial Consultant

Alastair Pringle, Ph. D.
Anheuser- Busch
Industrial Consultant

Robert Reynolds, Ph. D
Sigma Chemical Company
Industrial Consultant

David Corbin, Ph. D.
Monsanto
Industrial Consultant

TABLE OF CONTENTS

"BUGS" THAT PRODUCE DRUGS TO KILL "BUGS"

AT A GLANCE

Description:

This exercise will require three non-consecutive days for the students. The students will demonstrate how certain microorganisms produce antibiotics that inhibit the growth of other microorganisms. On the first day of the lab, students will streak an antibiotic-producing microbe onto a petri dish. After these incubate for 4 to 7 days, the students will streak 3 types of bacteria onto the dishes with the producer to see if the growth of these bacteria is inhibited near the producer. Results are observed on the third day. Some concepts addressed in this lab include adaptations that provide an advantage in competing for food, differences in sensitivity to antibiotics, and the use of sterile techniques in working with bacteria. It also demonstrates for students one way in which microorganisms are beneficial to human beings.

Time Requirements:

The students spend three lab periods on this exercise. The first two lab days are spaced 4 to 7 days apart. During the last lab period, students record and analyze their results. The actual activities do not require a full period — there is time for discussion and/or student work on questions and analysis of results each day.

Curriculum Placement:

This exercise can be used in any general biology course in conjunction with the following topics: an introduction to bacteria; competition among organisms and adaptations that enhance success in this competition; introduction to techniques involved in growing bacteria; biotechnology; human health and disease.

Equipment:

autoclave or pressure cooker
incubator at 32° C
bunsen burner
inoculating loop

Materials:

30 petri dishes (sterile, plastic)
nutrient agar to make 30 petri dishes
90 sterile applicators (swabs)
10 marking pens (1 per group)
3 sterile tubes with sterile water
one culture each of:
- *Escherichia coli*
- *Penicillium notatum*
- *Streptomyces griseus*
- *Bacillus subtilis*
- *Micrococcus luteus*

1 bio-hazard autoclave bag (optional)

Time Line for Antibiotic Lab:

- Order cultures of microorganisms so that they arrive one to two weeks before the lab.
- Three to five days before the lab: Teacher streaks at least two petri dishes per class, one with *Penicillium notatum* and one with *Streptomyces griseus.*
- At least one day before the lab: The teacher sterilizes nutrient agar and prepares petri dishes (one per student).
- Day one of lab: Students streak either *Penicillium notatum* or *Streptomyces griseus* (or no antibiotic producer as a control) onto their petri dishes.
- Day two of lab (5 to 7 days later): Teacher will prepare a suspension of each of the bacterial cultures (three) that the students will be using. Students will streak these three bacterial suspensions onto their dishes using sterile applicators.
- Day three of lab (1 to 2 days after day two): Students observe results and collect class data. Work on questions as time permits.

PREPARATIONS

I. Materials:

Per class
One culture each of:
Penicillium notatum
Streptomyces griseus
Escherichia col'i
Bacillus subtilis
Micrococcus luteus
3 sterile tubes of water (about 5 ml each) for each station (lab can be done with one or several stations)
test tube racks
5 petri dishes of nutrient agar (or more if several stations are going to be used)
2 bunsen burners
2 inoculating loops

For each group of 3 students

3 petri dishes containing nutrient agar
9 sterile applicators (swabs)

II. Prepare and Order Bacterial Cultures

Order the cultures you will need so they arrive at least one week before the lab (see list of supplies). When the cultures arrive, place them in the refrigerator. About 5 to7 days before the lab is scheduled, streak the antibiotic producers *P. notatum* and *S. griseus* onto petri dishes of nutrient agar (separate dishes for each). Generally, it is easier for students to work from a petri dish than a slant. You will need at least one dish of each antibiotic producer for each class (you can make more if you plan to have multiple stations for students to obtain these cultures). Incubate these dishes inverted at room temperature.

III. Preparing Nutrient Agar

When convenient, but at least one day before the lab, nutrient agar must be prepared, sterilized and poured into petri dishes. At the same time, you can sterilize at least 3 covered test tubes containing about 5 mL of water for each class. The number of test tubes needed, will depend on how many stations you want to set up in the classroom.

The recipe for most nutrient agar is to dissolve 23 grams of nutrient agar powder into 1 liter of distilled water (tap water will work.) Generally, figure on pouring 20-25 mL of nutrient agar into each dish. Prepare the agar in batches that only fill the flask half way to prevent boil-over. Once you have prepared the nutrient agar, cover the flasks with aluminum foil and place them in the autoclave/pressure cooker. Also, be sure to put your sample tubes of water in the sterilizer at the same time (caps should be on loosely).

Follow the instructions on your sterilizer to complete the sterilization process. Generally, you need 15 lbs of pressure and a temperature of 121° C for 15 minutes to achieve sterility.

Once the materials have been sterilized, the nutrient agar must be poured into the dishes before it solidifies. It is best to pour the agar when it has cooled enough to be held comfortably in your hand (45 - 50° C). Spread the plates out on the lab tables, lift the lid straight up and pour the agar into the dish. You may want to pour 20 ml of water into an empty dish to give you an idea of how much agar to pour into each dish. Once all of the dishes have been poured, let them set until they have solidified. Store the plates upside down until you are going to use them. If it is going to be several days until you use the plates, put them back into the plastic sleeve in which they where shipped and store them upside down in a refrigerator or a cool place.

IV. Day One

On the first day of the lab, be sure to have the 3 petri dishes and a marking pen available to each group of three students. You will need at least 2 stations — one with a petri dish of *P. notatum* and another with *S. griseus*. Each station will require one bunsen burner and an inoculating loop. The lab will clearly go faster if more than one station is set up for each of the antibiotic producers, in which case you will need to have streaked more petri dishes of each producer for each class.

Divide the class into groups of three students. At this point they can follow the instructions on the student pages. After deciding within their group which student will prepare a dish of which producer (or a control dish with no producer), the students must label and mark their dishes (on the outside of the bottom of the dish) using the template provided. They will then go to the appropriate station to streak their antibiotic producer onto their petri dishes. The streaked dishes should be inverted and incubated for 5 to 7 days at room temperature.

V. Preparing Test Bacteria

The day before the students begin the second part of the lab, you need to prepare the test bacteria cultures as follows. Get the three slants (*E. coil, B. subtilis, M. luteus*) from the refrigerator and sterilely streak each one onto a petri dish of nutrient agar. Incubate these dishes overnight at 32 - 37° C. This will give you a supply of actively growing bacteria for the next day.

VI. Day Two

On the second day of the student lab (5 to 7 days after the first day), the teacher must make a liquid suspension of the 3 bacterial cultures that were streaked onto petri dishes the day before. You will need at least one tube of liquid suspension of each culture. If you want to set up multiple stations for each bacterial sample, then more tubes must be prepared. To make the liquid suspension, use the tubes of sterile water you prepared earlier. For each bacterial culture, flame the inoculating loop, touch the loop to the agar surface to let it cool, and then pull the loop through the bacteria growing on the petri dishes you streaked yesterday. Try to get a visible clump of bacteria on the loop. Then open the sterile tube of water, insert the loop and swirl to knock the clump of bacteria off the loop, and re-cover the tube. Flame the loop again to sterilize. Repeat this to make as many liquid suspensions of each bacterial culture as you will need (one per class minimum, but more if you choose to have several stations for each bacterial sample in the classroom). Mix the tubes of water containing the bacteria to disperse them. Note—the B. subtilis does not disperse in water, but if you mix thoroughly, enough bacteria will be suspended to work well.

Distribute the tubes with the bacterial suspensions to the various stations for student use. Each student will need their petri dish and 3 sterile applicators. Emphasize to the students the need to work quickly and carefully with each culture. Have them remove the cover from the tube, dip the applicator into the bacterial suspension, and re-cover the tube. The bacteria must then be carefully streaked onto the dishes following the lines drawn from the template on day one. They need to begin their streak as close to the central line of antibiotic producer as possible without touching it and then move the applicator tip away from the producer toward the edge of the dish. They then will take their dish to the next station to streak the second bacterial culture and will repeat the procedure until they have streaked all of the test bacteria. At each station, have a flask or beaker containing 10% bleach for the students to dispose of their used applicators. The student who is working with the control dish will streak the dish in the same manner

as the others in the group. The dishes should be collected, inverted and incubated at 32° C overnight (37° C should also work; room temperature will probably take longer).

VII. Day Three

On the final day of the lab, the students will observe the results. By carefully comparing the growth of the bacteria on the dishes with the producers to growth on the control dish, the students should see how the growth of some of the bacteria were inhibited near the line of the antibiotic producer. Note that not all the cultures are equally sensitive to the different antibiotics. When the students are finished with their dishes, collect them for disposal. They can either be put into a disposable, autoclavable bag (see supplies list) and autoclaved or the dishes can be opened and soaked for 15 - 30 minutes with 10% bleach solution to disinfect them. After either procedure the dishes can be disposed of in the regular trash in plastic garbage bags.

SOURCES OF SUPPLIES

Sigma Chemical Company
P.O. Box 14508
St. Louis, MO 63178

Description	Stock Number	Quantity	Cost
Nutrient Agar	N 0394	250 g	$29.00
		500 g	$59.00

Carolina
Main Office and Laboratories
2700 York Road
Burlington, NC 27215

Description	Stock Number	Quantity	Cost
Stehle applicators (fabric-tipped swabs)	F6-15-5065	box of 200	$16.00
Petri dishes	F6-74-1350	case of 500	$90.00
Cultures of microorganisms— ordered as tubes (slants)			
Escherichia coli	F6-15-5065		$7.00
Bacillus subtilis	F6-15-4921		$7.00

Micrococcus luteus	F6-15-5155	$7.00
Streptomyces griseus	F6-15-5705	$7.00
Penicillium notatum (high yield)	F6-15-6155)	$7.00

TEACHER BACKGROUND

Most students are familiar with antibiotics, having taken them at one time or another. However, they probably do not know that microorganisms produced those antibiotics. This activity will demonstrate to the students that some microorganisms (antibiotic producers) have the ability to inhibit the growth of some, but not all, bacteria. The two antibiotic producing microorganisms used in this exercise are *Penicillium notatum* (a fungus) and *Streptomyces griseus* (a bacterium), which produce penicillin and streptomycin, respectively.

Penicillin and streptomycin are narrow spectrum antibiotics. This means they do not affect the growth of a wide variety of bacteria, but only a few. There are numerous ways in which antibiotics work. Penicillin inhibits the development of bacterial cell walls, while streptomycin inhibits the bacterial ribosomes. In both cases, these antibiotics are fairly specific to prokaryotes, but streptomycin can affect protein synthesis in mitochondria and chloroplasts.
The commercial production of antibiotics is an excellent example of biotechnology. Over 100,000 tons of antibiotics are produced every year, and the value of this production has been estimated to be 5 billion dollars per year. The production of these antibiotics on an industrial scale requires large fermentation tanks and extensive processing for the pure antibiotics. Many antibiotics are semisynthetic—that is, the antibiotic produced by the microbe is chemically modified to enhance its effectiveness.

There is a constant search underway for new antibiotics (about 8000 are discovered each year, but few are useful). This is because resistant strains of baceria constantly appear due to random mutation and genetic exchanges between bacteria. The overuse of antibiotics in medicine and the use of antibiotics in animal feed has led to many species of bacteria that are no longer sensitive to the antibiotics that once were effective in controlling their growth. The overuse of antibiotics in medicine, the use of antibiotics in animal feed, and the evolution of antibiotic-resistant strains of bacteria could be good topics for student reports or projects.

TEACHER HINTS & TROUBLESHOOTING

1. It is important that the antibiotic producers have sufficient time to grow on the student dishes before the test bacteria are streaked on the dishes. During their growth, they will produce the antibiotic and it will diffuse into the surrounding agar. Usually the surface of the antibiotic producers becomes "fuzzy" when they have had sufficient time to grow. It also seems that *S. griseus* requires more time to grow and produce its antibiotic (streptomycin) than does the *P. notatum*.

2. *Penicillium notatum* is actually a fungus. That is the reason the terms microbe, microorganism, or producer is used in this lab rather than bacteria.

3. When transferring the two antibiotic producers from slants to petri dishes, or when the students streak the producers onto their dishes from the ones you prepared, the growths are very "hard". When the loop is drawn over the surface of the growth, it does not appear to pick anything up, but assure the students that it really does. They may be disconcerted by not having any visible material on the loop.

4. *Micrococcus luteus* is very sensitive to penicillin, and sometimes no growth of this bacterium can be detected on the *Penicillium* dish. The control dish should assure the students that the bacteria really present in the liquid suspension and that it will grow visibly in the absence of antibiotics.

ANSWER KEY

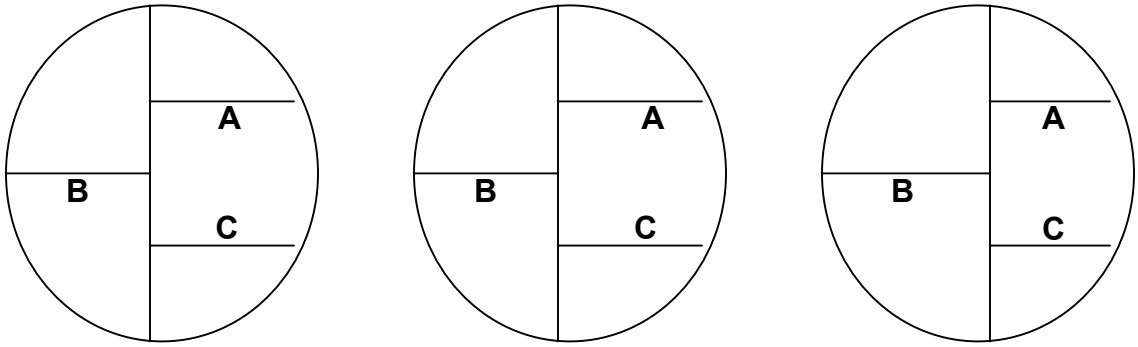
1. Record your predictions from Day 2 Procedure #4 in the space below.

   a. *Answers will vary, but students should predict that if the bacteria are sensitive to the antibiotic that they should not grow and form visible colonies.*

   b. *There will be various answers, but most students will recognize that the bacteria should grow normally and will form visible colonies on the dish.*

Results After Day 3:

2. Draw what your dish and your partners' dishes look like on the diagrams below.



Penicillium notatum Dish | *Streptomyces griseus* Dish | Control Dish

Diagrams will vary, but in general the students should see that P. notatum inhibits the growth of M. luteus and B. subtilis. M. luteus seems to be the most sensitive.
E. coli is not affected and should grow well right up to the producer streak.
S. griseus inhibits all three test bacteria, but E. coli is not nearly as sensitive as the other two.
On the control dish, all three bacteria should show good, even growth right up to the central line.

3. Bacteria A is *Micrococcus luteus*
Bacteria B is *Bacillus subtilis*
Bacteria C is *Escherichia coli*

Fill in Table A below for your group's results. When they are available, use data from the whole class to fill in Table B.
(+) means the antibiotic inhibits bacterial growth.
(-) means the antibiotic does not inhibit bacterial growth.

Table A — Group Results

Bacterial Culture Tested	*Penicillum* dish (penicillin)	*Streptomyces* dish (streptomycin)	Control dish (no antibiotic)
Micrococcus luteus (A)	+ +	+	-
Bacillus subtilis (B)	+ +	+	-
Escherichia coli (C)	-	+ (1/2)	-

Table B — Class Results

Bacterial Culture Tested	*Penicillum* dish (penicillin)	*Streptomyces* dish (streptomycin)	Control dish (no antibiotic)
Micrococcus luteus (A)	+ +	+	-
Bacillus subtilis (B)	+ +	+	-
Escherichia coli (C)	-	+ (1/2)	-

Analysis:

1. Dr. T. Mallard sent you bacteria culture C from one of her patients. In her notes to you, she states that she has started the patient on penicillin. What advice can you give Dr. Mallard? Explain your reasons for this advice.

   Penicillin is probably the wrong antibiotic for treating this infection. The experiment shows that bacteria C (E. coli) are not sensitive to penicillin. Streptomycin would probably be a better choice (although it is not extremely effective against E. coli either.

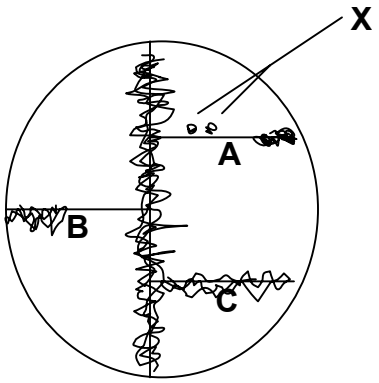
2. Culture B was isolated from some snacks at the Red Bird Pre-School. The effects of this bacterium is unknown. As a precaution, the hospital wants plenty of antibiotic on hand in case of an outbreak of infections due to culture B. What antibiotic would you recommend? Defend your recommendation.

   Students should recommend that either antibiotic could be used. Depending on the class results, one or the other may be judged to be the more effective.

3. What would the advantage to a microorganism for it to produce an antibiotic?

   Students may recognize that by secreting an antibiotic, the microbe reduces the ability of other microorganisms to grow nearby. This reduces competition for food and other resources.

4. You have performed another experiment very similar to the one you just completed. The results are shown below.



Which culture(s) are sensitive to penicillin? __Cultures A and B
Which culture(s) are resistant to penicillin? ___Culture C____

What could be some reasons for the appearance of the bacteria indicated by an "X"?

These could be a different type of bacteria that are contaminants on the dish, or some of the "A" bacteria could have been naturally resistant to the antibiotic.

5. What was the purpose of the control dish in the experiment you performed? (What do you learn from this dish? How would your analysis of the experiment have been different if you hadn't done the control, or if the control results had been different?)

   The control dish shows that all three of the test bacteria can grow on the agar. The control dish shows what the uninhibited growth of the bacteria looks like. It is used to compare with the growth of the bacteria on the experimental dishes (the ones with antibiotic producers).

Name_____

Date_____

"BUGS" THAT PRODUCE DRUGS TO KILL "BUGS"

BACKGROUND

Perhaps one of the most important medical advances of all time was the discovery of the antibiotic penicillin by Sir Alexander Fleming in 1928. Penicillin and the antibiotics discovered subsequently have been described as "miracle drugs", and the development of antibiotics to treat infectious diseases has had a tremendous impact on the practice of medicine. An antibiotic can be defined as a natural product produced by a particular microorganism that inhibits the growth of other microorganisms. For instance, penicillin is produced by the fungus, *Penicillium notatum*. Their usefulness is seen by the fact that over 100,000 tons of antibiotics are produced commercially each year.

In this lab exercise you will be working with two types of microbes that are known to produce antibiotics. *Penicillium notatum* is a fungus that produces penicillin, and the bacterium *Streptomyces griseus* produces streptomycin. As these microbes grow on the food in the petri dish, they produce the antibiotic which diffuses through the agar. Any bacteria that are sensitive to the antibiotic will not be able to grow near the microbes producing the antibiotic.

PURPOSE

1) To demonstrate the ability of some microbes to inhibit the growth of bacteria.
2) To show that not all bacteria are sensitive to particular antibiotics.

DAY ONE

You and your partners work in the lab of a major pharmaceutical company. You have been sent 3 samples of bacteria. Your assignment is to determine which of the bacteria are sensitive to penicillin and which are sensitive to streptomycin. Proceed carefully—someone's life may depend on your findings.

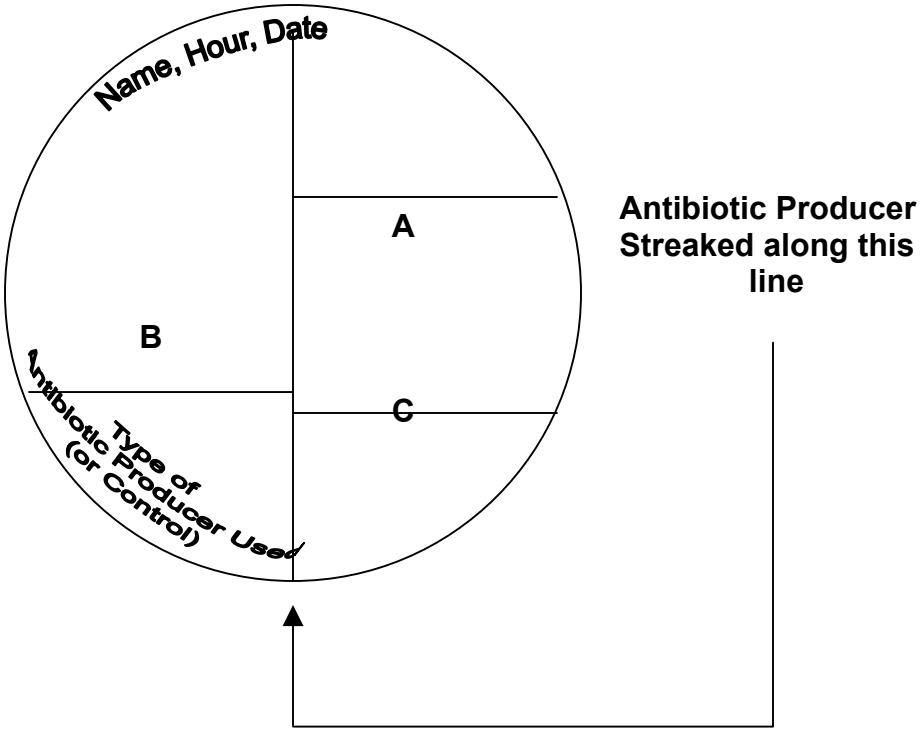
MATERIALS PER GROUP OF 3 STUDENTS:

3 Petri dishes with nutrient agar
1 marking pen
Access to: 1 culture of *Penicillium notatum*
1 culture of *Streptomyces griseus*
bunsen burner
inoculating loop

DAY 1 PROCEDURE

1. Work in groups of three. Within your group decide who will work with each antibiotic producer—*Penicillium notatum* or *Streptomyces griseus* and who will do the control (no antibiotic producer).

2. Each student needs one petri dish containing nutrient agar. NOTE: the contents of the petri dish are sterile—do NOT open the dish until you are required to do so. Place the petri dish upside down (the top side is the larger one—the agar is in the bottom of the dish) over the template shown below. Using the marking pen, draw the lines as they appear on the template onto the bottom of your petri dish. Then write your name, hour, date and type of microbe (Penicillium, Streptomyces, or control) on your dish (write on the bottom of the dish and use small letters as shown on the template).



3. Your teacher will demonstrate for you how to use an inoculating loop. Once you become familiar with the inoculating loop, you must streak the antibiotic-producing microbe onto the agar in your dish. Remember that you only want to put the producer microbe into your dish, so only open the dish when necessary as demonstrated by your teacher. The producer is spread on the surface of the agar along the central line that you drew that goes from one side of the dish to the other. (See the diagram above.)
   The member doing the control dish will not spread any microbe along the central line.

4. When you and your partners are finished, tape your three petri dishes together. Your teacher will give you instructions on where to put your plates. The microbes you streaked on the agar will need several days (4 to 7) to grow and produce their antibiotics.

DAY TWO

MATERIALS PER GROUP OF 3 STUDENTS

9 sterile applicators (cotton swabs)—3 for each student
Access to cultures of 3 different bacteria (A, B, C)
Tape

DAY TWO PROCEDURE

1. Your teacher will return your petri dishes, which should each have an obvious streak of the antibiotic producer growing down the center of the agar (not the control dish).

2. Today you are going to determine which of the sample bacteria are sensitive to the antibiotic produced by each of the two producers. You will need access to liquid suspensions of each of the three bacteria to be tested. At each station, use a sterile applicator to dip into the bacterial suspension and then streak the bacteria over the appropriate line on your petri dish. For example, sample A will be spread over line A. Open your petri dish only when necessary and replace the top as soon as you are done. Be sure to get as close to the growth of the producer as possible without touching the producer streak. The student doing the control dish will use the template line as a guide since no "producer" was streaked on this dish. Use the applicator to "paint" the test bacteria from the central producer streak towards the outer edge of the dish. NOTE: only use each applicator once. When finished, put the applicator into the container of bleach and leave it there to soak. This will kill the bacteria on the applicator. Repeat the above with the other two bacterial samples (B and C).

3. When you and your partners are finished applying all three bacterial samples to each of your dishes, tape your dishes together. Your teacher will tell you where to put them.

4. On the student data sheet (page 19), complete question 1. Predict what you think the petri dishes will look like:
(a) if the test bacteria are sensitive to the antibiotic made by the producer, and
(b) if the test bacteria are not sensitive to the antibiotic produced.

DAY THREE

DAY THREE PROCEDURE

1. Your teacher will return your petri dishes

2. Observe the growth of your bacteria. On the drawings on the data sheet, sketch the appearance of your dish and your partners' dishes. Then record your results in Table A

3. On the data table on the board, record whether the test bacteria are sensitive (+) or not sensitive (-) to the antibiotics produced on your group's two dishes. When all groups have reported their results, record this data in Table B

4. Answer the questions on the data sheet.

Name_____

Date_____

RESULTS AND ANALYSIS

1. Record your predictions from Day 2 Procedure #4 in the space below.

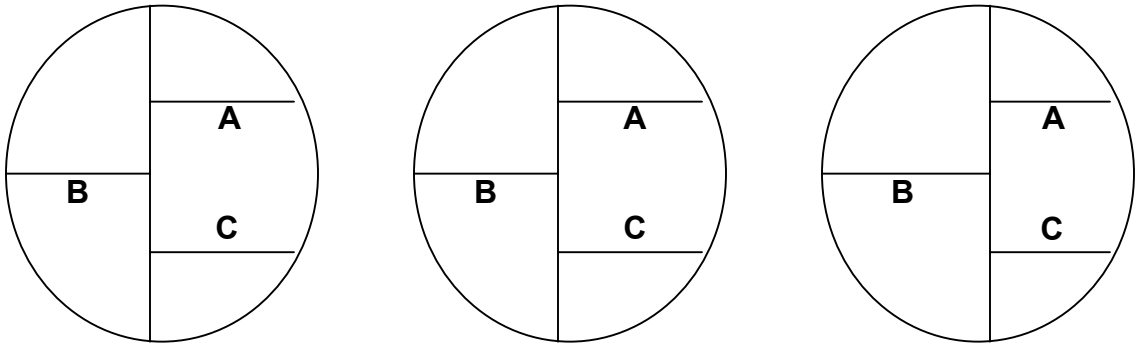
a.

b.

Results

After Day 3:

2. Draw what your dish and your partners' dishes look like on the diagrams below.



Penicillium notatum Dish

Streptomyces griseus Dish

Control Dish

3. Bacteria A is Micrococcus luteus
   Bacteria B is Bacillus subtilis
   Bacteria C is Escherichia coli

Fill in the Table A below for your group's results. When they are available, use data from the whole class to fill in Table B.
(+) means the antibiotic inhibits bacterial growth.
(-) means the antibiotic does not inhibit bacterial growth.

Table A — Group Results

Bacterial Culture Tested	***Penicillum* dish (penicillin)**	***Streptomyces* dish (streptomycin)**	**Control dish (no antibiotic)**
Micrococcus luteus (A)			
Bacillus subtilis (B)			
Escherichia coli (C)			

Table B — Class Results

Bacterial Culture Tested	***Penicillum* dish (penicillin)**	***Streptomyces* dish (streptomycin)**	**Control dish (no antibiotic)**
Micrococcus luteus (A)			
Bacillus subtilis (B)			
Escherichia coli (C)			

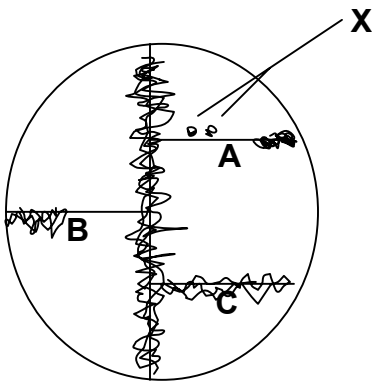
Analysis:

1. Dr. T. Mallard sent you bacteria culture C from one of her patients. In her notes to you, she states that she has started the patient on penicillin. What advice can you give Dr. Mallard? Explain your reasons for this advice.

2. Culture B was isolated from some snacks at the Red Bird Pre-School. the effects of this bacteria are unknown. As a precaution, the hospital wants plenty of antibiotic on hand in case of an outbreak of infections due to culture B. What antibiotic would you recommend? Defend your recommendation.

3. What would the advantage to a microorganism for it to produce an antibiotic?

4. You have performed another experiment very similar to the one you just completed.
   The results are shown



   Which cultures are sensitive to penicillin? _____
   Which cultures are resistant to penicillin? _____
   What could be some reasons for the appearance of the bacteria indicated by an "X"?

5. What was the purpose of the control dish in the experiment you performed? (What do you learn from this dish? How would your analysis of the experiment have been different if you hadn't done the control, or if the control results had been different?)