BIOLOGY 163 LABORATORY

THE EFFECT OF ANTIBIOTICS ON THE GROWTH OF *Escherichia coli* B

(Revised Fall 2009)

Bacteria are single-celled prokaryotic organisms. As bacterial cells take in nutrients from their surroundings and convert it into cellular material, they grow to a point at which each cell divides into two cells. Each resulting cell grows and then divides in the same manner as the original cell. This method of reproduction is called **binary fission**. Under optimal conditions (e.g., sufficient nutrients and proper temperature) this process can occur quite rapidly.

When bacterial cells are first added to fresh medium, there may be a **lag phase** before growth begins. During the lag phase bacteria reorganize their cellular machinery and this results in a delay in growth. Once this occurs, cells divide rapidly in what is called the **exponential phase** of growth. In an old culture the cells have used all the nutrients and have stopped dividing. This phase when there is little change in the number of cells is called the **stationary phase**.

The *relative* number of cells in a broth culture can be quantified using spectrophotometry. As the number of cells increases, so does the turbidity (cloudiness) of the suspension. As tubidity increases, the amount of light able to be transmitted through the sample decreases, and the optical density (absorbance) of the sample increases. Therefore, an increase in absorbance (easily measured with a spectrophotometer) is indicative of an increase in the total number of bacteria in the culture.

Antibiotics are chemical substances (natural or synthetic) that are able to destroy or kill bacterial cells, or inhibit their growth. Most antibiotics act by inhibiting the formation of a particular type of macromolecule or structure in the microbial cell. Some types inhibit cell wall (peptidoglycan) synthesis, ultimately resulting in cell lysis. Other types inhibit protein or nucleic acid synthesis, effectively halting cell growth.

During this laboratory, you will use spectrophotometry to measure the growth (or *population density*) of broth cultures of *E. coli* before and after the addition of the common antibiotics penicillin and streptomycin. Cultures treated with an antibiotic will be compared to untreated control cultures. Prior to adding the antibiotic you should discuss the range of possible outcomes (i.e., develop a hypothesis). Can the data you collect be used to infer something about the mechanism of action of these two antibiotics?

Materials

You will work in teams comprised of sides of laboratory tables. Each team will require the following equipment and materials (some of which may be shared with the other group at your lab bench):

- Spec-20 spectrophotometer w/ light-proof hood
- 3 Nephelo culture flasks w/ 50 ml PC broth
- Overnight broth culture of *E. coli*
- Vial w/ antibiotic (25,000 I.U.)
- Vial w/ sterile H_2O
- Sterile disposable pipettes and inoculating loops
- Phase-contrast microscope w/ slides
- Marking pen and labeling tape

Procedure

Follow the procedure carefully as written below. Use of the Spectronic 20 spectrophotometer is described in the appendix at the end of the laboratory handout. You should read this section carefully *before* you begin the exercise.

Before inoculating your cultures, calibrate the Spec-20 as described in Appendix A. Label one Nephelo culture flask "CONTROL" and the other "TREATMENT".

t = 0 min: Using a sterile 1 ml pipette, inoculate 1.0 ml of an overnight culture of *E. coli* B into each of the two labeled Nephelo culture flasks. Immediately measure the O.D. (absorbance) of both cultures at 540 nm (Appendix A). Record your measurements in Table 1. As soon as you have measured the O.D., place the cultures in the 37^{o}C incubator set at 200 rpm.

t = 20 min: Measure the O.D. of both cultures.

t = 40 min: Measure the O.D. of both cultures.

t = 60 min: Measure the O.D. of both cultures. Make microscopic observations of both cultures (Appendix B).

t = 80 min: Measure the O.D. of both cultures. As soon as you have measured the O.D., add 0.5 ml of a sterile aqueous solution of Penicillin *or* Streptomycin (as assigned by your instructor) at 25,000 international units to the TREATMENT flask, and 0.5 ml of sterile H_2O to the CONTROL flask. (*Why do this?*) Return your cultures to the incubator as quickly as possible.

t = 100 min: Measure the O.D. of both cultures.

t = 120 min: Measure the O.D. of both cultures.

t = 140 min: Measure the O.D. of both cultures. Make microscopic observations of both cultures.

t = 160 min: Measure the O.D. of both cultures. Be sure all data is entered into the class data sheet. Clean up as directed.

WHEN YOU ARE FINISHED WITH YOUR EXPERIMENT, PLEASE DO THE FOLLOWING:

1. Turn off the Spec-20.
2. Remove labels from your CONTROL and TREATMENT flasks. Place the flasks near the sink at the front of the lab.
3. Be sure that all materials are returned to the trays at each table. *Please leave things as you found them!*
4. Wash your hands thoroughly with soap and water.

Results

Table 1. Optical density (absorbance) and microscopic observations of CONTROL and TREATMENT cultures over a 160 minute time period.

TIME (min)	ABSORBANCE @ 540 nm		MICROSCOPIC OBSERVATIONS @ 400 X	
	CONTROL	TREATMENT	CONTROL	TREATMENT
0			---	---
20			---	---
40			---	---
60				
80			---	---
ANTIBIOTIC ADDED				
100			---	---
120			---	---
140				
160			---	---

APPENDIX A
Use of the Spectronic-20 Spectrophotometer

Calibration of Spec-20
Insure that the wavelength of the spectrophotometer is set at 540 nm. Cover the sample well with the index card and, using the **left hand knob**, set the **absorbance** (bottom scale) reading to "infinity". Remove the index card, and place the tube of the Nephelo culture flask labeled "BLANK" into the sample well. Orient the flask so that the large cap points toward the right side of the machine. Cover the flask completely with the light-proof hood, and, using the **right hand knob**, set the absorbance reading to "0". You are now ready to take an experimental reading.

Measuring O.D. with the Spec-20
Before each reading, recalibrate the spectrophotometer as described in the previous section. Place the flask labeled "CONTROL" in the spectrophotometer, cover it with the light-proof hood, and record the reading on the absorbance scale. **Do not touch any knobs while taking a reading**. Remove the "CONTROL" flask and repeat the process with the flask labeled "TREATMENT." Return the flasks to the incubator as quickly as possible.

APPENDIX B
Making Microscopic Observations

Using a sterilized inoculating loop, transfer a drop of culture to a clean glass slide and cover the drop with a coverslip. Observe the slide with the phase-contrast microscope using standard microscopic techniques. Although it is difficult to get an accurate estimate of bacterial numbers using this technique, you may be able to get an impression of the relative density of bacteria in the culture at any given time. You should also look carefully at the arrangement of the cells in each culture. Do the cells exist separately from one another? Do they form chains or clusters? Is there any difference before and after the addition of the antibioltic?

ASSIGNMENT

1. Using class data provided by your instructor, present the results of this study in a manner suitable for publication in a scientific paper. (You may wish to consult “Working with Statistics” and the "Guide to Writing Scientific Papers for ideas and assistance.)

2. Use your results to *concisely* address the following questions (i.e., be brief and to the point):

    - In comparing the treated cultures to the control culture, what can you conclude about the effect of each antibiotic on the relative number of cells in suspension?
    - What does your answer to the previous question suggest about the specific mechanism of action of penicillin? How about streptomycin? (*Feel free to consult outside sources, but be sure to cite them appropriately!*)