Effects of Nutrition and Temperature on the Population Growth of Bacteria

Objectives

- Examine the effects of temperature and nutrition on the growth of *E.coli* cultures
- Measure bacterial growth by taking optical density readings on a spectrophotometer
- Analyze the results graphically

Introduction

A **population** is a group of individuals of the same species living together in a certain area. The size and structure of any population changes over time due to a variety of factors including changing rates of birth, death, and migration; nutritional resources, space and environmental conditions. Ecologists study these changes in order to learn more about how populations of different species interact with each other and with their environment, as well as how to manage the growth of populations of special interest to humans, such as crop species or disease organisms.

Escherichia coli is the model organism for bacteria. Bacteria are ideal for population studies because of their rapid reproduction, their small size and the fact that a spectrophotometer can "measure" *E.coli* population size.

Under optimal conditions, a bacterial population can reproduce itself in about 20 minutes; this is called the doubling time. This fast growth period is only one phase of the growth cycle of a batch of bacteria in a closed system. The first phase of the growth cycle is the **lag phase** when the bacteria are put into fresh culture. A period of time passes while the bacteria adjust to the new environment, increase in size and prepare to reproduce. The **log phase** follows where exponential growth occurs. Unlimited resources and optimal conditions allow for bacteria to reproduce at their doubling rate. As resources are used up, wastes build and space becomes limited, the bacterial growth slows and remains steady in the **stationary phase**. As stationary phase continues, resources decrease further and wastes increase until the population can no longer support itself and it decreases dramatically in the **death phase**, the reverse of the exponential growth in the log phase.

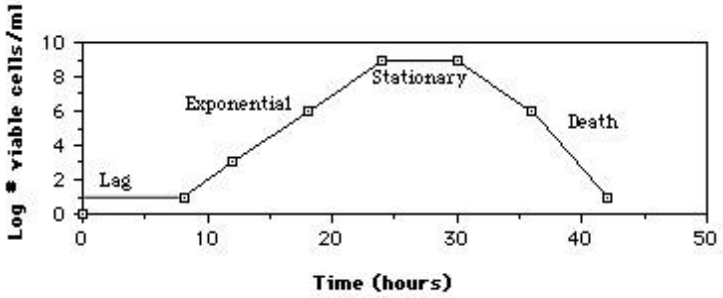

Figure 1. Typical bacterial culture when grown in a closed system.
http://www.textbookofbacteriology.net/growth.html

The Problem

You have just begun an internship in a research lab. This lab works with the model organism for bacteria, *Escherichia coli*. The researchers in the lab want to culture as much *E. coli* as possible as quickly as possible to keep the supply equal to their high demand. The lab has two kinds of bacterial media on which to grow the *E. coli*, nutrient broth and yeast extract. Your job is to figure out which media will produce a larger population of *E. coli* in the shortest amount of time.

The first thing you do is to research the two types of media. You find that nutrient broth is composed of beef broth and peptone. Peptone is an enzyme derived digest of protein and provides nitrogen, amino acids, and vitamins. It is distinguished by low cystine and tryptophan content. Yeast extract contains yeast extract, tryptone and salt. Yeast extract is the water-soluble portion of autolyzed yeast and it is often used to supply B-complex vitamins in bacteriological media as well as flavor to snack foods and pet foods. Tryptone is a digest of the milk protein casein; it provides nitrogen, amino acids and vitamins. Both media seem like good potential culture sources for *E. coli* because they contain nitrogen, amino acids and vitamins. However, you remember from general biology that tryptophan is one of the essential amino acids for humans. All twenty amino acids are necessary to build proteins but the essential amino acids cannot be made by an organism; they must be consumed in the diet. You don't know if *E. coli* requires tryptophan for its normal growth or not, so you decide to look up the nutritional requirements of *E.coli*.

The website http://www.textbookofbacteriology.net/nutgro_2.html tells you that in fact *E.coli* does not need any special growth factors and can synthesize all its essential chemical needs including amino acids and vitamins from a complex medium like either beef broth or yeast extract. A complex medium is one in which the exact chemical composition is not known because it is made from biological sources such as blood, milk, yeast or beef broth. It contains tiny amounts of many different elements that may be necessary to sustain and grow *E. coli* or other bacteria. You are left, however, without knowing which media is better for growing *E. coli*. To decide, you'll set up an experiment growing the *E. coli* in both media and see which culture produces a larger population of *E. coli*.

You continue on with your reading and see that *E. coli* is a common inhabitant of mammalian intestinal systems including our own. The optimum temperature to culture *E. coli* should probably be similar to the human body temperature of 37°C. As a control, you decide to culture the two media at both 37° C and room temperature of about 21º C. The experiment is taking shape. You'll culture the bacteria in a shaking water bath to aerate the solutions continually; one bath set at room temperature and the other one at 37º C.

You will culture four populations of *E.coli* under the following conditions:

Flask a - nutrient broth, 21° C
Flask b - nutrient broth, 37° C
Flask c - yeast extract, 21° C
Flask d - yeast extract, 37° C

Confident that you have a solid plan, you relax and start to set up your materials.

Note: *E.coli* culture cannot be poured directly down the sink; please use the *E.coli* waste jars on your table for any *E.coli* culture that you no longer need. Once a tube or flask is emptied of *E.coli*, it may be rinsed and that water may go down the sink.

Procedure:

Setup:

1. Turn on the spectrophotometer and let it warm up. Set the wavelength to 590 nm.
2. Wipe down your table area using ethanol and a paper towel.
3. Label 4 - 125 ml flasks "a", "b", "c", "d", and your group symbol with tape towards the top of the flask.
4. Label 4 spectrophotometer test tubes "a", "b", "c", "d", and your group name with tape at the top of the tubes.
5. Label 4 – green pi-pumps "a", "b", "c", "d" with tape.
6. Label 1 spectrophotometer test tube "BN" for blank nutrient broth and one test tube "BY" for blank yeast extract with tape at the top of the tubes.
7. Put on gloves. This *E.coli* strain is not virulent but it is best to work with sterile technique to reduce the chance of adding other contaminating organisms to your cultures. Safety glasses are available to protect your eyes from splashes.
8. Fill the "BN" test tube 2/3 full with nutrient broth media using a sterile plastic pipet. Fill the "BY" test tube 2/3 full with yeast extract media. You need to keep both blanks for the entire experiment.
9. Measure 50 ml of nutrient broth solution using the graduated cylinder and pour it into flask "a". Repeat with flask "b". Rinse this graduated cylinder with water in the sink.
10. Measure 50 ml of yeast extract solution and pour it into flask "c". Repeat with flask "d".
11. Take the microcentrifuge tube of concentrated *E. coli* cells from the ice bucket and shake it. Open the tube and draw up the *E. coli* cells in the sterile plastic pipette. Let a couple of drops fall back into the microcentrifuge tube to be sure you have full drops of culture with no air bubbles. Put 5 drops of *E. coli* cells into each of your 4 flasks. Add the *E. coli* cells slowly and carefully so that each flask receives the same amount of cells.
12. Place flasks "a" and "c" in the room temperature water bath. Place flasks "b" and "d" in the 37°C water bath. Turn on the shakers and let the flasks acclimate to temperature for about 5 minutes.

spectrophotometer spec·tro·pho·tom·e·ter (spĕk'trō-fō-tŏm'ĭ-tər) n.

An instrument for measuring the intensity of light of a definite wavelength transmitted (or absorbed) by a substance or a solution, thus providing a measure of the amount of material in the solution absorbing the light.

Dictionary.com. *Merriam-Webster's Medical Dictionary*. Merriam-Webster, Inc.

http://dictionary.reference.com/browse/spectrophotometer (accessed: August 09, 2011).

Figure 1. GENESYS 20 Spectrophotometer

Starting up the instrument

When you turn on your GENESYS 20 spectrophotometer, it performs its power-on sequence. This sequence includes checking the software revision, initializing the filter wheel and the monochromator. The power-up sequence takes about 2 minutes to complete. Allow the instrument to warm up for 30 minutes before using it.

Note: *Be sure that the cell holder is empty before turning on the instrument.*

Absorbance measurements

1. Press **nm ▲** or **nm ▼** to select the wavelength (in nanometers).
   Note: *Holding either key will cause the wavelength to change more quickly.*

2. Wipe the blank and insert it into the cell holder and close the sample door.

3. Press **0 ABS/100%T** to set the blank to 0 Absorbance.

4. Remove your blank and insert a sample into the cell holder. The sample measurement appears on the LCD display. Do not hesitate to ask for more instruction if you are not sure you are using the machine properly.

5. Fingerprints on the test tubes can alter the optical density readings so wipe down your tubes with a Kim wipe every time you put them in the spectrophotometer.

Note: Questions from this lab will be on the lab practical possibly including the use of pipettes and the spectrophotometer. Every student must know how to take samples, blank the spectrophotometer and find the O.D. so take turns using all of the equipment.

Take your samples and O.D. readings:

1. Your TA will explain how to use the spectrophotometer and you can review the directions on the previous page. Blank your spectrophotometer with the "BN" tube so you are ready to read the O.D. of tubes "a" and "b".
2. When you are ready to begin sampling, start your timer. One student should remove flasks "a" and "b" from their water baths. Swirl flask "a" well; it's important to swirl the contents of the flask before sampling because the cells tend to settle quickly. Remove about 5 ml (does not have to be exact) of solution using a sterile graduated pipette and green pi-pump and place the solution into test tube "a". Save the pipette and use it each time for flask "a". Repeat the procedure with flask "b" using another pipette. Take the O.D. readings of tubes "a" and "b". Pour the 5 ml sample that you just obtained back into the flask it came from and replace the stopper. Return flasks "a" and "b" to their proper water bath.
3. Repeat the procedure with flasks "c" and "d", blanking the spectrophotometer with the "BY" tube first. Record all 4 of these readings as time 0. Once you start your timers, let them run throughout the experiment without stopping. Each time you take a sample reading, use the same "time" even though it takes 5-10 minutes to take all four samples. This will make the graphing easier. There is no need to rinse the test tubes between readings.

Continue the sampling:

4. At about 20 minute intervals, remove your 4 flasks and take them back to your bench for sampling and O.D. readings. Remember to blank the machine each time the solution you are sampling changes. Continue taking readings for 120 minutes (7 samples) throughout the class.

Analysis:

5. While your cultures are incubating, fill in the Bacterial Population Data Table in the lab manual.
6. Graph the $O.D_{590}$ (dependent variable on the y-axis) vs. time (independent variable on the x-axis) on regular graph paper or on Excel using the computers in the lab. The maximum optical density you will record is ~1.00 and the maximum time will be 120 minutes. Use these numbers to set up the scales of your graph. Start to plot your results as you receive them. Graph each culture separately so that there will be four lines showing optical density over time for each of the four *E.coli* populations (see the example on the pre-lab). Each line should be distinguished by different colors which should be shown in the legend.
7. Highlight one 20 minute interval on your graph in which your fastest growing culture is in its log or exponential phase.

Lab Report

Your lab report must be typed; hand-drawn graphs are acceptable but computer generated tables and graphs are preferable. This lab report is due at the beginning of lab one week after you perform this lab. Although you work together with other students, your lab report must be completely your own words. Any ideas or quotes from others must be referenced. See Appendix A on *How to Write a Lab Report* as well as any handout from your TA with directions.

The results section of your lab report must include:

1. the Bacterial Population Data Table
2. the graph you created for the Analysis Section including a title, labeled axes showing units and legend

The discussion section of your lab report must include answers to the questions below *based on the results of your experiment and your graphed data*:

1. What answer will you give to your boss as to which media at what temperature will produce *E.coli* at the fastest rate?
2. Suppose the heating element on your shaking water bath breaks and you can only grow the bacteria at room temperature rather than at 37°C. Will there be a significant difference between a culture grown in yeast extract and a culture grown in nutrient broth at room temperature? How do you know?
3. If you run out of yeast extract and have to grow *E.coli* on nutrient broth medium, at what temperature, room temperature or 37°C, will you grow it to get faster population growth? Does the difference in temperature make sense in terms of what you know about the natural habitat of *E. coli*? Explain why.
4. Overall, was the choice of media or the choice of temperature more important in this experiment to produce the fastest growing population of *E.coli*?
5. How would the shape of your fastest growing *E.coli* growth curve change if the experiment continued for a substantially longer period of time and why?
6. Is your internship boss happy with your experiment and its results and is she prepared to offer you a full time job with benefits? If your experiment did not produce a good result, explain what went wrong and how you might revise it.

Bacterial Population Data Table								
O.D. 590 or 610								
Flask	Culture	0 min	20 min	40 min	60 min	80 min	100 min	120 min
a	NB at 21°C							
b	NB at 37°C							
c	YE at 21°C							
d	YE at 37°C							

Table 1. Absorbance readings for each culture at each sampling time

Clean Up

Remove all tape from flasks and test tubes. Please place all *E.coli* cultures into the *E.coli* waste jars on your tables. Rinse used flasks, test tubes and beakers and place them into the "dirty glassware" containers next to the sink. Blank solutions may go down the sink. Place the pipettes in the pipette waste containers. Wash down your table area again with ethanol. Place your gloves in the trash. Wash your hands.

Reference

http://www.textbookofbacteriology.net/growth.html

Honor Pledge

Although students work together in groups during lab, use the same data and discuss the analysis of their results, the report that each student writes must be his or her original work. Please sign and date the following honor pledge to give your word that the lab report you submit is completely your own work. Attach the signed honor pledge to the front of your lab report.

Adding sterilized water to the ferns in the Petri dishes.

You do not need to understand the reason for this activity today. You are preparing fern plants to be used during the Plant Diversity Lab in two weeks.

1. Take a Petri dish of c-fern gametophytes from the black flat. Put some tape on the bottom and label the dish with your group name and TA name.

2. Open the sterile pipet package and draw up water from the bottle of sterile water on the tray to the top mark of the pipet – this is 1 ml. Repeat 2 times for a total of 3 ml of water; add this water to the dish.

3. Swirl the water around to cover all surfaces of the media and to wet all ferns.

4. Return the Petri dish to the black flat, put the cover on and have the light near the flat to maintain temperature of about 28°C.

5. Don't tape the plates together.

6. There is a dissecting microscope nearby in case you want to look at the ferns under the microscope.

HONOR PLEDGE

- **I pledge that this work is entirely my own.**
- **I understand that plagiarism is copying words or original thoughts without proper acknowledgement of the source.**
- **I understand that this includes copying straight from the lab manual, a partner or other student, other lab reports, the internet, texts, etc.**
- **I understand that plagiarism will result in a zero for the report, and that other consequences may follow.**

Print your name: _____

Sign: _____

Date: _____