BIOL 111L: Cell Biology Lab
Week 13 and 14 - Bacteria Lab **Name**_____

Pages 1 – 2 MUST be completed BEFORE you come to lab the week of April 22 – 24. Since we will be working with Bacteria in lab you will not be able to have your books, computers or phones out on the lab benches during lab.

Introduction

Bacteria are single-celled organisms that are found virtually everywhere in and around us. As biologists, we work with bacteria in the lab for many different purposes - from researching the characteristics of an infectious organism that causes illness to using genetically engineered bacteria to produce a specific protein. Most of what we know about the basic functions of cells, such as DNA replication and expression of genes into proteins, was first studied in bacteria. This short lab series is designed to give you exposure to working safely with bacteria in a lab setting, to test factors that influence growth of bacteria, and to demonstrate the usefulness of bacteria in the modern biology lab.

Lab Safety

Read the biosafety document and answer the following questions:

1. What are 7 things you must do when you enter the lab?

2. What must you do before you leave the lab?

Model organisms

Resources to help answer the following questions:

> National institute of General Medical Sciences. Using Model organisms to Study Health and Disease. Available from: http://www.nigms.nih.gov/Education/Pages/modelorg_factsheet.aspx

Nobel laureate, Jacques Monod, said, "Once we understand the biology of Escherichia coli, we will understand the biology of an elephant."

1. What does Monod's statement mean with respect to using model organisms in experiments?

2. What are some features that make model organisms desirable to work with in biological sciences?

Antibiotic resistance

Resources to help answer the following questions:

Genetic Science Learning Center. "What Is An Antibiotic?." Learn.Genetics. http://learn.genetics.utah.edu/content/microbiome/antibiotics/

Genetic Science Learning Center. "Antibiotic Resistance." Learn.Genetics. http://learn.genetics.utah.edu/content/microbiome/resistance/

Alliance for the Prudent Use of Antibiotics. Tufts University. General Background: About Antibiotic Resistance. Available from: http://www.tufts.edu/med/apua/about_issue/about_antibioticres.shtml

1. What is an antibiotic (also called antibiotic agent)?

2. How do antibiotics work (in general)? Why do antibiotics affect bacterial cells but not human cells?

3. What does it mean for an organism to be antibiotic resistant?

Bacterial growth

Even though bacteria seem very different from us, we actually share many of the same basic needs for survival and growth.

1. Come up with at least 3 things that bacteria need to live.

2. How do bacterial cells reproduce?

Lab Packet - Bacteria

Learning Objectives

1. Basic understanding of bacteria as model organisms
2. Basic understanding of antibiotic resistance in bacteria
3. Demonstrate understanding of how environmental conditions affect growth of cells
4. Build on experimental design experiences, data analysis, and communicating results

Organism: *Chromobacterium violaceum*

Chromobacterium violaceum (Fig. 1) is a common bacterium found in many different water and soil environments around the world. It has been studied most extensively for its signature dark-violet pigment. The pigment acts as an antibiotic, and may also be useful in treating colorectal cancers (Kodach 2006).

A pitcher plant strain of *C. violaceum* was cultured by Paul Melchior's group of MSUM and NHCC students from the phytotelmata (fluid) of the carnivorous plant *Sarracenia purpurea*, commonly known as the northern pitcher plant. The phytotelmata was collected from plants found at Beckman Lake bog within the Cedar Creek Ecosystem Science Reserve just north of the Twin Cities in Minnesota. Cultured solutions revealed a large number and variety of bacteria living within the fluid of the plant. It was also discovered that the pitcher plant strain is "naturally" resistant to ampicillin (amp), an antibiotic, at 22°C but sensitive to ampicillin if grown at 37°C.

Figure 1. An isolation plate of *C. violaceum*. Colonies are dark-violet with a metallic sheen.

Obtain a sample of bacteria and prepare a wet mount slide. What do you see?

Week of April 22 – 24

Design an experiment

1. Learn how to culture bacteria – plates AND run the experiment
2. **Come in and immediately put backpacks on shelves and then disinfect your lab table!**
3. Work in groups of 4 to design an experiment with *C. violaceum.*
4. Possible questions for you to choose from:
    a. Is *C.* violaceum resistant to antibiotics? (3 antibiotics available)
    b. Does light affect the growth of *C. violaceum*? (Light options: UV, incandescent, dark)
    c. Does temperature affect the growth of *C. violaceum*? (Available temps: 22C, 37C, fridge)
    d. Does light affect *C. violaceum's* ability to resist antibiotics?
    e. Does temp affect antibiotic resistance in *C. violaceum*?
    f. What are the effects of different sugar solutions on the growth of *C. violaceum*?
    g. What are the effects of different salt solutions on the growth of *C. violaceum*?
5. Make sure to plan appropriate controls as well as the manipulation of a variable. Use words and sketches to design your experiment below. Have your experiment approved by your instructor.
6. Supplies: plates (LB), broth, disposable sticks, disinfectant, tubes, cultures of *C. violaceum*
7. Set up plates of experimental units
8. Set up corresponding controls for experiment
9. Allow 1-2 days to grow (if different timing set up a time with your instructor to move the plates)
10. Instructor or TA moves plates to refrigerator

Your Experimental Design:

Question:

Hypotheses

Null:

Alternative:

Materials:

Sketch of your experimental design (make sure to include the number of samples you will use): (*hint: one plate can contain up to 4 treatments, including a control)

Data Table: (you will be measuring percent coverage of the bacterial plate in each of the treatment areas of each plate)

Instructor/TA approval of design: _____

If your experimental design is approved, you may gather your supplies and set up your experiment – Your group MUST first get a demonstration of how to plate your bacteria before you can get started.

Group received demonstration – Instructor/TA signature:_____

Week of April 29 – May 1

Collect data and analyze results

1. Follow the lab's safety procedures for working with bacteria.
2. Gather your *C. violaceum* plates and measure the percent coverage of the experimental area of your plate; this will represent growth of bacteria in each of your treatments. You will need a transparency with centimeter squares for this measurement.
3. Record your data in your data table.
4. Once you have analyzed your data, create a mini-presentation on PowerPoint to communicate your results to the rest of the lab.
    a. You will present your methods, results, and discussion **today in lab**
    b. Digital cameras are available for you to take pictures of your plates
    c. You will use a table to show percent coverage
    d. Be able to discuss the environmental variable you chose and the effects of that variable on cell growth

<u>Presentation Checklist</u>

___Title – should be descriptive and accurately portray your experiment.

___Question and Hypotheses – be sure to include your question and hypotheses in your presentation.

___Methods – what was your experimental design? How did you control for your variables?

___Results – include a table of your results, explain the results

___Conclusion – how does your experimental variable affect cell growth? Include references – textbook, peer-reviewed literature (these will need to be found BEFORE presentation day!). What are future considerations for this research?

___Literature Cited – include the full citation for your sources

Biol 111L - Group Research Oral Presentation Rubric Lab Section: _____

Names: _____

Evaluator: _____

Title

Does the title accurately portray the main topic of the presentation?

☐ Yes (1.5 pts) ☐ No

Question and Hypotheses

Is the question and are the hypotheses clearly communicated?

☐ Yes (1.5 pts) ☐ No

Methods

Experimental Design: Is the general design clearly and efficiently communicated?

☐ Yes ☐ Somewhat ☐ No

Variables: Are the methods for controlling and/or measuring variables clearly and efficiently communicated?

☐ Yes ☐ Somewhat ☐ No

Results

Data Summary: Are the raw data summarized into figures and/or tables in an efficient, clear, and correct manner?

☐ Yes ☐ Somewhat ☐ No

Interpretation: Are the graphs and/or tables clearly explained?

☐ Yes ☐ Somewhat ☐ No

Conclusions

Summary: Are the results summarized into a few main "take home" messages?

☐ Yes ☐ Somewhat ☐ No

Significance: Are the results placed into a larger scientific context?

☐ Yes ☐ Somewhat ☐ No

Future Research: Are recommendations made for improving this experiment or pursuing related research?

☐ Yes ☐ Somewhat ☐ No

Literature Cited

Are the references present, and appropriately cited?

☐ Yes (1.5 pts) ☐ No

Overall Presentation

Do all group members participate equally in the presentation?

☐ Yes (1.5 pts) ☐ No

Is the presentation well-organized and well-timed?

☐ Yes (1.5 pts) ☐ No

Are questions fielded adequately?

☐ Yes (1.5 pts) ☐ No

Total: (_____ Yes × 3 pts) + (_____ Somewhat × 1.5 pts) + (_____ No × 0) = _____ out of 30 pts