Techniques for Studying Prokaryotic Cells (Bacteria)

Introduction: In this lab, we will be learning basic techniques for culturing bacterial cells, and doing research with them. Specifically, we will examine one variable to determine how it affects bacterial cell survival and growth.

Materials:

|  |  |  |  |
| --- | --- | --- | --- |
| 1. Petri dishes (2 per group) | 1. Nutrient agar powder | 1. Balance | 1. Water |
| 1. Glass Flask | 1. Microwave | 1. Graduated cylinder | 1. Candle (flame source) |
| 1. Matches or lighter | 1. Inoculating loop for each group | 1. Masking tape for labeling | 1. 5 small test tubes per group |
| 1. Masking tape (for labeling) |  |  |  |

Procedure:

**Day 1: Pouring Agar Plates**

1. Follow instructions found on the nutrient agar container, but scale down your measurements to make 100 ml in your glass flask.
2. Microwave your flask (10-20 seconds at a time), then remove from the microwave and swirl. Repeat until liquefied and a translucent amber color.
3. Pour 2 plates following teacher instructions.
   1. Avoid contaminating plates
      1. Use clamshell technique!
      2. Don’t touch agar!
      3. Flame the lip of the flask before pouring!
      4. Don’t breathe all over the agar or let too much air blow in!
4. Label your plates with a group identifier. Allow the plates to dry undisturbed until next class.

**Day 2: Inoculation and Testing**

Part 1: Serial dilutions

For this lab, we will test how effective a particular substance is at killing bacteria. Since the concentration of the substance is critical to its effectiveness as an antibacterial agent, we will test several concentrations by performing a **serial dilution**.

Steps to performing a serial dilution:

1. Set out 5 test tubes and label them (with tape) as follows:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Tube # | 1 | 2 | 3 | 4 | 5 |
| Concentration  (compared to stock solution) | 100% | 50% | 25% | 12.5% | 6.25% |

1. You will be given a substance to test (for example bleach, vinegar, or isopropyl alcohol).
2. The initial substance you will be given is called a stock solution. Measure 10 ml of stock solution with a graduated cylinder.
3. Pour this stock solution into the test tube labeled “100%.”
4. Precisely measure and remove 5 ml of the solution from this 100% solution test tube and pour it into your 50% test tube.
5. Now, precisely measure and add 5 ml of water to the test tube labeled “50%.” Notice that now your solution is one half water and one half stock solution, so it is half as concentrated (i.e. 50%). Swirl the solution to ensure it is evenly mixed.
6. Next, precisely measure and remove 5 ml of the solution from this 50% solution test tube and pour it into your 25% test tube.
7. Now, precisely measure and add 5 ml of water to the test tube labeled “25%.” Notice that your solution is half as concentrated as the previous solution. Swirl the solution to ensure it is evenly mixed.
8. Continue with this procedure, removing 5 ml from the 25% test tube and adding it to the 12.5% test tube. Then dilute with 5 ml of water.
9. Finally, remove 5 ml from the 12.5% test tube and adding it to the 6.25% test tube. Then dilute with 5 ml of water.

\*Notice that each subsequent test tube is half as concentrated as the previous test tube.

Part 2: Inoculating Plates

Steps:

1. Decide on a source of bacteria you will use (pond water is recommended) . Please note that this will likely consist of many different species of bacteria (not a pure culture).
2. Flame the inoculating loop to sterilize it.
3. Pick up some of your bacteria with the loop and quickly spread evenly over the entire surface of the agar (use clamshell technique etc.). WARNING: Be careful to NOT dig into the agar!
4. Close the Petri dish

Part 3: Testing an antibacterial substance

Steps:

1. One of your inoculated Petri dishes will serve as your control group and the other will be your experimental group. Label each accordingly with tape.
2. Although each group made 5 dilutions of the antibacterial substance, each group will only test 1 of the dilutions and we will share data at the end. Ask your teacher which dilution your group should do.
3. Your control group dish will receive no antibacterial substance. Instead you will add precisely 1 ml of water to the dish and spread this with your inoculating loop (after sterilizing the loop, using aseptic technique).
4. Your experimental group dish will receive 1 ml of your assigned dilution of the antibacterial substance. Use the same technique to spread this substance over the dish with your inoculating loop (after sterilizing the loop, using aseptic technique).
5. After treatment, store your plates (taped together, upside down). Let them incubate for a day or two at room temperature.

**Day 3: Data Collection and Mini Lab Report**

1. After allowing for incubation, count the number of bacterial colonies on each of your plates.
2. Create a data table to record this data (put this in the raw data section of your report)
3. Collect Class Data (put this in the raw data section of your report)
4. We will process the data together as a class.
5. Finish the rest of your report.

## Here is a skeleton outline of your lab report format… (note: this is a smaller version of the report format you will be using for your Internal Assessments) I. Design

**A. Problem –**

**B. Hypothesis** (When appropriate)

**C. Variables**

**1. Independent Variable**

**2. Dependent Variable-**

**3. Controlled Variables**

**D. Method (Procedure)-**

**1. Materials**

**2. Procedure (step by step)**

# II. Data Collection and Processing

**A. Raw Data** –

# B. Data Processing

**C. Presentation** **of Processed Data**–

# III. Conclusion & Evaluation

**A. Conclusion** –

**B. Evaluation of Procedure** –

**C. Improving the Investigation**