Bacteria Pool Party

Introduction

Prove to students that bacteria aren't just "bumps on a log"! Perform an activity which shows the swimming ability of motile bacteria and their attraction to nutrients in semi-solid agar.

Concepts

- Bacterial motility
- Chemotaxis

Materials

Agar, non-nutrient, 2.0 g
Cultures of your favorite motile bacteria *(E. coli, S. marcescens, B. subtilis,* etc.*)*
Dextrose, 1.0 g
DL-Methionine, 0.38 g
EDTA, 0.22 g
Potassium phosphate, dibasic, 1.8 g
Nutrient agar
Distilled water, 1000 mL
Autoclave or pressure cooker
Erlenmeyer flask, 2000-mL
Inoculating loops
Petri/culture dishes
Wax pencils or permanent markers

Safety Precautions

Always wear safety goggles when working with chemicals, glassware, or heat. Although the bacteria used for this activity are considered to be non-pathogenic, it is extremely important to practice aseptic/sterile techniques when working with all microorganisms. Finally, it is extremely important to sterilize all prepared media (used or unused) prior to disposal to prevent growth of any pathogenic or non-pathogenic organisms. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

1. Upon receipt of bacterial culture(s), use an inoculating loop and sterile technique to make streak plates of the bacteria. Use regular nutrient agar and not the agar media used later in this activity. Allow these plates to grow up for 2 to 3 days prior to this activity.
2. Prepare special media for *Bacteria Pool Party* prior to the lab by mixing 2.0 grams of agar and 1 L distilled or deionized water and heating to a boil in a 2000-mL Erlenmeyer flask.
3. Sterilize the agar solution (covered loosely with aluminum foil) in an autoclave or pressure cooker following the equipment directions carefully.
4. After allowing the agar solution to cool slightly, add the enrichments (0.22 g EDTA, 1.0 g dextrose, 0.38 g DL-methionine, and 1.8 g potassium phosphate dibasic) and stir to dissolve.
5. Allow agar to cool to approximately 50 °C before dispensing into Petri dishes. (One liter of media is sufficient to prepare 50 standard [15 × 100 mm] Petri dishes or 125 small [15 × 60 mm] Petri dishes.)
6. Allow the media in the Petri dishes to cool to room temperature and store the plates in a refrigerator, right-side up (this is necessary as this special media is very soft) until ready to use.
7. Allow the media to warm up to room temperature just prior to class.

Procedure

1. Remove the Petri dishes from the refrigerator and allow them to warm to room temperature.
2. Have each group of students "pick" 2 or 3 whole colonies of bacteria from a streak plate using an inoculating loop and sterile technique.
3. Inoculate the bacteria into the center of the plate, making sure that most of the cells are embedded in the media instead of on top. This will be easy because the media is so soft. The area of bacteria should be about the size of a pencil eraser.
4. Allow the cultures to grow up for 2–3 days. If the particular bacteria requires a temperature higher than 25–30 °C, an incubator will be required. Remember to store the plates right-side up.

Publication No. 508
032317

5. Observe the culture over time—there should be a visible "ring" of cells which has moved out from the original point of inoculation.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. All prepared media (used or unused) should be sterilized in an autoclave or pressure cooker prior to disposal. Sterilization of the media and disposable culture dishes can also be achieved by soaking the plates in a 10% bleach solution.

Tips

- As with all demonstrations or activities, the instructor should practice this demonstration before presenting it in order to become familiar with the procedures and outcomes. The use of *Serratia marcescens* provides excellent results for this actiity due to the red pigment it produces in optimal growth conditions. This pigment will still be observed in the center of the plate where the cells were initially placed, but will not be observed in the ring of cells which is proceeding toward the edge of the plates.
- As a simple extension of this activity, it is easy to culture bacteria from the "ring" on standard nutrient agar. A more in depth extension of this activity would be to compare the level of activity of different types of bacteria, or to change the energy source in the media (for example, replace the dextrose in the media with maltose or lactose). Also, it is possible to isolate non-motile mutants from the center of the plate by this method.

Discussion

Motile bacteria swim in a succession of runs and tumbles. A run is defined as a period of swimming or movement in a straight line. A tumble occurs when the clockwise or counterclockwise rotation of the bacterial flagellum reverses abruptly, causing the direction of swimming to randomly change. When conditions are favorable, each bacterium runs until it reaches unfavorable conditions, at which time it undergoes a tumble to change direction. This "swimming" is not observed in most bacterial media because it has a high concentration of nutrients and is too hard. The media used in this experiment is soft and has minimal amounts of nutrients and energy sources, thus it not only allows "swimming," but encourages it. This soft media environment more closely emulates a natural bacterial environment than does nutrient agar or other growth media. In this activity, the ring of bacteria forms as the bacteria move out from the center of the plate in search of a food source. This swimming in search of an energy source and other nutrients is called chemotaxis. As the food in each location is used up, the bacteria continue to migrate outward.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Content Standards: Grades 5–8
Content Standard C: Life Science, structure and function in living systems
Content Standards: Grades 9–12
Content Standard C: Life Science, the cell, interdependence of organisms

Reference

Adler, J. Science. 1966, 153, 708–716.

Materials for *Bacteria Pool Party* are available from Flinn Scientific, Inc.

Catalog No.	Description
M0106	DL-Methionine, 200 g
A0257	Agar, Bacteriological Grade, 25 g
E0016	Ethylenediaminetetraacetic Acid, EDTA, 25 g
LM1013	Serratia marcescens
N0092	Nutrient Agar, 23 g

Consult the Flinn Scientific website for current prices.