GLOBAL EDITION

Microbiology

A Laboratory Manual

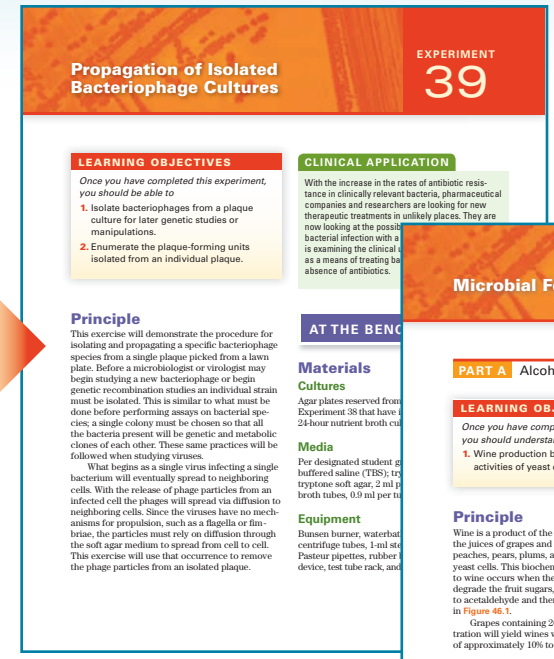
ELEVENTH EDITION

Cappuccino • Welsh

Pearson

A flexible approach to the **modern** microbiology lab

NEW! "Propagation of Isolated Bacteriophage Cultures" experiment has been added to the **Eleventh Edition**. This experiment (39) guides students to isolate bacteriophages for genetic manipulation, an important technique in current clinical research as a possible way to treat antibiotic-resistant bacterial infections.



EXPERIMENT 39

Propagation of Isolated Bacteriophage Cultures

LEARNING OBJECTIVES

Once you have completed this experiment, you should be able to

1. Isolate bacteriophages from a plaque culture for later genetic studies or manipulations.
2. Enumerate the plaque-forming units isolated from an individual plaque.

CLINICAL APPLICATION

With the increase in the rates of antibiotic resistance in clinically relevant bacteria, pharmaceutical companies and researchers are looking for new therapeutic treatments in unlikely places. They are now looking at the possibl bacterial infection with a is examining the clinical as a means of treating ba absence of antibiotics.

Principle

This exercise will demonstrate the procedure for isolating and propagating a specific bacteriophage species from a single plaque picked from a lawn plate. Before a microbiologist or virologist may begin studying a new bacteriophage or begin genetic recombination studies an individual strain must be isolated. This is similar to what must be done before performing assays on bacterial species; a single colony must be chosen so that all the bacteria present will be genetic and metabolic clones of each other. These same practices will be followed when studying viruses.

What begins as a single virus infecting a single bacterium will eventually spread to neighboring cells. With the release of phage particles from an infected cell the phages will spread via diffusion to neighboring cells. Since the viruses have no mechanisms for propulsion, such as a flagella or fimbriae, the particles must rely on diffusion through the soft agar medium to spread from cell to cell. This exercise will use that occurrence to remove the phage particles from an isolated plaque.

AT THE BENC

Materials

Cultures

Agar plates reserved from Experiment 38 that have i 24-hour nutrient broth cu

Media

Per designated student g buffered saline (TBS); try tryptone soft agar, 2 ml p broth tubes, 0.9 ml per tu

Equipment

Bunsen burner, waterbat centrifuge tubes, 1-ml ste Pasteur pipettes, rubber device, test tube rack, and

EXPERIMENT 46

Microbial Fermentation

PART A Alcohol Fermentation

LEARNING OBJECTIVE

Once you have completed this experiment, you should understand

1. Wine production by the fermentative activities of yeast cells.

Principle

Wine is a product of the natural fermentation of the juices of grapes and other fruits, including peaches, pears, plums, and apples, by the action of yeast cells. This biochemical conversion of juice to wine occurs when the yeast cells enzymatically degrade the fruit sugars, fructose and glucose, first to acetaldehyde and then to alcohol, as illustrated in Figure 46.1.

Grapes containing 20% to 30% sugar concentration will yield wines with an alcohol content of approximately 10% to 15%. Also present in grapes are acids and minerals whose concentrations are increased in the finished product and that are responsible for the characteristic tastes and bouquets of different wines. For red wine, the crushed grapes must be fermented with their skins to allow extraction of their color into the juice. White wine is produced from the juice of white grapes.

The commercial production of wine is a long and exacting process. First, the grapes are crushed or pressed to express the juice, which is called **must**. Potassium metabisulfite is added to the must to retard the growth of acetic acid bacteria, molds, and wild yeast that are endogenous to grapes in the vineyard. A wine-producing strain of yeast, *Saccharomyces cerevisiae* var. *ellipsoideus*, is used to inoculate the must, which is then incubated for 3 to 5 days under aerobic conditions at 21°C to 32°C. This is followed by an anaerobic incubation period. The wine is then aged for 1 year to 5 years in aging tanks or wooden barrels. During this time, the wine is clarified of any turbidity, thereby producing volatile esters that are responsible for characteristic flavors. The clarified product is then filtered, pasteurized at 60°C for 30 minutes, and bottled.

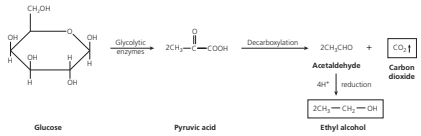


Figure 46.1 Biochemical pathway for alcohol production

REVISED EXPERIMENTS include options for alternate media, making the experiments affordable and accessible to all sizes of lab programs. Experiment 46 now includes both wine and lactic acid fermentation, looking at the production of wine and yogurt.

AT THE BENCH

Materials

Cultures

48- to 72-hour nutrient broth cultures (50 ml per 250-ml Erlenmeyer flask) of *Staphylococcus aureus* BSL-2 *and Bacillus cereus*; 72- to 96-hour Sabouraud broth cultures (50 ml per 250-ml Erlenmeyer flask) of *Aspergillus niger* and *Saccharomyces cerevisiae*.

Media

Per designated student group (pairs or groups of four): five nutrient agar plates, five Sabouraud agar plates, and one 10-ml tube of nutrient broth.

Equipment

Microincinerator or Bunsen burner, 800-ml beaker (waterbath), tripod and wire gauze screen with heat-resistant pad, thermometer, sterile test tubes, glassware marking pencil, and inoculating loop.

Procedure Lab One

1. Label the covers of each of the nutrient agar and Sabouraud agar plates, indicating the experimental heat temperatures to be used: 25°C (control), 40°C, 60°C, 80°C, and 100°C.
2. Score the underside of all plates with a glassware marking pencil into two sections. On the nutrient agar plates, label one section *S. aureus* BSL-2 and the other *B. cereus*. On the Sabouraud agar plates, label one section *A. niger* and the second *S. cerevisiae*.
3. Using aseptic technique, inoculate the nutrient agar and Sabouraud agar plates labeled 25°C by making a single-line loop inoculation of each test organism in its respective section of the plate.
4. Using a sterile pipette and mechanical pipetter, transfer 10 ml of each culture to four sterile test tubes labeled with the name of the organism and the temperature (40°C, 60°C, 80°C, and 100°C).
5. Set up the waterbath as illustrated in Figure 40.2, inserting the thermometer in an uncapped tube of nutrient broth.

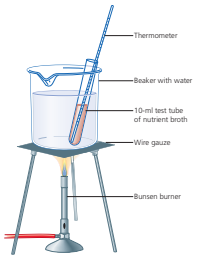


Figure 40.2 Waterbath for moist heat experiment

6. Slowly heat the water to 40°C; check the thermometer frequently to ensure that it does not exceed the desired temperature. Place the four cultures of the experimental organisms into the beaker and maintain the temperature at 40°C for 10 minutes. Remove the cultures and aseptically inoculate each organism in its appropri- ate section on the
7. Raise the waterbat repeat Step 6 for th plates labeled 60°C
8. Raise the waterbat repeat Step 6 for th plates labeled 80°C
9. Raise the waterbat repeat Step 6 for th plates labeled 100°
10. Incubate the nutrie inverted position fo and the Sabouraud 5 days at 25°C in a

Procedure L

1. Observe all plates f the test organisms
2. Record your result the Lab Report.

NEW! BioSafety Levels (BSLs) alert students to appropriate safety techniques. The organisms within this manual are mostly BSL-1 organisms, with any BSL-2 organisms now marked within the text. The Eleventh Edition also reflects the most up to date safety protocols from governing bodies such as the EPA, ASM, and AOAC, better preparing students for professional lab work.

TIPS FOR SUCCESS

- Gram stain your unknown culture first and then determine which tests would be useful in identifying your bacteria. For example, the oxidase test and the citrate test would be of no use in identifying a Gram positive cocci bacteria.
- Since many of the tests utilize agars that are similar in appearance, be sure to label all tubes and plates to ensure that results are collected for the correct test.

NEW! Tips for Success appear throughout the experiments and draw attention to common mistakes and stumbling blocks in the lab. Each tip explains why specific techniques are necessary to yield accurate results and helps guide students on how to perform crucial procedural steps correctly.

Microbiology: A Laboratory Manual, Global Edition

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