Exercise 9

EXERCISE 9

Microbes in the Environment

Whatever is worth doing at all is worth doing well.

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Objectives

After completing this exercise, you should be able to:

1. Describe why agar is used in culture media.
2. Prepare nutrient broth and nutrient agar.
3. Compare bacterial growth on solid and liquid culture media.
4. Describe colony morphology using accepted descriptive terms.

Background

Microbes are everywhere; they are found in the water we drink, the air we breathe, and the earth on which we walk. They live in and on our bodies. Microbes occupy ecological niches on all forms of life and in most environments. In most situations, the ubiquitous microorganisms are harmless. However, in microbiology, work must be done carefully to avoid contaminating sterile media and materials with these microbes.

In this exercise, we will attempt to culture (grow) some microbes from the environment. When a medium is selected for culturing bacteria, macronutrients, an energy source, and any necessary growth factors must be provided. A medium whose exact chemical composition is known is called a **chemically defined medium** (Table 9.1).

Most chemoheterotrophic bacteria are routinely grown on **complex media**—that is, media for which the exact chemical composition varies slightly from batch to batch. Organic carbon, energy, and nitrogen sources are usually supplied by protein in the form of meat extracts and partially digested proteins called *peptones*. **Nutrient broth** is a commonly used liquid complex medium. When agar is added, it becomes a solid medium, called **nutrient agar** (Table 9.2).

Agar, an extract from marine red algae, has some unique properties that make it useful in culture media. Few microbes can degrade agar so it remains solid during microbial growth. It liquefies at 100°C and remains in a liquid state until cooled to 40°C. Once the agar has solidified, it can be incubated at temperatures of up to 100°C and remain solid.

Media must be sterilized after preparation. The most common method of sterilizing culture media that are heat stable is **steam sterilization,** or **autoclaving,** using steam under pressure. During this process, material to be sterilized is placed in the autoclave and heated to 121°C at 15 pounds of pressure (15 psi) for 15 minutes.

Culture media can be prepared in various forms, depending on the desired use. **Petri plates** containing solid media provide a large surface area for examination of colonies. The microbes will be **inoculated,** or intentionally introduced, onto nutrient agar and into nutrient broth. The bacteria that are inoculated into culture

Table 9.1

Glucose–Minimal Salts Broth

Ingredient	Amount/100 ml
Glucose	0.5 g
Sodium chloride (NaCl)	0.5 g
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	0.1 g
Dipotassium phosphate (K_2HPO_4)	0.1 g
Magnesium sulfate ($MgSO_4$)	0.02 g
Distilled water	100 ml

Table 9.2

Nutrient Agar

Ingredient	Amount/100 ml
Peptone	0.5 g
Beef extract	0.3 g
Sodium chloride (NaCl)	0.8 g
Agar	1.5 g
Distilled water	100 ml

media increase in number during an **incubation period.** After suitable incubation, liquid media become **turbid,** or cloudy, due to bacterial growth. On solid media, colonies will be visible to the naked eye. A **colony** is a population of cells that arises from a single bacterial cell. A colony may arise from a group of the same microbes attached to one another, which is therefore called a **colony-forming unit.** Although many species of bacteria give rise to colonies that appear similar, each colony that appears different is usually a different species. (See Color Plate VI.1.)

Materials

250-ml Erlenmeyer flask with cap or plug

100-ml graduated cylinder

Distilled water

Nutrient broth powder

Agar

Glass stirring rod

5-ml pipette

Propipette or pipette bulb

Test tubes with caps (3)

Sterile Petri dishes (4)

Balance

Weighing paper or dish

Autoclave gloves

Hot plate

Tube containing sterile cotton swabs

Tube containing sterile water

Demonstration

Use of the autoclave

Techniques Required

Pipetting, Appendix A

Procedure

First Period

1. Preparing culture media
   a. Prepare 100 ml of nutrient broth in a 250-ml flask. Using the graduated cylinder, add 100 ml of distilled water to the flask. Read the preparation instructions on the nutrient broth bottle. Calculate the amount of nutrient broth powder needed for 100 ml. If the amount needed for 1000 ml is _____ grams, then _____ grams are needed for 100 ml. Weigh out the required amount and add it to the flask. Stir with a glass rod until the powder is dissolved.
   b. Attach a bulb or Propipette to the 5-ml pipette. Pipette 5 ml of the nutrient broth into each test tube and cap each tube. Label the tubes "nutrient broth." Place two in the To Be Autoclaved rack. Label the remaining tube "not sterilized," and incubate it at room temperature until the next period.
   c. Add agar (1.5% w/v) to the remaining 85 ml of nutrient broth. What quantity of agar will you need to add? _____
   d. Bring the broth to a boil and continue boiling carefully until all the agar is dissolved. *Be careful:* Do not let the solution boil over. Stir often to prevent burning and boiling over.
   e. Stopper the flask, label it "nutrient agar," and place the flask and tube in the To Be Autoclaved basket.
   f. Listen to the instructor's demonstration of the use of the autoclave.
   g. After autoclaving, allow the flasks and tube to cool to room temperature, or proceed to part 2. What effect does the agar have on the culture medium? _____
2. Pouring plates
   Transfer the melted sterile nutrient agar flasks to a 45°C water bath. Allow the flask of nutrient agar to cool to about 45°C (warm to the touch). If the agar has solidified, it will have to be reheated to liquefy it. To what temperature will it have to be heated? _____
   The sterile nutrient agar must be poured into Petri dishes *aseptically*—that is, without letting microbes into the nutrient medium. *Read the following procedure before beginning* so that you can work quickly and efficiently.
   a. Set four sterile, unopened Petri dishes in front of you with the cover (larger half) on top. Have a lighted laboratory burner within reach on your workbench.

> ⚠ **Keep the burner away from your hair and in the center of the bench.**

   b. Holding the flask at an angle, remove the stopper with the fourth and fifth fingers of your other hand. Heat the mouth of the flask by passing it through the flame three times (Figure 9.1a). Why is it necessary to keep the flask at an angle through this procedure? _____

c. Remove the cover from the first dish with the hand holding the plug. Quickly and neatly pour melted nutrient agar into the dish until the bottom is just covered to a depth of approximately 5 mm (Figure 9.1b). Keep the flask at an angle, and replace the dish cover; move on to the next plate until all the agar is poured.

d. When all the agar is poured, gently swirl the agar in each dish to cover any empty spaces; do not allow the agar to touch the dish covers.

e. To decrease condensation, leave the Petri plate covers slightly ajar for about 15 minutes until the agar solidifies.

f. Place the empty flask in the discard area.

3. Culturing microbes from the environment

a. Design your own experiment. The purpose is to sample your environment and your body. Use your imagination. Here are some suggestions:

1. You may use the lab, a washroom, or any place on campus for the environment.
2. One nutrient agar plate might be left open to the air for 30 to 60 minutes.
3. Inoculate a plate from an environmental surface such as the floor or workbench by wetting a cotton swab in sterile water, swabbing the environmental surface, and then swabbing the surface of the agar. Why is the swab first moistened in sterile water?

_____

_____

After inoculation, the swab should be discarded in the container of disinfectant.

b. Inoculate two plates from the environment. Inoculate one nutrient broth tube using a swab as described in step 3 of 3a. After swabbing the agar surface, place the swab in the nutrient broth and leave it there during incubation. You may need to break off part of the wooden handle to fit the swab into the nutrient broth.

c. The plates and tube should be incubated at the approximate temperature of the environment sampled.

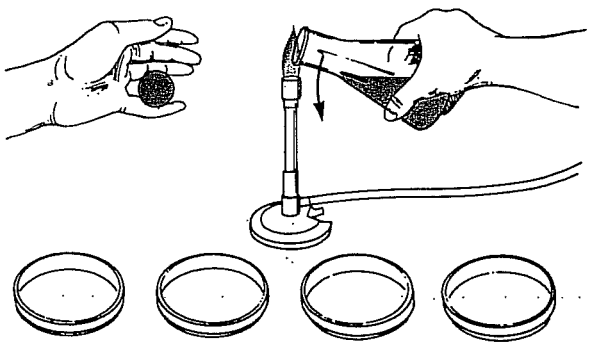
d. Inoculate two plates from your body. You could:

1. Place a hair on the agar.
2. Obtain an inoculum by swabbing (see step 3 of 3a) part of your body with a wet swab.
3. Touch the plate with your fingers.

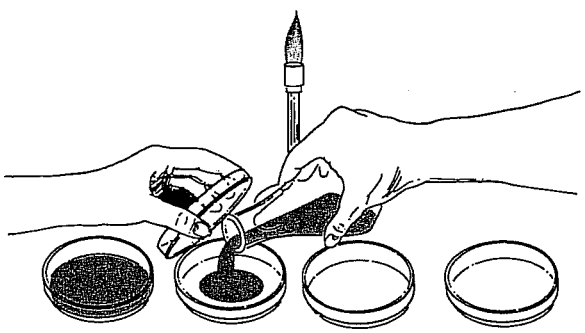
e. Incubate bacteria from your body at or close to your body temperature. What is human body temperature? _____°C

f. Incubate all plates inverted, so water will condense in the lid instead of on the surface of the agar. Why is condensation on the agar undesirable? _____

_____



(a) Remove the stopper, and flame the mouth of the flask.



(b) Remove the cover from one dish, and pour nutrient agar into the dish bottom.

Figure 9.1
Petri plate pouring.

g. Incubate all inoculated media until the next laboratory period.

Second Period

1. Observe and describe the resulting growth on the plates. Note each different-appearing colony, and describe the colony pigment (color) and morphology using the characteristics given in Figure 9.2. Determine the approximate number of each type of colony. When many colonies are present, record TNTC (too numerous to count) as the number of colonies.
2. Describe the appearance of the nutrient broth labeled "not sterile" and the broth you inoculated. Are they uniformly cloudy or turbid? _____ Look for clumps of microbial cells, called **flocculent.** Is there a membrane, or **pellicle,** across the surface of the broth? _____ See whether microbial cells have settled on the bottom of the tube, forming a **sediment.** (See Color Plate X.1.)
3. Save one turbid broth in the refrigerator for Exercise 11. Discard the plates and remaining tubes properly.

Place contaminated plates in the biohazard bag for autoclaving.

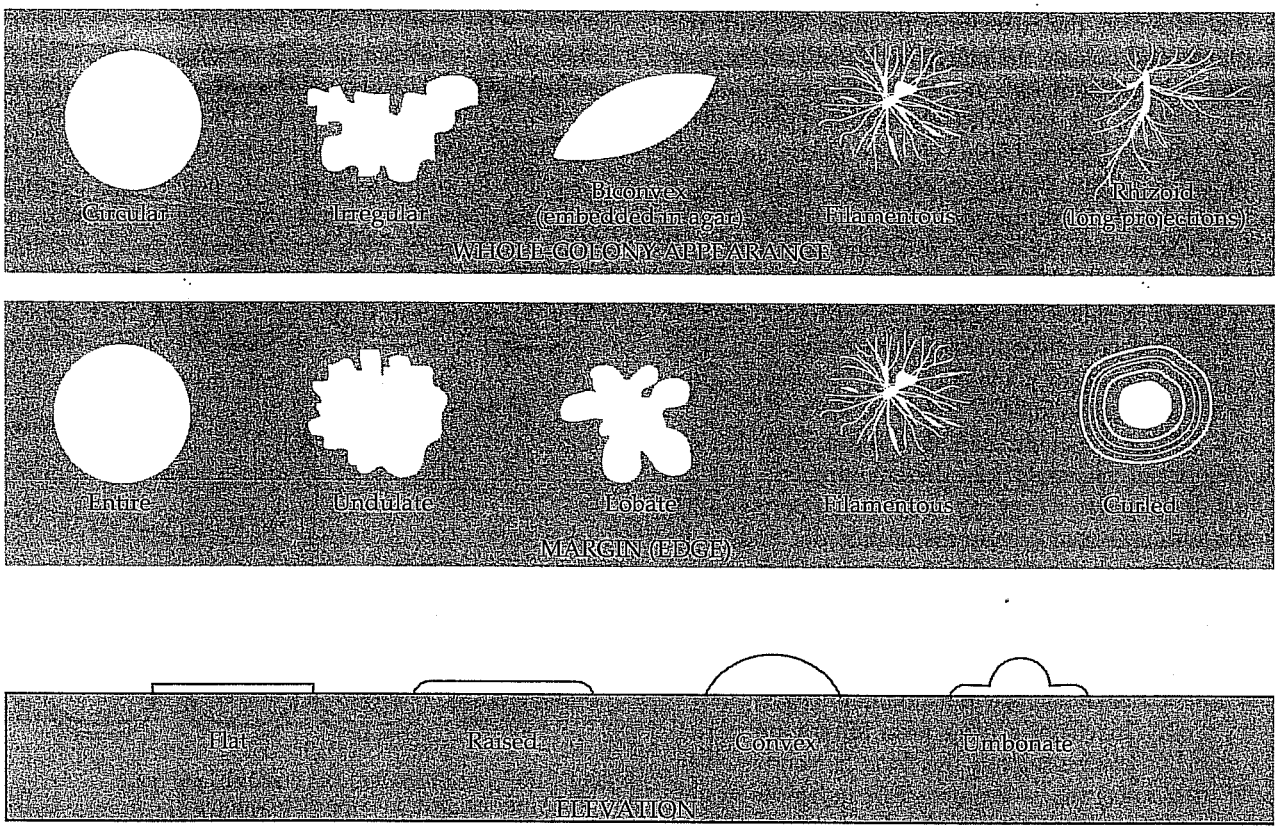


Figure 9.2
Colony descriptions.

Exercise 9

Microbes in the Environment

LABORATORY REPORT

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Fill in the following table with descriptions of the bacterial colonies. Use a separate line for each different-appearing colony. Observe your classmates' plates if you didn't inoculate four plates.

	Colony Description					
	Diameter	Whole-Colony Appearance	Margin	Elevation	Pigment	Number of this Type
Area sampled: _____						
Incubated at _____°C for _____ days						

Area sampled: _____						
Incubated at _____°C for _____ days						

Area sampled: _____						
Incubated at _____°C for _____ days						

	Colony Description					
	Diameter	Whole-Colony Appearance	Margin	Elevation	Pigment	Number of this Type
Area sampled: _____						
Incubated at _____°C for _____ days						

Nutrient broths: Incubated at _____°C for _____ days

	Not-Sterilized Broth	Sterilized Broth Not Inoculated	Inoculated Broth Area Sampled: _____
Turbidity			
Flocculent present			
Sediment present			
Pellicle present			
Color			

Questions

1. How can you tell whether there is bacterial growth in the nutrient broth? _____

2. What is the minimum number of different bacteria present on one of your plates? _____

   How do you know? _____

3. What is the value of Petri plates in microbiology? _____

4. What are bacteria using for nutrients in nutrient agar? _____

_____

What is the purpose of the agar? _____

_____

5. Which environment has the most total bacteria? _____ The least? _____ Provide a reason for the differences in total bacteria in these two places. _____

_____

_____

6. Which environment has the most different bacteria? _____ The least? _____

Provide a reason for the differences in bacteria in these two places. _____

_____

_____

Critical Thinking

1. Why is agar preferable to gelatin as a solidifying agent in culture media?

2. Did all the organisms living in or on the environments sampled grow on your nutrient agar? Briefly explain.

3. How could you determine whether the turbidity in your nutrient broth tube was from a mixture of different microbes or from the growth of only one kind of microbe?