Exercise 3
CULTURE OF MICROORGANISMS & MEDIA PREPARATION

Introduction

A. Culture of Microorganisms

In nature, microorganisms exist as mixed populations of many widely differing types; however, most knowledge of microbes has come from the study of isolated species growing under controlled conditions in an artificial environment. This environment, known as the **culture medium**, is typically maintained free from contamination by other organisms, and must provide conditions necessary for growth including; moisture, essential nutrients, proper pH, osmotic pressure, atmosphere, etc. New types of **media** are constantly being developed and old recipes revised in an effort to isolate and identify bacteria; however, essentially all culture media can be divided into two basic categories; 1) liquid or **broth media**, and 2) **solid media**.

1. Broth Media

Broth media are liquid at room temperature and are typically contained within tubes or flasks. There are numerous recipes for broth culture media depending on the types of organisms you wish to grow. Growth may appear as:

a. **Turbidity** - cloudiness in the liquid,
b. **Pellicle formation** - a thin coat of cells floating on top of the broth,
c. **Sediment** - a deposit of cells at the tube bottom that will swirl upward if the tube is tapped gently. Many bacteria will maintain their characteristic shape and arrangement better in broth media than on solid media. Because all cells are in contact with the nutrients available (are suspended in it), growth may occur more readily in broth media than on solid media.

2. Solid Media

Solid media are made by adding a solidifying agent (usually agar) to what would otherwise be broth media. Agar is a complex polysaccharide produced by marine algae and is ideal for laboratory use because it becomes soluble in liquds at temperatures of 97-100^{o} C, and solidifies at around 42^{o} C. This means media containing agar will be solid at room temperature and will remain solid even at relatively high incubation temperatures. Most microorganisms do not use agar as a nutrient source, therefore it retains its solidifying effect despite metabolic activity. Since media containing agar behave as liquids when hot, and are usually solid at room temperature, they may be placed into a variety of containers as follows:

a. **Agar tubes (deeps)** - Media containing about 1% agar will solidify at the bottom of glass culture tubes maintained in a vertical position. Such tubes are called deeps, and can be used to test the ability of bacteria to catabolize nutrients under anaerobic conditions since air is not readily able to penetrate the media.

b. **Agar slants (slopes)** - Agar slants or slopes are formed when tubes of molten agar-containing media are placed at an angle during cooling. The tube contents then harden with a slanted or sloped surface that can easily be inoculated. Agar slants are commonly used to maintain stock cultures because they provide a fairly broad surface for growth and observation, but do not dry out as fast as will agar plates.

c. **Streak plates** - Molten agar media poured into Petri plates will cool to form broad, thin slabs of solid media known as streak plates. These can be inoculated with mixed cultures, and colonies of individual types can be easily separated for isolation and identification.

d. **Pour plates** – Molten agar media can be cooled slightly, bacteria and virus particles can be added, and the mixture poured into a Petri plate (often over a layer of bottom agar). Bacteria growing within the agar can support virus reproduction, and free virions can be collected on wire loops stabbed into the plaques that form (clear areas in the agar surface).

Culture media can also be categorized on the basis of composition and the type of organisms supported. Thus they may be categorized as either 1) **complex** or 2) **defined** media.

1. **Complex media**

   Complex media are those that provide all the nutrients necessary for the growth of micro-organisms, but in crude form, i.e., the exact chemical composition of such media is unknown and varies slightly from one batch to the next. Complex media commonly contain extracts of yeast, meats, or vegetable materials such as soybeans and/or digests of proteins from such sources. Energy, carbon, nitrogen and sulfur requirements are provided by the proteins or protein breakdown products (peptones, proteoses, etc.) present in such media. Vitamins and other essential growth requirements are also available. Some examples of complex media include nutrient broth/agar, trypticase soy broth, tryptic soy agar, brain heart infusion, and potato dextrose agar. Remember that **agar is not itself a nutrient**, but may be added to a nutrient mixture as a solidifying agent.

2. **Defined media** (Chemically defined media)

   Defined media or chemically defined media are those for which the exact chemical composition is known. These contain nutrients in relatively pure chemical form and in specified amounts. Defined media often contain long lists of ingredients and are more time consuming to prepare than are complex media. Two examples of defined media are the *Pseudomonas* and *Azotobacter* enrichment media used in the culture of bacteria from soil. The concentration of each constituent is indicated in grams per liter.

***Pseudomonas* Enrichment Medium**		***Azotobacter* Enrichment Medium**	
Sodium Benzoate	1.00 g	Mannitol	10.0 g
$MgSO_4$ (magnesium sulfate)	0.05 g	Mg SO_4 (Magnesium sulfate)	0.1 g
$FeCl_3$ (Ferric chloride)	0.05 g	$FeCl_3$ (ferric chloride)	0.02g
$CaCl_2$ (Calcium chloride)	0.005 g	NaCl (sodium chloride)	0.2 g
NH_4Cl (ammonium chloride)	1.00 g	H_2MoO_4 (Molybdic acid)	.002 g
Buffer Solution (see below)	33.00 ml	$CaCO_3$ (Calcium carbonate)	10.0 g
(1M Na_2HPO_4 and 1 M KH_2PO_4 - pH 6.8)		K_2HPO_4 (Potassium phosphate)	0.5 g
Distilled water to equal	1000 ml.	Distilled water to equal	1000 ml.

(For solid media, add purified agar (noble agar) to equal 1.5% of the above solutions.)

Other specific recipes for microbiological media may be found in the *Bergey's Manual of Systematic Bacteriology* (first and second editions) and *BMSAB*, in commercially available catalogs such as the *Difco Manual*, in books or periodicals dealing with the culture of specific microbes, and from a variety of on-line sources.

B. Media Preparation

In this laboratory, students are usually provided with various forms of media that have been prepared ahead of time by a laboratory technician. All of our culture media are **sterilized**, i.e. rendered free of any viable cells, prior to use. During this exercise you will have an opportunity to make your own culture medium and to observe some of the techniques used in media preparation.

The medium used for this exercise is a dehydrated form of a complex solid medium (Nutrient agar) that can be used for the culture of microorganisms not requiring vitamins, minerals or other growth factors. The directions for the preparation of this medium are written on the side of the container and should be followed accurately.

Procedure:

A. Media Preparation

1. Working in small groups (2-3 students), prepare 100 milliliters (100mL) of nutrient agar according to the directions given on the container and the instructions given in class.

2. Have each student in your group prepare one agar plate by carefully pouring the slightly cooled liquid agar into the bottom of a sterile Petri plate. Be careful not to touch the sides or top of the plate while pouring. Make certain each plate contains a layer of medium not more than 5 mm thick over the bottom surface.

3. Cover the plates and allow them to cool thoroughly without disturbance in order to preserve a smooth surface on the agar.

4. Use the remaining medium to prepare agar slants by pouring hot liquid agar (approximately 15 mL) into large culture tubes, capping these tubes, and placing them on the slant board provided. **Please do not waste media!**

Note: Media prepared for student use is normally sterilized by autoclaving, a step omitted from this exercise in the interest of saving time. The agar slants made during this laboratory will be autoclaved and reslanted before they are made available for class use.

B. Medium Exposure and Incubation

1. Take your agar plate home or to a location of your choice (permission may be required under some circumstances). Indoor locations are preferable.

2. Place the plate agar-side-down on a stable surface, carefully remove the lid, and place it to one side, face down and supported by the plate edge.

3. Expose the agar surface to the air in the selected environment for approximately one hour and then replace the lid. Avoid exposing the agar for longer periods of time as this can result in desiccation of the medium and restricted growth.

4. Record specific information relating to the exposure (location, temperature, wind, etc.) on the worksheet provided. Return the closed plate to the laboratory and place it in a lab drawer for incubation at room temperature.

Questions:

1. What advantage is there to growing microorganisms in broth or liquid media?

2. What general type of organism might you expect to produce a pellicle growth form, a clouded medium, or sediment at the tube bottom? (Refer to the section of your textbook covering oxygen requirements and microbial growth.)

3. What is agar, and why is it an ideal solidifying agent for microbiological media?

4. In what ways do defined media differ from complex media?

5. Why are culture media normally sterilized prior to use?

6. How many different types of bacteria appear to be growing on the plate you exposed to air?

Name_____ **Lab Section**_____

WORKSHEET
Exercise 3
Culture of Microorganisms & Media Preparation

Goals: _____
_____

Hypothesis: _____
_____

Materials & Methods:
Nutrient agar was prepared using the procedure described and exposed to air as specified here:
_____
_____

Incubation temperature: _____ Duration of incubation: _____

Data & Results:
Total number of colonies observed: _____

Morphological features of four different looking colonies (if you have that many):

	Colony A	Colony B
Form:		
Margin:		
Elevation:		
Surface Texture:		
Optical Character:		
Pigmentation:		
Size (mm):		
Other:		

	Colony C	Colony D
Form:		
Margin:		
Elevation:		
Surface Texture:		
Optical Character:		
Pigmentation:		
Size (mm):		
Other:		

Conclusions:

Was your hypothesis correct? _____ Explain. _____

_____

_____

_____

What does your data tell you about the environment sampled? _____

_____

_____

_____