Minot State University
Biol 142
Microbiology
Lab Exercise I

Part 1
PREPARATION AND USE OF GROWTH MEDIA

MEDIA

Microorganisms require specific nutrients and growing environments which can often be replicated in the laboratory. One of the most commonly used media types is called **nutrient broth** (liquid form) or **nutrient agar** (solid form). Nutrient broth is mostly water in which beef extract is dissolved. Beef extract is prepared by soaking beef in water, allowing soluble carbohydrates, nitrogenous substances, vitamins and minerals to leach into the water. The nutrients are then concentrated into a powder by dehydration. Some nutrients are added to the beef extract. These include small chains of proteins, made by partial breakdown of large proteins. These chains of amino acids are called **peptones**. Most of the bacterial strains we will use in this course grow well on nutrient broth/agar. Media used to grow some bacterial strains, yeasts, molds etc may contain additional nutrients, different amounts of nutrients or may be made to be more acidic or basic.

Solid media are prepared by adding **agar**, usually at about 1.5%. Agar is a polysaccharide (chain of sugar molecules) isolated from seaweed. It is added in power form to liquid media, dissolved by heating and the mixture (broth + agar) and allowed to cool. Agar solidifies at ~40 degrees C. and melts at ~100 degrees C so it can be used as a solid media at very high incubation temperatures. Agar itself is not a nutrient.

As mentioned above, not all organisms can be grown in the lab using simple media like nutrient broth/agar. Organisms with complex nutritional requirements must be grown on special media with specific added nutrients. Some media are designed to support the growth of one organism while suppressing the growth of another. These media are called **selective media**. Other media are made with specific indicators that allow differentiation of similar organisms on the usually on the basis of specific biochemical properties of one of the organisms. These are called **differential media**. Media containing special nutrients to allow the growth of specific rare organisms that are present in very low numbers is called **enrichment media**.

Media can be dispensed into a number of different types of containers, depending on the use intended. Broth media is usually used in flasks or bottles, whereas solid media can be dispensed into tubes (slants\slopes, stabs) or into plates of various sizes.

STERILIZATION

Sterilization refers to any procedure that eliminates life forms. In the microbiology lab we use sterilization to kill microorganisms---after we have observed an experiment using them, and to prevent contamination of newly made media/plates etc. Liquid media (with or without added agar) is prepared in an **autoclave**, which is much like a household pressure cooker. The autoclave allows temperatures of water/liquids to exceed boiling (100 degrees C). In fact some heat resistant bacteria require prolonged temperatures of 120 degrees C. Media is thus prepared in an autoclave, which keeps liquids under sufficient pressure to reach these temperatures. Liquids that cannot be heated can be sterilized by other means such as by **filtration**. Filters of various sized pores can be used. A good cut off for filtration of bacteria is 2 μM pore size. Finally **irradiation** is occasionally used to kill microorganisms on surfaces such as foods. Irradiation has recently been put into practice to kill bacteria in meats sold by grocery store.

MEDIA PREPARATION EXERCISE

Procedure

Media will have been prepared for you by measuring out dry ingredients, adding them to water and sterilizing by autoclave.

Recipe for nutrient agar: (1 liter)

Peptone	5 grams (g)
Beef extract	3 g
Agar	15 g
Distilled water	1000 milliliters (ml)

Autoclave (120 degrees C for 15 minutes)

Each student will carefully pour one plate of nutrient agar. Pour liquid until it just covers the plate. Let sit undisturbed for the duration of the lab.

1. Describe what happens to the plate.

2. Take the plate home and "sample your environment" (see part 2) at home. Leave the plate at room temperature and make daily observations until next lab period.

3. Solution exercise:
Modify the recipe for nutrient (agar above) to make:
a) 5 liters
b) 200 milliliters

***Think about altering recipes for cookies or pancakes or anything!

Part 2
WIDESPREAD DISTRIBUTION OF MICROORGANISMS

Microorganisms are the most wildly distributed of all living things. They are found in soil, air, and water. They live both on and in you. They can be found living at the polar ice caps, and in the near-boiling hot springs of Yellowstone National Park. Most of these organisms are harmless and many are *essential* for life as we know it.

This exercise is designed to illustrate that "we are not alone"---that microorganisms are virtually everywhere we live, work, study, eat, and sleep. We will also compare some household "disinfectants" to see whether they are effective in eliminating microorganisms in our environment.

Each group will sample several areas of their choice to show whether microorganisms are present there. In a separate experiment each group will choose 2 common cleaners to do "before and after" testing for disinfectant efficiency.

Materials

4 small Nutrient agar plates/pair of students
2 large Nutrient agar plates/pair of students
Sterile cotton swabs
Household cleaners
Incubator
Permanent marking pen

Procedure

1. Expose (remove the lid) one of the small plates to air as soon as possible.

2. Use 3 small plates to "sample your environment" by *swabbing* a surface and *streaking* the contents of the swab onto the plate.

3. Use 2 large plates to test the effectiveness of some of the household cleaners provided.
    - **a.** Streak onto half of one of the large plates from a surface/orifice/body part as you did in step 2.
    - **b.** Clean the surface/orifice/body part with an appropriate cleaner used as directed or as you normally do.
    - **c.** Streak onto the other half of one of the large plates after cleaning the surface/orifice/body part.
    - **d. Make sure you have thought of the necessary *controls* for your experiment.**

Design an accurate method for keeping track of what is on each of the 6 plates! Place all 6 plates in the 37 degree incubator, lid side down. Dr. Super will remove in 24-48 hours and place in the fridge.

Observations will be made at the next lab session.