Preparation of Media for Bacterial Growth

Introduction

The growth of bacteria in the research, teaching or clinical laboratory is of great importance. This is because research labs may need the bacteria to perform a specific task, the teaching lab needs the bacteria for learning and/or the clinical lab functions to identify disease-causing bacteria for appropriate treatment. Bacteria, however, are a bit finicky when it comes to growing on artificial (man-made) media.

Not all bacteria grow optimally on the same kind of medium, nor do all bacteria grow optimally at the same temperature (but that's another experiment). Although the growth of bacteria on different kinds of media will be studied in a later experiment, the preparation of media so bacteria can be "planted" to grow is the focus of this experiment. Additionally, this experiment will supply the student with the basic laboratory skills for media preparation.

Experimental

Materials

Cotton batting	Electronic pan balance	Tryptic soy agar	Simmonds's citrate agar
Cheesecloth	Weighing boats	Mueller-Hinton agar	Voges-Proskauer agar[5]
Autoclave	Nutrient agar[1]	Nitrate agar[3]	Methyl red agar[6]
Petri dishes	Macconkey agar[2]	Tryptic nitrate agar[4]	wide mouth flask
Indelible marker	Refrigerator	Disinfectant	Paper towels

Recipes
[1]4 grams of nutrient broth plus 7 g agar powder; [2]2.5 grams bile salts, 10 g peptone, 5 g lactose, 7.5 g agar and a TINY pinch of bromocresol purple; [3]1.5 g Beef extract, 2.5 g peptone, 0.5 g KNO_3 and 6 g agar; [4]10 g peptone, 0.5 g dextrose, 1 g Na_2HPO_4, 0.5 g KNO_3 and 7.5 g agar; [5]7 g peptone, 5 g K_2HPO_4, 5 g dextrose and 7.5 g agar; [6]6 g agar, 3.5 g peptone, 2.5 g K_2HPO_4 and 2.5 g dextrose. Dissolve each recipe in enough water to make 500 mL of each media.

Skill #1: Operating the Electronic Pan Balance and Hydrating the Media Mixture

Your professor will demonstrate the use of an electronic pan balance. You need only be concerned with two buttons: On/Off and "TARE". "On/Off" is self-explanatory; "TARE" is not. Suffice it to say that "TARE" balances the balance at "0". When using the balance, first turn it on and second, "TARE" the instrument. After the instrument is balanced at "0", place a weighing boat on the pan and press "TARE", again. When the LED reads "0.0000", it is time to mass your agar and other reagents for your respective recipes. For those agars for which you do not find recipes, read the sides of the bottles in which they are dispensed.

> NOTE: if "U" shows up in the lower right corner of the LED, DO NOT record the value -- "U" means unstable – just as soon as the "U" disappears, record your value.

When massing each individual reagent, use a different boat for each reagent. The boats are disposable -- throw them away after you have used each boat once. Obtain a wide-mouthed flask and pour the powders into the flask through a paper-towel funnel. Dispose of the funnel as instructed by your instructor. Add enough distilled water to the solid mixture to bring the water level to the 500 mL mark on the flask. Obtain a piece of cotton batting approximately 8" on a side and place it on a piece of cheese-cloth that is about 9" on a side. Make a plug out of the two pieces of material and place it in the neck of the flask with the cheese-cloth between the cotton and the neck of the flask.

Skill #2: Sterilizing and Melting the Medium

Place the plugged flask in the autoclave. Pour enough water beneath the rack that it just comes up to the lip inside the chamber. Close the lid by hooking the claw over the copper bar with a snap. Press "reset", make certain that the exhaust is set to "Slow -- liquids" and set the timer for 15 minutes.

Skill #3: Labeling Petri Dishes

While your media is sterilizing in the autoclave, obtain a package of 20 petri dishes. Open the package and place all petri dishes on your disinfected lab bench

with the small portion UP. Write the date, the kind of agar and your initials on a small portion of the plate with indelible marker. Next turn all the petri dishes over so that the big portion is UP. Wait until the autoclave is done sterilizing before proceeding with the petri dishes.

Skill #4: Cooling the Agar and Pouring Agar Plates

When the autoclave is done (i.e., light is out, pressure is at "0" instead of at 15 psi and temp is < 121°C), open the door carefully while wearing autoclave gloves. Open the door from the side so you do not get "blasted" by hot steam. Once the initial release of steam is done, open the door the rest of the way. Remove your flask, while wearing autoclave gloves, of the now melted and sterile agar. Place your flask of HOT agar in the water bath. (The water bath is set at about 48°C. This will allow the agar to cool, but not to go less than 40°C when it will solidify). Let it set for about 30 minutes. Once the thirty minutes have passed, remove the warm agar from the water bath, remove the plug from the opening of the flask and begin pouring the mixture into the small portion of the petri dish. You need only pour about 0.5 - 0.75 cm of agar into the plate. Cover the plate before advancing to the next plate. Repeat the procedure until all plates have melted agar in them. Dispose of the left-over agar and rinse your flask as directed by your instructor. Let the agar plates cool until solid, then turn them upside down for storage in the refrigerator. These plates will be used throughout the semester.

REFERENCES

1._____: **DIFCO Manual: Dehydrated Culture Media and Reagents, 10th Edition**. (Difco Laboratories: Detroit) ©1984, Pp. 543, 546, 616, 1023.