Experiment 3

PREPARATION OF NUTRIENT AGAR

Bacteriological media come an a wide range of types. Nutrient Agar is a **complex** medium because it contains ingredients with contain unknown amounts or types of nutrients. Nutrient Agar contains Beef Extract (0.3%), Peptone (0.5%) and Agar (1.5%) in water. Beef extract is the commercially prepared dehydrated form of autolysed beef and is supplied in the form of a paste. Peptone is casein (milk protein) that has been digested with the enzyme pepsin. Peptone is dehydrated and supplied as a powder. Peptone and Beef Extract contain a mixture of amino acids and peptides. Beef Extract also contains water soluble digest products of all other macromolecules (nucleic acids, fats, polysaccharides) as well as vitamins and trace minerals. Although we know and can define Beef Extract in these terms, each bach can not be chemically defined. There are many media ingredients which are complex: yeast extract, tryptone, and others. The advantage of complex media is that they support the growth of a wide range of microbes.

Agar is purified from red algae in which it is an accessory polysaccharide (poly-galacturonic acid) of their cell walls. Agar is added to microbiological media only as a **solidification agent**. Agar for most purposes has no nutrient value. Agar is an excellent solidification agent because it dissolves at near boiling but solidifies at 45°C. Thus, one can prepare molten (liquid) agar at 45°C, mix cells with it, then allow it to solidify thereby trapping living cells. Below 45°C agar is a solid and remains so as the temperature is raised melting only when >95°C is obtained.

In this experiment each student will prepare 200 ml of Nutrient Agar to be used in Experiment 4. In subsequent experiments, the media ingredients can be found in the Appendix. It is important for you to know how each medium works: what is the energy source? what is the carbon source? what is the nitrogen source? does the medium have selective or differential ingredients? why can only some types of bacteria grow on the particular medium?

MATERIALS

1. Electronic or beam balances.
2. Weigh boats, tongue depressors.
3. Tripods, asbestos wire-gauze, asbestos gloves.
4. 10 ml nonsterile pipettes.
5. pH paper or pH meter with standard buffers.
6. 4 13x100 mm screw capped culture tubes.
7. Graduated Cylinder, 250 ml.
8. 2 500ml Erlenmeyer Flasks
9. Beef Extract, Peptone, Agar.
10. 3 N HCl, 3 N KOH.
11. 16 x 150 mm screw cap culture tubes.

12. Nonabsorbent cotton and gauze to make cotton stoppers.

Nutrient Agar

Beef Extract:	0.3%	
Peptone:		0.5%
Agar:		1.5%

PROCEDURE

1. You will be making 200 ml of Nutrient Agar. To weigh out Beef Extract, first tare a tongue depressor, then dip it into the Beef Extract and weigh. Adjust the amount of Beef Extract until the correct amount is obtained. Be sure to be careful not to get Beef Extract on to the balance! You need to weight out enough Beef Extract to get a 0.3% solution. Place the tongue depressor into the flask, beef extract side down.

2. Tare a weigh boat and weigh out enough Peptone and add that to the flask.

3. Add 200 ml of distilled water and swirl to dissolve the peptone and beef extract. Check the pH, it should be 7.0.

4. Tare a weigh boat and weigh out enough Agar and add that to the flask.

5. With a bunsen burner, tripod, asbestos wire-gauze, heat the medium to boiling to dissolve the agar. CAREFUL: 1) keep the rotating the flasks to prevent the agar from cooking onto the bottom of the flask and 2) watch out: boiling agar can froth and boil out all over the lab bench. As soon as it begins to boil take it off the heat and put it on to the bench. Allow it to cool a few minutes.

6. While the agar is still warm, but not hot, pipette 3 ml each into 4 13x100 mm screw cap culture tubes.

7. Label the flask and your tubes with your name.

8. After preparation of your medium, the instructor will take you to the autoclave.

9. Place your media in the autoclave with those of the rest of the class.

10. After discussion of the parts of the autoclave, autoclave the medium for 20 minutes.

11. The media will be saved and used in Experiment 4.

QUESTIONS

1. What are the functions of each ingredient in the medium.

2. What is the buffer in Nutrient Agar? Why do media have buffers?

3. What is the function of the nitrogen source? sulfur source? phosphorus source?