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, Peptone and Agar 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 batch 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^{o}C. Thus, one can prepare molten (liquid) agar at 45^{o}C, mix cells with it, and then allow it to solidify thereby trapping living cells. Below 45^{o}C agar is a solid and remains so as the temperature is raised melting only when >95^{o}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. Hot plate.
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

PROCEDURE

1. You will be making 200 ml of Nutrient Agar by adding 6.2 g of Nutrient Agar in 200 ml of distilled water. **HINT:** Make sure to tare a weight boat before weighting the agar.

2. Check the pH, it should be 7.0.

3. Heat the medium to boiling to dissolve the agar. **CAREFUL:** 1) keep 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.

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

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

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

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

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

9. 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?