

PRACTICAL NO 9

Preparation of basic liquid Medium (broth) for routine Cultivation of Bacteria

Bacteria are often cultivated in liquid broth (media lacking agar)

Materials: Peptone 5g, Beef extract 3 g, distilled water 1 L, 0.1 N HCl, 0.1 N NaOH, pressure cooker, 1 L beaker measuring cylinder, non-absorbent cotton, test tube and pH paper.

Procedure: Take the weighed amounts of peptone and beef extract and mix in 50 ml of distilled water and heat it to dissolve the contents. Add more distilled water to make it to 1 L. Adjust the pH to 7 using pH papers by adding either acid or alkali as the case may be. Take this into the test tube and apply cotton plug, sterilize at 15 lbs pressure for 15 mts in pressure cooker. Allow the pressure cooker to cool, remove the nutrient broth tubes and store at room temp and cover with butter paper.

Preparation of Basic Solid Medium

Liquid broth media containing nutrients are usually solidified by the addition of agar.

Eg. Potato Dextrose agar medium, Nutrient agar medium.

A) Preparation of Potato Dextrose Agar Medium: Used in isolation and maintenance of common fungi.

Materials: Peeled potatoes - 200g, Dextrose - 20 g, Agar - 20 g, Distilled water 1 L, beaker 1L, 250 ml conical flasks, knife, muslin cloth, measuring cylinder, cotton non-absorbent, pressure cooker.

Procedure

1. Take 500 ml of distilled water in 1L beaker and add 200g of peeled and sliced potato boil the potatoes till they become soft.
2. Filter the contents of the beaker through muslin cloth and squeeze out all liquid
3. Add the dextrose dissolved in water to this extract.
4. Adjust the pH of medium to 6 to 6.5 using 0.1 N HCl or 0.1N NaOH as the case maybe
5. Add the dissolved agar to dextrose-potato extract and make the volume to 1lt and dispense 200ml each to 5 conical flask and plug with non absorbent cotton. Sterilise the flasks at 15 lbs pressure for 15 mts in a pressure cooker.

6. Allow the pressure cooker to cool, "Remove the conical flask and store at room temperature. Allow the flask to cool until the flask can be held by hand.
7. Prepare agar plate by pouring the media into Petri-dish quickly. Using aseptic condition, allow the media in Petri-dish to solidify to produce the agar plate.

INTRODUCTION TO PLANT PATHOGENS

PRACTICALS