

PRACTICAL NO 10

Preparation of Nutrient Agar Medium:

Used for the maintenance and isolation of bacteria.

Materials: peptone - 5g, beef extract - 3g, Agar - 20g, distilled water - 1lt, Petri-dish, 1lt beaker, 250 ml conical flasks, measuring cylinder, non absorbent cotton, pressure cooker and hot plate.

Procedure

1. Dissolve the weighed amounts of peptone and beef extract into 500 ml of water.
2. Heat and dissolve the chemicals and adjust the pH of medium to 7 by adding 0.1N HCl or 0.1N NaOH.
3. Weigh 20g agar and dissolve in 500 ml of distilled water in another beaker
4. Mix the dissolved agar with chemical solution and make up the vol. to 1lt.
5. Dispense 200 ml each into 5 conical flasks.
6. Plug the flask with non absorbent cotton and sterilise at 15 lbs pressure for 15 mts in a Pressure cooker.
7. Allow the cooker to cool, remove the conical flask and store at room temp, or
8. Allow the flask to sufficiently cool and prepare agar plates by pouring media into Petri-dish under aseptic condition; allow the media with Petri-dish to solidify.

Precautions

1. Don't pour the media over 2/3 of flask capacity.
2. Cotton plug must be loose while autoclaving.
3. Don't pour media to Petri-plate when the medium is too hot since it produces condensation of water on underside of Petri plate lid and thus can fall on to agar surface and may lead to contamination and spreading of colonies.
4. Pour medium quickly to avoid contamination by air-pores and close lid down as soon as possible.
5. Perform the pouring of medium in inoculation chamber fitted with U. V. lamp with filtered air.
6. Pouring should be performed near the flame.

Observation

- After sterilization of medium observe the medium in conical flask and plate for solidification.
- After incubation period of 24-48 hrs for nutrient agar medium and 7 days for PDA observe the growth of any microbe on the surface of the medium.

Exercise

1. Prepare 500 ml nutrient agar medium.
2. Prepare 500 ml potato dextrose agar medium

Dr. Muhammad Naveed Aslam
INTRODUCTION TO PLANT PATHOGENS
PRACTICALS

INTRODUCTION TO PLANT PATHOGENS

PRACTICALS