EXPERIMENT NO-7

DATE-12.8.15

AIM OF THE EXPERIMENT

Preparation of culture media.

THEORY

Media may be classified as natural and synthetic and they may be either solid or liquid. Natural media eg- nutrient contains organic matter derived from some animal and vegetative sources and are often best for growth purpose. Synthetic media eg- czapek's media are prepared from chemical substance obtainable in a state of purity. They are therefore of standard compositions at whatever kind and place they are made up, there is a good advantage of description and diagonistic purpose.

Fungi and bacteria are cultured in both solid and liquid media. Solid media have an advantage in being more easily handled without danger of spoiling and with less degree of contamination than liquid media. They are also essential for isolating organisms.

SOLID CULTURE MEDIA

AGAR MEDIA

They are usually prepared in tube or ~~stracts~~ slants. These are prepared by filling the tube one-third after sterilization allowing the liquid agar to solidify when the tubes are kept in inclined position.

The agar is dissolved by heating with the medium for that and have more preferably 15-20 lbs pressure by autoclave and the medium is then filled through a thin layer of dump absorbent cotton wool. It is then run into test tube by means of a dropping funnel arrange before use. The tubes

are plugged by cotton and sterilized in the usual way. Ordinary agar contains soluble impurities and when required for special purpose the pure form should be employed and washed in running water for 12-24 hours before use.

The following are the agar media which have found satisfactory for various plant pathogen –

i) Czapeck's Agar Media

Nutrient Broth

It contains the following substances –

- Sodium nitrate – 25 gm
- Potassium hydrogen phosphate (K_2HPO_4) – 1 gm
- Potassium chloride (KCl) – 0.5 gm
- Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) – 0.5 gm
- Ferrous sulphate ($FeSO_4$) – 0.01 gm
- Sucrose – 30 gm
- Agar – 15 gm
- Distilled water – 1000 cc

PROCEDURE

15 gm of agar-agar is weighted and added 1000 cc of distilled water (heated). To this cocoid like liquid 25 gm of sodium nitrate, 1 gm of potassium hydrogen phosphate, 5 gm of potassium chloride, 0.5 gm of magnesium sulphate, 0.01 gm of ferrous sulphate and 30 gm of sucrose added. This mixture is sterilized in the autoclave at 15 lbs pressure and then make ready for experiment.

ii) POTATO DEXTROSE AGAR MEDIA

This media is very satisfactory for the growth of many pathogenic fungi and bacteria. It consists of

- Potato tubers – 200 gm
- Agar agar – 20 gm
- Dextrose – 20 gm
- Distilled water – 1000 cc

PROCEDURE–

To prepare potato dextrose agar (PDA) media 200 gm of peeled potato are taken in a conical flask, and 500 cc of distilled water is added to it. In another flask 20 gm of agar is taken in 500 cc of distilled water. The flasks are plugged with cotton and kept in autoclave for steaming. After steaming off for about half an hour the flasks are taken out. The potato extracts are filtered and the filtrates is then added to the melted agar. The 20 gm of dextrose is added to the mixed solution and steamed for sometime. Some clean test tubes are taken in a little quantity of the liquid media is poured in each tube. The tube is then plugged with cotton plugs and are placed inside the autoclave. The autoclave is started and the outlet of the steam is kept open so that the steam can come out through the outlet. After about 12 hr steaming outlet is closed. Then the pressure will rise which will be indicated in the pressure gauze. Then it is switched off and the pressure steadly come down to zero and the test tubes are taken out. When they are cooled to some extent they are kept in a slanting position so that the liquid media can't touch the cotton plug. When the liquid media in the test tubes are

cooled, it remains in solid state and the preparation is taken to completed.

iii) Richard's Agar Media

The following are the composition of the medium –

- Potassium nitrate (KNO_3) – 10 gm
- Potassium dihydrogen phosphate ($K_2H_2PO_4$) – 5 gm
- Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) – 2.5 gm
- Ferric chloride ($FeCl_3$) – 0.02 gm
- Sucrose – 5 gm
- Agar agar – 15 gm
- Distilled water – 1000 cc

All the chemicals are dissolve in 500 ml of distilled water in a flask. Agar is dissolve in 500 ml of water in a separate flask by heating. Both the solution are mixed together and the volume is made up to 1000 cc. The sterilization is as usual

LIQUID CULTURE MEDIA

For many of the experiments liquid nutrient media are necessary. There are a number of liquid media under different names with different composition. Some more useful ones are as follows –

1) Richard's SOLUTION-

This media is the best suitable for the growth of fungi. Its composition are as follows –

- Sucrose – 50 gm
- Potassium nitrate – 10 gm
- Potassium dihydrogen phosphate – 5 gm

- Magnesium sulphate – 2.5 gm
- Ferric chloride – 0.2 gm
- Distilled water – 1000 ml

ii) Czapeck's CULTURE SOLUTION

The following are the composition of the solution –

- $MgSO_4 \cdot 7H_2O$ – 0.5 gm
- K_2HPO_4 – 10 gm
- KCl – 0.5 gm
- $FeSO_4$ – 0.1 gm
- $NaNO_3$ – 2 gm
- Sucrose – 30 gm
- D/H_2O – 1000 ml

All the above mentioned chemicals are dissolved in water. In order to avoid precipitation, each of the ingredient is dissolved in small amount of water first and then those solution are mixed together. Volume is made upto 1000 ml, by adding required amount of water. The use of K_2HPO_4 instead of $K_2H_2PO_4$ produce a high pH and is therefore desirable when bacteria are to be cultivated. The sterilization is done as usual manner.