Aim 19

To Prepare the Culture Media for Microorganisms

Introduction

Microorganisms required nutrients for the growth and maintenance. The medium in which culture (population of microorganisms) grows on or in nutrient in lab condition is called culture medium. For microbiological work, numbers of media are available. The most common of component used in basic are beef extract (source of organic nitrogen, carbon, vitamins and inorganic salts), amino acid-rich peptone (semi digestive proteins) and carbon, nitrogen etc. According to the growth of specific types of microorganisms, basic culture medium may be modified by supplementing some chemicals to the medium.

Nutrients present in liquid growth media are called broths. Addition of agar cause solidification of media. Agar is produced from red algae. It is a complex polysaccharide consisting of 3, 6-unhydro-L-galactose and D-galactopyranose, free of nitrogen. It liquefies on heating at 96°C and on cooling at 40-45 °C is converted into a jell like substance. Liquid agar media can be poured into the test tubes or Petri plates. The test tube are either allowed to cool and hardens in an upright position producing agar deep tubes or for producing agar slants by allowing to harden the media in a slanted position.

A. COMPOSITION OF SOME COMMONLY USED MEDIA

1. Nutrient broth (pH 7.0)

Beef extract	3 g
Peptone	5 g
NaCl	5 g
Distilled water	1.0 litre

Add 15g agar/litre of nutrient agar medium.
(For cultivation of bacteria)

2. Glucose broth (pH 7.3)

Peptone	10 g
Glucose	5 g
NaCl	5 g
Distilled water	1.0 litre

(For cultivation of bacteria)

3. Luria Bertani (LB) medium (pH 7.0)

Bacto yeast extract	5 g
Bactro-tryptone	10 g
NaCl	10 g
Distilled water	1.0 litre

For LB agar medium add 15 g/litre of agar in LB broth.
(LB broth is complex medium for rapid growth of members of Enterobacteriaceae e.g *E. coli*).

4. Czapek- Dox agar (pH 7.3)

Sodium nitrate	2 g
Dipotassium hydrogen phosphate	1 g
Potassium chloride	0.5 g
Magnesium sulphate	0.5 g
Ferrous sulphate	0.01 g
Sucrose	30 g
Agar	15 g
Distilled water	1.0 litre

(For cultivation of molds)

5. Malt extract medium

Malt extract	15 g
Ammonium chloride	1 g
Dipotassium hydrogen phosphate	1 g
0.1 N citric acid	15 ml
Agar	15 g
Distilled water	1.0 litre

For fungal medium, 30 g malt extract and agar 12 g for one liter of the medium is used.

(For cultivation of yeasts and acetobacter)

6. Eosin methylene blue (EMB) agar (pH 7.2)

Peptone	10 g
Lactose	5 g
Dipotassium hydrogen phosphate	2 g
Eosin Y	0.4 g
Methylene blue	0.065 g
Agar	15 g
Distilled water	1.0 liter

This medium is used to differentiate non-lactose fermenting gram-negative bacteria from lactose fermenting gram-negative bacteria. Differentiation of *E. coli* and *Aerobacter aerogenes* is carried out by this medium.

7. Sabouraud agar (pH 5.6)

Peptone	10 g
Dextrose	40 g
Agar	15 g
Distilled water	1.0 liter

8. Beef peptone agar(pH 7.2)

Beef extract	3 g
Peptone	5 g
Dextrose	10 g
Yeast extracts	5 g
Agar	15.0 g
Distilled water	1.0 liter

(For cultivation of pathogenic fungi)

9. MacConkey' s agar medium (pH 7.1)

Peptone	20 g
Lactose	10 g
Sodium chloride	5 g
Bile salt	1.5 g
Neutral red solution (1%)	10 ml
Crystal violet	0.001 g
Agar	13.5 g
Distilled water	1.0 liter

(For determining the number of coli forms in water and faeces)

10. Brilliant green lactose bile broth (pH 7.4)

Peptone	10 g
Lactose	10 g
Bile	20 g
Brilliant green (0.1 % in water)	13 ml
Distilled water	1.0 litre

11. Endo agar (pH 7.5)

Peptone	10 g
Lactose	10 g
Dipotassium hydrogen phosphate	3.5 g
Sodium sulphate	2.5 g
Basic fuchsin	0.4 g
Agar	15 g
Distilled water	1.0 litter

(For checking the presence of Coli forms)

12. Glycerol yeast extract agar(pH 7.0)

Glycerol	5 ml
Yeast extract	2 g
Dipotassium hydrogen phosphate	1 g
Agar	15 g
Distilled water	1.0 litter

(For the cultivation of Actinomycetes)

B. PREPARATION OF MEDIA (LIQUID MEDIA- NUTRIENT BROTH)

1. Add 5 g peptone, 3 g beef extract, 5 g NaCl into 500 ml of deionized water. Shake until all the solutes have dissolved.
2. Set the pH to 7.0 with 5 N NaOH.
3. Add deionized water to make the final volume of the solution 1.0 liter.
4. Pour the 100 ml medium in 250 ml flasks, 200 ml in 500 ml flask and 10 ml per tube.
5. Cap them with cotton plug.
6. Sterilize medium by autoclaving at 15 lbs pressure for 30 minutes.
7. Allow the autoclave to cool.
8. Take out nutrient broth flasks and tubes, store at low temperature (cover with aluminum foil) for further use.

Precautions

1. During autoclaving cotton plug are to be kept loose.
2. The medium should be stored at low temperature in dust free environment.

C. PREPARATION OF NUTRIENT AGAR MEDIUM.

Nutrient broth in test tube

Nutriant broth in flask

Procedure

1. Prepare 200 ml of medium as describe above in step 1-2 in flasks
2. Before autoclaving add 3.0 g agar to medium.
3. Add 1 g peptone, 1 g NaCl and 0.6 g beef extract in 100 ml of water and mix till all solutes get dissolved for agar slants/ agar deep tubes.

4. Add 3 g of agar in slowly in 50 ml of hot water to dissolve it.
5. Mix agar with an above nutrients solution.
6. With deionised water make the final volume 200 ml.
7. Pour 7 to 8 ml of medium in each test tube/flask.
8. Plug the test tube and flask containing a medium.
9. Sterilize the medium at 121°C, 15 lbs pressure for 20 minutes in an autoclave.
10. Allow the tube to cool in an upright position (for agar deep tubes) and slanting position (for agar slants).
11. When the flasks having medium is ejected from the autoclave, shake it well to distribute the melted agar uniformly throughout the solution.
12. Leave the medium to cool at 50°C.
13. Under aseptic conditions, quickly pour the medium into the petri plates.
14. Invert the plates, when the medium gets hardened completely, store them at 4°C.
15. If the plates are fresh, they will sweat when incubated at 37°C. This increases the chance of contamination. So, the plates should be removed from storage 1-2 hours before they are used.

Agar slants Agar deep tube Agar plate

Precautions

1. During autoclaving, the cotton plug should be loosed.
2. The medium should not be more than half of the capacity of the container used for autoclaving.

3. Media should not be poured to Petri plates when it is too hot as it produces much-condensed water vapors on the lid of the Petri plate that can fall on the agar surface and may cause culture contamination.
4. Use laminar air flow hood for pouring.
5. The rim of the flask should be flamed, before pouring media into the Petri plate.
6. Pouring should be done quickly to avoid contamination.
7. Slants/deep tubes/ Petri plates are always stored at low temperature or in dust free environment.

Advantage of Agar Plate

1. Provides a maximum surface area for growth.
2. The number of bacteria present in the sample can be evaluated.
3. Cells can be easily isolated.

Limitations

1. Difficulty in handling.
2. Media get rapidly dry.
3. The Large surface area exposed to contamination.

Advantage of Agar Slants over Agar Plates

1. Easy to handle and store.
2. Occupies less space.
3. Same colony characteristics can be seen
4. Media do not get dry rapidly.
5. Fewer chances of contamination.

Limitations

1. Less surface area for growth.
2. Individual colonies cannot be seen.