PREPARATION OF CULTURE MEDIA

Culture Media

Types of culture media

1. Basic /Simple / All purpose media

It is a media that supports the growth of micro-organisms that do not require special nutrients.

Uses :

. To prepare enriched media
. To maintain stock cultures of control bacterial strains
. To subculture pathogenic bacteria from selective/differential medium prior to performing biochemical or serological tests.

Eg. Nutrient Broth
Nutrient Agar

2. Enriched media

Media that are enriched with whole blood, lyzed blood, serum,special extracts or vitamins to support the growth of pathogenic bacteria.

Eg. Blood Agar
Chocolate Agar

3. Enrichment media

Fluid media that increases the numbers of a pathogen by containing enrichments and/or substances that discourage the multiplication of unwanted bacteria.

Eg. Selenite F broth media
Alkaline peptone water

4. Selective media

Media which contain substances (Eg. Antibiotics) that prevent or slow down the growth of bacteria other than pathogens for which the media are intended.

Eg. Modified Thayer –Martin Agar
Salmonella-Shigella(SS) agar

5. Differential media

Media to which indicator substances are added to differentiate bacteria.

Eg. TCBS Agar differentiates sucrose fermenting yellow colonies of *Vibrio cholerae* to non-sucrose fermenting blue colonies other Vibrio species.

Note: Most differential media distinguish between bacteria by an indicator which changes color when acid is produced following carbohydrate fermentation.

6. Transport media

Media containing ingredients to prevent the overgrowth of commensals and ensure the survival of pathogenic bacteria when specimens can not be cultured soon after collection.

EG. Amies transport media
Stuart media
Kelly-Blair media

Choice of culture media

The selection culture media will depend on:

1. The major pathogens to be isolated, their growth requirements and the features by which they are recognized.
2. Whether the specimens being cultured are from sterile sites or from sites having normal microbial flora.
3. The cost, availability and stability of media.
4. The training and experience of laboratory staff in preparing, using and controlling culture media.

Forms of culture media

1. Solid culture media
2. Semisolid culture media
3. Fluid culture media

1. Solid culture media

Plate cultures in petri dishes. Stab/slope cultures in tubes and bottles

Uses: Description of bacterial colonies

- Size : diameter in mm
- Out line : circular, entire, wavy, indented
- Elevation: flat, raised, low convex and dome shaped.
- Transparency: transparent, opaque, and translucent.
- Surface: smooth (mucoid) and shiny, rough and dull.
- Color: colorless, white, pink, and pigmented
- changes in medium

Eg. Hemolysis in Blood Agar

Blackening of medium due to hydrogen sulfide production.

2. Semisolid culture media

Uses:

. as an enrichment media

. as motility media

3. Fluid culture media

Bacterial growth in fluid media is shown by a turbidity in the medium.

Uses :

. as an enrichment media

. as biochemical testing media

. as blood culture media

Common ingredients of culture media

Peptone
Meat/Beef extract
Yeast extract
Mineral salts
Carbohydrates
Agar & Water

1.Peptone: Hydrolyzed product of animal and plant proteins: Free amino acids, peptides and proteoses(large sized peptides). It provides nitrogen; as well carbohydrates, nucleic acid fractions,minerals and vitamins.

Peptone is an enzymatic digest of animal protein. Peptone was first introduced in 1914 and became the standard Peptone for the preparation of bacteriological culture media. The nutritive value of Peptone is largely dependent on the amino acid content that supplies essential nitrogen. Peptone contains only a negligible quantity of proteoses and more complex constituents.

Applications

Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi. Peptone has also been utilized as a nitrogen source in cell culture media formulations.

2.Meat/Beef extract:

Beef Extract is a nutritive ingredient in many classical culture media, including Antibiotic Assay media, and several media recommended for standard methods applications.

Beef Extract is a mixture of peptides and amino acids, nucleotide fractions, organic acids, minerals and some vitamins. "Its function can therefore be described as complementing the nutritive properties of peptone by contributing minerals, phosphates, energy sources and those essential factors missing from peptone. Beef Extract is not exposed to the harsh treatment used for protein hydrolysis, so it can provide some of the nutrients lost during peptone manufacture.

Beef Extract is derived from infusion of beef and provides an undefined source of nutrients.

Applications

Beef Extract is intended to replace aqueous infusion of meat in microbiological culture media.

Beef Extract is frequently used at a concentration of 0.3 to 1.0% in culture media, although concentrations may vary depending on the nutritional requirements for the medium formulation.

Beef Extract was used in media for early studies of non-sporulating anaerobes of the intestinal tract and as a stock broth in the study of nutritional needs of streptococci.

3.Yeast extract:

Yeast extract is a complex and widely used hydrolysate of yeasts. It provides nitrogenous compounds, carbon, sulfur, trace nutrients, vitamin B complex and other important growth factors, which are essential for the growth of diverse microorganisms.

There are two different types of yeast extracts - the hydrolyzed yeast extract, also called yeast peptone, and the autolyzed yeast. The hydrolyzed yeast extract is produced by digestion of exogeneous enzymes or acid to hydrolyze the proteins. A yeast autolysate or

yeast autolysate extract is made by fermentation of yeast to a concentration level where the yeast dies and the cells walls break. The proteases from the yeast itself start the digestion of the proteins and split them into peptides and amino acids. The insoluble portion is removed.

The yeast extract contains glutamate. The "glutamate" refers to the content of the amino acid glutamate – which is in fact naturally present in yeast and yeast extract, but also occurs in many other foodstuffs. Glutamate represents approximately 5% of yeast extract. In a product with yeast extract, where the ingredient is only present in small quantities, the average glutamate content is less than 1%.

4.Mineral salts: these are: Sulfates as a source of sulfur.
Phosphates as a source of phosphorus.
Sodium chloride
Other elements.

5.Carbohydrates: Simple and complex sugars are a source of carbon and energy.
.Assist in the differentiation of bacteria.
Eg. Sucrose in TCBS agar differentiates vibro species.
Lactose in MacConkey agar differentiates enterobacteria.
Preparation of commonly used Media:

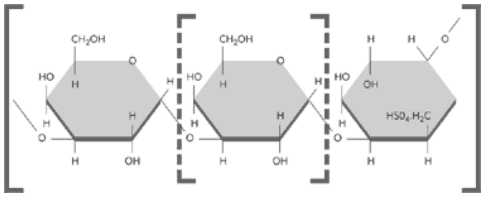
6.Agar Agar
Agar is an inert polysaccharide of seaweed.It is not metabolized by micro-organism.
Throughout history into modern times, agar has been chiefly used as an ingredient in desserts throughout Asia and also as a solid substrate to contain culture media for microbiological work. The agar was discovered in the late 1650s or early 1660s by Minoya Tarozaemon in Japan, where it is called **Kanten.**

In the natural state, agar occurs as structural carbohydrate in the cell walls of agarophytes algae, probably existing in the form of its calcium salt or a mixture of calcium and magnesium salts. It is a complex mixture of polysaccharides composed of two major fractions:

Agarose, a neutral polymer, and
Agaropectin, a charged, sulfated polymer.

Agarose - gelling fraction, is a neutral linear molecule essentially free of sulfates, consisting of chains of repeating alternate units of β-1,3-linked- D-galactose and α-1,4-linked 3,6-anhydro-L-galactose.

Agaropectin- non gelling fraction, is a sulfated polysaccharide (3% to 10% sulfate), composed of agarose and varying percentages of ester sulfate, D-glucuronic acid, **and** small amounts of pyruvic acid. The proportion of these two polymers varies according to the species of seaweed. Agarose normally represents at least two-thirds of the natural agar-agar.



Properties

Agar-agar may come in several forms: powdered, flakes, bars and threads. Available as yellowish powder.

Agar-agar is insoluble in cold water, but soluble in water and other solvents at temperatures between 95^{O} and 100^{O} C. It dissolves readily in boiling water and sets to a firm gel at concentrations as low as 0.50%.

The melting point of agar is 85-90 ^{O} C, whereas the solidifying point is in between 32-45 ^{O} C depending upon the concentration.

Specifications

Appearance	Yellowish Powder
Particle size	100 Mesh
Moisture content	Max. 18%
Water absorption	Max. 75 cc
Acid insoluble ash	Max. 0.5%
Total Ash	Max. 6.5%
Foreign organic material	Max. 1%
Foreign insoluble material	Max. 1%
pH	6.8-7.0
Gelatin	Negative
Gel Strength 1.5% sol at 20^{O}C	700-1000gm/cm^{2}
Viscosity 1.5% solution at 60^{O}C	10-100cps
Melting Point	85-95 ^{O}C
Setting Point	32-45 ^{O}C
Solubility	Boiling water
Arsenic	Max. 3ppm
Lead	Max. 10 ppm
Heavy metals	Max. 10 ppm
Starch	Negative

7. Water: Deionized or distilled water must be used in the preparation of culture media.

Commonly used culture media in microbiological laboratory:

Nutrient Broth:

Peptic digest of animal tissue	5gm
Sodium chloride	5gm
Beef extract	1.5 gm
Yeast Extract	1.5 gm
Distilled water	1000ml
pH	7.4

Mix the contents boil to dissolve. Autoclave at 15 lb pressure at 121^{O}C for 20 minutes. Dispense in sterile test tubes and use.

Nutrient Agar:

Peptone	10gm
Sodium chloride	5gm
Beef extract	4gm
Agar	20 gm
Distilled water	1000ml
pH	7.4

Mix the contents boil to dissolve. Autoclave at 15lb pressure at 121^{O}C for 20 minutes. Dispense in sterile petridish and use.

Blood Agar:

Prepare nutrient agar. Cool it to 40-50^{O}C. And add 5-10% defibrinated blood collected in sterilized flask.

MacConkey's Agar:

Peptone	5gm
Bile salt (Sodium taurocholate)	5gm
Sodium chloride	5gm
Neutral Red (2% in 50% ethanol)	3.5ml
Crystal violet	0.001gm
Lactose	10gm
Agar	20gm
Distilled water	1000ml
pH	7.4

Mix the contents boil to dissolve. Autoclave at 15 lb pressure at 121^{O}C for 20 minutes. Dispense in sterile petridish and use.

Brilliant Green Agar:

Yeast Extract	3gm
Peptone	10gm
Sodium chloride	5gm
Lactose	10gm
Sucrose	10gm
Phenol Red	0.08gm
Brilliant Green	0.125gm

Agar	20gm
Distilled water	1000ml
pH	7.4

Mix the contents boil to dissolve. Autoclave at 15 lb pressure at 115^{O}C for 15 minutes. Dispense in sterile petridish and use.

Eosin Methylene Blue Agar:

Peptone	10gm
Sucrose	5gm
Lactose	5gm
Di-potassium phosphate	2gm
Eosin Y	0.4gm
Methylene Blue	0.065gm
Agar	13.5gm
Distilled water	1000ml
pH	7.2

Mix the contents boil to dissolve the medium completely. Cool to 50OC and shake the medium in order to oxidize the methylene blue (i.e., to restore it's blue colour) and to suspend the flocculent precipitate, which is an essential part of the medium. Dispense and sterilize by autoclaving at 15 lb pressure at 121^{O}C for 15 minutes.

Exercise:

1. Why lactose fermenting bacterial colonies appear pink red on MacConkey's agar?
2. How the metallic sheen is developed by growth of *Escherichia coli* on EMB agar.
3. What makes MacConkey's agar selective for enteric bacteria.
4. What concentration of NaCl is required for the growth of bacteria of veterinary & medical importance.
5. What is halophillic bacteria?
6. What do you understand by fastidious bacteria?
7. Name the reducing agents used in anaerobic media for development of anaerobiosis.

Reference

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