Lab: 1 **Practical bacterial physiology**

General Components of Culture Media

When culturing bacteria, it is very important to provide similar environmental and nutritional conditions that exist in its natural habitat. Hence, an artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat. Most often, a culture medium contains water, a source of carbon and energy, source of nitrogen, trace elements and some growth factors. Besides these, the pH of the medium must be set accordingly. Some of the ingredient of culture media include water, agar, peptone, casein hydrolysate, meat extract, yeast extract and malt extract.

The composition in media would be under the following:

1-Nutrients: proteins/peptide/amino

2-Energy: carbohydrates (glucose)

3-Minerals: essential: Ca, Mg, Fe, K, Cl.

4-Trace metals: Phosphate, sulphate etcetera

5-Buffering agents: e.g., phosphate, acetates

6-Indicator for pH change: bromo -cresol purple, phenyl red

7-Selective agent: antimicrobial agents e.g., penicillin,

8-Gelling agent: Agar

9-Water

CLASSIFICATION OF CULTURE MEDIA

The concept of classification of culture is broad. Some studies identified that culture media can be classified into at least three groups based on consistency, ingredients, and function. Some other studies classified media into four. Adding chemical composition.

Classification based on chemical composition

On this bases, cultural media are classified as:

1-natural, 2-semi-synthetic, 3-synthetic media.

1. Natural/empirical media: these are media in which the exact chemical composition is not known. E.g., milk, urine diluted blood, vegetable juices, meat extract, extract, tomato juice, blood etc.

2. Semi-synthetic media: these are media with partially known and partially obscure chemical components.

E.g., potato dextrose agar (PDA), Czapek-Dox agar, oat meat agar (OMA), corn meal agar (CMA), beef peptone agar and nutrient agar.

3. Synthetic media: these are media composed of the substances that are chemically known. These media are very useful in studying the physiology, metabolic and nutritional requirement of microbes.

E.g., Mineral glucose medium, Richard's solution, Raulins medium etc.

Classification based on consistency

Culture media are classified as solid, semi solid, liquid, and biphasic.

Solid media: Colony morphology, pigmentation, hemolysis can be appreciated.

Eg: Nutrient agar, Blood agar

Semi solid medium Motility medium

Liquid media: it does not contain agar. It is used for; inoculum preparation,

Blood culture, and for the isolation of pathogens from a mixture.

Eg: Nutrient broth

Biphasic: this comprises of both liquid and solid medium in the same bottle.

Based on the constituents/ ingredients

Here media are classified as simple, complex, synthetic or defined, Special media.

a. Simple media / basal media: these are basically simple media that supports most non-fastidious bacteria.

E.g., Peptone water, nutrient broth/agar

b. Complex media

Media other than basal media. They have added ingredients. They provide special nutrients.

c. Synthetic or defined media

Media prepared from pure chemical substances and its exact composition is known

Eg: peptone water – 1% peptone + 0.5% NaCl in water

d. Special media

Enrichment media: these are liquid media used for the isolation of pathogens from a mixed culture. It is incorporated with inhibitory substances to suppress the unwanted organism.

Eg: i. Selenite F Broth – for the isolation of Salmonella, Shigella.

ii. Alkaline Peptone Water – for *Vibrio cholerae*

Enriched media: Addition of extra nutrients in the form of blood, serum, egg yolk etc. to basal medium makes them enriched media. It is usually in solid form. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar,Loeffler's serum slope are few of the enriched media.

Selective media: are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or combination of these.

Eg:

MacConkey's medium : for

gram negative bacteria

Thiosulphate-citrate-bile salts (TCBS) – for

Vibrio cholerae

Lowenstein-Jensen (LJ) medium – for

Mycobacterium tuberculosis

Wilson and Blair medium – for

Salmonella typhi

Potassium tellurite medium – for

Diphtheria bacilli

Indicator media

These media contain an indicator which changes its color when a bacterium grows in them.

Eg: Blood agar, Mac Conkey's medium, and Christensen's urease medium

Differential media

A media which has substances incorporated in it enabling it to distinguish between bacteria.

Eg: MacConkey's medium, Neutral red, Taurocholate

MacConkey medium distinguish between lactose fermenters & non lactose fermenters.

Lactose fermenters – Pink colonies

Non lactose fermenters – colorless colonies

Transport media

Media used for transporting the samples. Delicate organisms may not survive the time taken for transporting the specimen without a transport media.

Eg: Stuart's medium – non nutrient soft agar gel containing a reducing agent: Gonococci

Buffered glycerol saline – enteric bacilli

Alkaline peptone water: V. cholerae

Oxygen

Aerobic media

For culturing :

Bacillus cereus*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis

Anaerobic media

for culturing :

Clostridium perfringens*, *Clostridium botulinum

Eg: Robertson's cooked meat medium, Thioglycolate medium.

General Components of Nutrient agar \broth

Nutrient agar, a widely used culture medium in microbiology, typically consists of peptone, beef extract, agar, and sodium chloride. Peptone provides a source of organic nitrogen, while beef extract supplies essential nutrients like carbon, vitamins, and minerals. Agar acts

as a solidifying agent, and sodium chloride maintains the osmotic balance of the medium.

- **Peptone:**

A protein hydrolysate that serves as a source of amino acids and peptides, which are essential for microbial growth.

- **Beef Extract:**

Provides a complex mixture of nutrients, including amino acids, peptides, vitamins, and minerals, which support microbial growth.

- **Agar:**

A polysaccharide derived from seaweed that acts as a gelling agent, solidifying the medium and allowing for the growth of microorganisms on its surface.

- **Sodium Chloride (NaCl):**

Maintains the osmotic balance of the medium, preventing excessive water loss or gain by the microorganisms.

- **Distilled Water:**

Provides the liquid medium for the other components to be dissolved in.

Some formulations may also include yeast extract, which provides additional vitamins and growth factors.

Nutrient Agar Media Preparation

Component	Quantity in grams for 100 ml	Quantity in grams for 1000 ml
Beef extract	0.3 g	3 g
Peptone	0.5 g	5 g
Sodium Chloride	0.5 g	5 g
Agar	1.5 g	15 g
Distilled water	100 ml	1000 ml

General Components of Brain heart infusion agar \broth

Brain Heart Infusion (BHI) agar is a nutrient-rich medium used to cultivate a wide variety of microorganisms, including fastidious bacteria, yeasts, and molds. Its key components include infusions from animal tissues, peptones, dextrose, sodium chloride, disodium phosphate, and agar.

- Brain and Heart Infusions: These provide a rich source of nutrients, including amino acids, vitamins, and growth factors. The infusions are typically made from calf brain and beef or pig heart.
- Proteose Peptone: This is a protein digest that serves as a source of nitrogen, amino acids, and other essential nutrients.
- Dextrose: A sugar that provides a source of energy for microbial growth.

- **Sodium Chloride: Helps maintain the osmotic balance of the medium.**
- **Disodium Phosphate: Acts as a buffer to maintain a stable pH.**
- **Agar: A solidifying agent that provides a solid surface for microbial growth.**

In addition to these basic components, BHI agar can be supplemented with other ingredients to enhance growth or select for specific microorganisms. For example, blood is often added to support the growth of fastidious bacteria and fungi. Antibiotics like penicillin and streptomycin can also be added to inhibit bacterial growth and allow fungi to grow selectively.

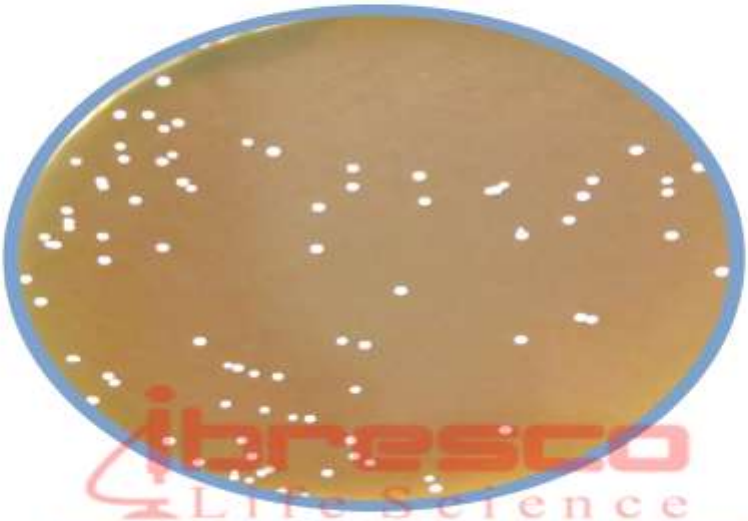
Ingredients	Quantity Required for 1000 mL (g)
Protease peptone	10.00
Calf brain infusion	200.00
Sodium chloride	5.00
Dextrose	2.00
Beef heart infusion	250.00
Agar	15.00
Disodium phosphate	2.50
Human blood	50.00

MRS (de Man, Rogosa, and Sharpe) agar is a culture medium specifically designed for the growth of Lactobacillus and other lactic acid bacteria. It contains a rich nutrient base with peptones, beef extract, and yeast extract providing nitrogen, vitamins, and amino acids. Glucose serves as the primary fermentable carbohydrate, while sodium acetate and ammonium citrate act as selective agents. Additionally, polysorbate 80 (Tween 80), magnesium sulfate, and manganese sulfate are included to support the growth of these bacteria.

- **Peptones: Provide nitrogen, amino acids, and other essential nutrients.**
- **Beef extract: Another source of nitrogen, vitamins, and growth factors.**
- **Yeast extract: Serves as a source of vitamins and growth factors.**
- **Glucose: The main fermentable carbohydrate.**
- **Sodium acetate: An energy source and selective agent.**
- **Ammonium citrate: Another selective agent.**
- **Polysorbate 80 (Tween 80): A surfactant that facilitates nutrient uptake by Lactobacillus.**
- **Dipotassium phosphate: A buffer that helps maintain the pH.**
- **Magnesium sulfate: Provides magnesium ions which are essential for bacterial metabolism.**
- **Manganese sulfate: Provides manganese ions which are essential for bacterial metabolism.**
- **Agar: The solidifying agent**

MRS Agar

de Man, Rogosta, Sharpe Agar



MRS Agar Media

- Proteose peptone
- Yeast extract
- Glucose
- Potassium dihydrogen phosphate

Basic media composition

- Meat extract
- Polysorbate 80
- Magnesium sulfate
- Manganese sulfate

Enhances lactic acid bacteria growth

- Sodium acetate
- Ammonium citrate
- Agar

Slightly inhibits contaminant growth

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Technical Data

Lactobacillus MRS Agar

M641

Lactobacillus MRS Agar is recommended for cultivation of all *Lactobacillus* species.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Beef extract	10.000
Yeast extract	5.000
Dextrose	20.000
Polysorbate 80	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium phosphate	2.000
Agar	12.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 67.15 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.