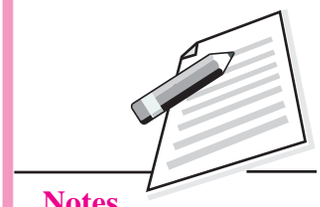

Notes

9

BACTERIAL CULTURE MEDIA

9.1 INTRODUCTION

Why bacteria have to be grown (cultured) in the laboratory on artificial culture media?

1. One of the most important reasons being its utility in diagnosing infectious diseases. Isolating an organism from sites in body normally known to be sterile is an indication of its role in the disease process. Indeed, isolating an organism from the clinical specimen is the first step in proving its role as an etiologic agent.
2. Culturing bacteria is also the initial step in studying its morphology and its identification.
3. Bacteria have to be cultured in order to obtain antigens from developing serological assays or vaccines.
4. Certain genetic studies and manipulations of the cells also need that bacteria be cultured in vitro.
5. Culturing bacteria also provide a reliable way estimating their numbers (viable count).
6. Culturing on solid media is another convenient way of separating bacteria in mixtures.

This lesson deals with culture media.

OBJECTIVES

After reading this lesson, you will be able to:

- enlist the common ingreditents of culture medium
- describe about history of culture medium in brief

Notes

- classify the culture media
- describe the preparation and storage of Culture media

When culturing bacteria, it is very important to provide similar environmental and nutritional conditions that exist in its natural habitat. Most culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors. Besides these, optimum pH, oxygen tension and osmolarity too have to be taken into consideration. Some of the ingredients of culture media include:

While tap water is suitable for culture media, it must not be used if it contains high amount of minerals. In such situations**, distilled or demineralised water** should be used. **Peptone** is a byproduct of protein digestion. Proteins are often obtained from heart muscle, casein, fibrin or soya flour and is digested using proteolytic enzymes such as pepsin, trypsin or papain. The final product contains peptones, proteoses and amino acids besides a variety of inorganic salts including phosphates, potassium and magnesium. **Casein hydrolysate** is obtained from hydrolysis of milk protein casein using HCl or trypsin. **Meat extract** is obtained by hot water extraction of lean beef and then concentrated by evaporation. **Yeast extract** is prepared from washed cells of bakers' yeast and contains wide range of amino acids, growth factors and inorganic salts.

9.2 BRIEF HISTORY

Robert Koch realized the importance of solid media and used potato pieces to grow bacteria and agar was used to solidify culture media. Before the use of agar, attempts were made to use gelatin as solidifying agent. Gelatin had some inherent problems; it existed as liquid at normal incubating temperatures (35–37^{o}C) and was digested by certain bacteria.

Classification

Bacterial culture media can be classified in at least three ways; Based on consistency, based on nutritional component and based on its functional use.

1. Classification based on consistency

- liquid media
- semi-solid media
- solid media

Liquid media

In liquid medium, bacteria grow producing turbidity/ surface pellicle (Vibrio & Bacillus)/ granular deposits (Streptococci). Culturing bacteria in liquid media

has some drawbacks. Properties of bacteria are not visible in liquid media and presence of more than one type of bacteria cannot be detected.

Solid media

Any liquid medium can be rendered solid by the addition of certain solidifying agents. Agar agar (simply called agar) is the most commonly used solidifying agent. It is an unbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera Gelidium. Agar is composed of two long-chain polysaccharides (70% agarose and 30% agarapectin). It melts at 95°C and solidifies at 42ºC, doesn't contribute any nutritive property, it is not hydrolysed by most bacteria and is usually free from growth promoting or growth retarding substances. Agar is available as powders. New Zealand agar and Japanese agar are most commonly used at concentration of 2% and 4% respectively to make a solid agarmedium.

Semi-solid media

Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility (U-tube and Cragie's tube). Certain transport media such as Stuart's and Amies media are semi-solid in consistency. Hugh & Leifson's oxidation fermentation test medium as well as mannitol motility medium are also semi-solid.

Biphasic media

Sometimes, a culture system comprises of both liquid and solid medium in the same bottle. This is known as biphasic medium (Castaneda system for blood culture). The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply tilted to allow the liquid to flow over the solid medium. This obviates the need for frequent opening of the culture bottle to subculture.

Other solidifying agents

Besides agar, egg yolk and serum too can be used to solidify culture media. Serum containing medium such as Loeffler's serum slope and egg containing media such as Lowenstein Jensen (LJ) medium and Dorset egg medium are solidified as well as disinfected by a process of inspissation.

INTEXT QUESTIONS 9.1

1. The by-product of protein digestion is .....
2. ..... is the most commonly used solidifying agent

Notes

3. Culture system having both liquid & solid medium in the same container is called as .....

4. ..... media are useful in demonstrating bacterial motility

2. Classification based on nutritional component

Media can be classified as simple, complex and synthetic (or defined). Those bacteria that are able to grow with minimal requirements are said to non-fastidious and those that require extra nutrients are said to be fastidious. Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria.

Complex media such as blood agar have ingredients whose exact components are difficult to estimate.

Synthetic or defined media such as Davis & Mingioli medium are specially prepared media for research purposes where the composition of every component is well known.

3. Classification based on functional use or application

Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal medium

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

Blood agar is prepared by adding 5-10% (by volume) to a basal medium such as nutrient agar or other blood agar bases. Since blood cannot be sterilized, it has to be collected aseptically from the animal. Animals have to be bled and the blood is collected in sterile containers with anticoagulant or glass beads. While sheep blood is preferred, blood from rabbit, horse and ox can also be collected. Human blood must be avoided since it may contain inhibitory substances including antibiotics. After the blood agar base is autoclaved, blood is added to the medium at temperature just above the solidifying point of agar. The mixture is then poured on to the plates and allowed to solidify. Blood agar is useful in demonstrating hemolytic properties of certain bacteria.

Chocolate agar is also known as heated blood agar or lysed blood agar. The procedure is similar to that of blood agar preparation except that the blood is added while the molten blood agar base is still hot. This lyses the blood cells and releases their contents into the medium. This process turns the medium brown, hence the name. This medium is especially useful in growing *Hemophilus* sp and

Neisseria sp. Serum for medium can be obtained from animal blood but must be filtered through membrane or seitz filter before use.

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

Thayer Martin Agar used to recover N. *gonorrhoeae* contains Vancomycin, Colistin and Nystatin.Mannitol Salt Agar and Salt Milk Agar used to recover S.*aureus* contain 10% NaCl. Potassium tellurite medium used to recover C.*diphtheriae* contains 0.04% potassium tellurite. McConkey's Agar used for Enterobacteriaceae members contains Bile salt that inhibits most gram positive bacteria. Pseudosel Agar (Cetrimide Agar) used to recover P.*aeruginosa* contains cetrimide. Crystal Violet Blood Agar used to recover S.*pyogenes* contains 0.0002% crystal violet. Lowenstein Jensen Medium used to recover M.*tuberculosis* is made selective by incorporating malachite green. Wilson & Blair's Agar for recovering S.*typhi* is rendered selective by the addition of dye Brilliant green. TCBS Agar and Monsur's Tellurite Taurocholate Gelatin Agar used for isolating V. *cholerae* from fecal specimens have elevated pH (8.5-5.6), which inhibits most other bacteria.

Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water are used to recover pathogens from fecal specimens.

Differential/Indicator media

Differential media or indicator media distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism.

When a particular substrate (carbohydrate) is incorporated into a medium and a mixture of bacteria inoculated on it, only that bacterium that can ferment it produces acid. This change in pH is detected by using a pH indicator incorporated in the medium and the bacterium that can ferment the sugar appears in a different colour. This approach is used in MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

MacConkey's agar is the most commonly used media to culture and identify gram negative bacilli (especially enterobacteriaceae members). It contains bile salts (selective agent), lactose (sugar), peptone and neutral red (pH indicator),

Notes

agar and water. Those bacteria that can ferment lactose produce pink coloured colonies where non-lactose fermenting colonies produce colourless colonies.

Similarly, *Vibrio cholerae* produces yellow coloured colonies on sucrose containing TCBS medium.Reduction of potassium tellurite to metallic tellurium by *Corynebacterium diphtheriae* results in production of black coloured colonies on KT agar. Production of H_2S by *Salmonella typhi* results in production of black coloured colonies on Wilson & Blair's medium.

Enterococcus fecalis produces black coloured colonies on bile esculin agar due to reduction of esculin to esculetin.

Transport media

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the viability of all organisms in the specimen without altering their concentration . Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Cary Blair medium and Venkatraman Ramakrishnan medium are used to transport feces from suspected cholera patients. Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery. Pike's medium is used to transport streptococci from throat specimens.

Anaerobic media

Anaerobic bacteria need reduced oxidation –reduction potential and extra nutrients. Such media may be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced.

- Robertson cooked meat that is commonly used to grow *Clostridium* spps medium .
- Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.
- Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colourless.

Preparation and Storage of Culture Media

Care must be taken to adjust the pH of the medium before autoclaving. Various pH indicators that are in use include phenol red, neutral red, bromothymol blue, bromocresol purple etc. Dehydrated media are commercially available and must be reconstituted as per manufacturers' recommendation. Most culture media are sterililized by autoclaving. Certain media that contain heat labile components like glucose, antibiotics, urea, serum, blood are not autoclaved. These components are filtered and may be added separately after the medium is autoclaved. Certain highly selective media such as Wilson and Blair's medium and TCBS agar need not be sterilized. It is imperative that a representation from each lot be tested for performance and contamination before use. Once prepared, media may be held at 4-5^{o}C in the refrigerator for 1-2 weeks. Certain liquid media in screw capped bottles or tubes or cotton plugged can be held at room temperature for weeks.

INTEXT QUESTIONS 9.2

1. Bacteria that require extra nutrients for growth are called as .....organism
2. Blood agar is a type of ..... media
3. Chocolate agar is specially useful in growing .....
4. Media used to inhibit commensals are ..... media
5. Robertson Cooked meat is commonly used to grow ..... species

WHAT YOU HAVE LEARNT

- Culture Media are used in diagnosing infections diseases.
- Culture of bacteria is carried out for studying its morphology and its identification.
- Most culture media contains water, a source of carbon and energy, source of nitrogen, trace elements and some growth factors, optimum pH, oxygen and osmolarity.
- Based on consistency culture media is classified as liquid, semi-solid and solid media.
- Agar is used for solidifying liquid media into solid media.
- Semi-solid media are useful in demonstrating bacterial motility.
- Biphasic media comprises of both liquid and solid medium in the same bottle.

- Based on nutritional component, culture media are classified as simple, complex and synthetic.
- Bacteria that grow with minimum requirements are called non-fastidions.
- Bacteria that require extra nutrients are called fastidious.
- Based on functional use or application, culture media are classified as Basal media, Enriched media, Blood sugar, chocolate agar, selective & enrichment media.
- Enrichment media serves to inhibit commensals in clinical specimen.
- Differential media/indicator media distinguish are microorganism from another growing on the same media.
- Transport media prevent drying of specimen and maintain viability of organisms in the specimen.

TERMINAL QUESTIONS

1. Classify Culture media
2. Describe the preparation & storage of culture media
3. Explain transport & differential media

ANSWERS TO INTEXT QUETIONS

9.1

1. Peptone
2. Agar
3. Biphasic medium
4. Semi solid

9.2

1. Fastidious
2. Enriched
3. Hemophilus & Neisseria
4. Enrichment
5. Clostridium species