Dr. Rima Kumari: Date: 14/07/2020
Online class and e- content for B.Sc. Ist year students

Date and Time	Online class medium	E. content topic
08/07/2020 12:30 p.m to 01.20 p.m	Via Google meet Link: Meeting URL: https://meet.google.com/kcj-wera-fnd	Micro-organism: Culture media preparation

Microbiology

Culture media:

A culture medium is a solid or liquid preparation of nutrient or combination of nutrients used to grow, transport, and store microorganisms. Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory, and this is possible only if suitable culture media are available. To be effective, the medium must contain all the nutrients the microorganism requires for growth. Although all microorganisms require energy sources, carbon, nitrogen, phosphorus, sulphur and various minerals, the precise composition of a satisfactory medium depends on the microbial species one is trying to grow because nutritional requirements vary so greatly. Knowledge about microorganism's natural habitat often is useful in selecting an appropriate culture medium because its nutritional requirements reflect its natural surroundings.

Preparation of a micro-organism culture medium:

In preparing a culture medium for any microorganism, the primary goal is to provide a balanced mixture of the required nutrients at concentrations that will permit good growth. Additionally, the culturing of microorganisms requires careful control of various environmental factors which normally are maintained within narrow limits. Microbiological culture media, however, consist of various nutrient substances supporting the growth of particular types of microorganisms. Some media contain solutions of inorganic salts and may be supplemented with one or more organic compounds. Other media are prepared from complex ingredients such as extracts or digests of plant and animal tissues. Culture media would, thereinafter, be called 'media' (sing, medium).

Types of Culture Medium:

Following are some important types of culture medium:

- **Non-Synthetic Media (or Complex Media):**

A medium in which the exact chemical composition of each of the constituents is not known with certainty is referred to as non-synthetic medium, undefined medium, or complex medium. Potato-Dextrose-Agar (GM-25), Soil-Extract-Agar (SM-1), Oatmeal-Agar (GM-24), Malt-Extract-Agar (GM-19b), Waksmans medium (GM- 40) are some of the most widely used non-synthetic media. For convenience, the undefined chemical composition medium (complex medium) used to grow either Escherichia coli or Leuconostoc mesenteroides is as follows.

Glucose …….. 15g
Yeast extract……..…… 5g
Peptone…… ….. 5g
KH_2PO_4 …… 2g
Distilled water…….. 1,000 ml
pH…… 7

Non-synthetic media often employ digests of caesin (milk protein), soybeans, beef, yeast cells, or any of a number of highly nutritious but chemically undefined substances. Such digests are available commercially in powdered form and can be weighed out rapidly and dissolved in distilled water to prepare a medium.

- ## Synthetic Media (or Defined Media):

A medium in which only pure chemicals in definite concentrations are used is called synthetic medium or defined medium. On account of their known chemical compositions these media are useful for nutritional and metabolic studies. Czakek's Dox medium (GM-9) and Richard's solution (GM-27) are the most widely used synthetic media.

For example, a defined medium used for *Escherichia coli* is as follows:

K_2HPO_4 …… 7 g
KH_2PO_4 …….. 2 g
NH4)2SO4 ……. 1 g
MgSO4 …… 0.1 g
CaCl2 ……. 0.02 g
Glucose……. 4-10 g

Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo)…….…… 2-10 µm each
Distilled water…….. 1000 ml
pH…… 7

- **Solid Media:**

Solid media may either always remain solid (e.g., potato slices, coagulated blood serum, coagulated egg) or be liquefied (e.g., nutrient-agar medium, nutrient-gelatin medium, potato dextrose-agar medium). Liquefiable solid media are prepared by adding suitable amount of gelatin or agar to the liquid medium to remain solid when cooled but become liquid when warmed or vice-versa.

Agar is a complex polysaccharide (carbohydrate) consisting of 3, 6-anhydro-L-galactose and D-galactopyranose, free of nitrogen, produced from various red algae belonging to genera Gelidium, Gracilaria, Gigartina and Pterocladia. It liquefies on heating to 96°C and hardens into a jelly on cooling to 40-45°C.

The solidified medium kept in a Petri dish provides an artificial environment suitable for a rapid growth of fungi. While in liquefied state, solid media can be taken in test tubes, which are either allowed to cool and harden in a slanted position producing agar slants or allowed to harden in the upright position producing agar deep tubes.

4. Liquid Media:

Liquid media remain in liquid form and are called liquid broth (i.e., media lacking agar). Bacteria, in contrast to fungi, are often cultured in liquid broth. Nutrient broth, glucose broth, beef extract, skimmed milk, peptone solution are examples of liquid media. The most commonly used liquid media in bacteriological laboratory are beef extract (a beef derivative which is a source of organic carbon, nitrogen, vitamins and inorganic salts) and peptone solution (a semidigested protein). These may be modified in a variety of ways by adding some specific chemicals or materials to provide a medium suitable for cultivation or demonstration of a reaction for specific types or groups of bacteria.

5. Semi-Solid Media:

Semi-solid media contain a smaller amount (0.5% or less) of agar which imparts a “custard consistency”. Example: Cystine trypticase-agar medium.

6. Special Media:

(i) Enrichment Media:

Enrichment culture is that in which the growth of a particular microorganism is favoured as against a mixed population by adjusting the nutritional

requirements and environmental factors. The so grown microbial population in the medium is called enrichment culture.

(ii) Selective Media:

A selective medium is one which prevents or retards the growth of unwanted microorganisms while permits and promotes the growth of wanted microorganisms to form distinctive colonies.
The selective action of the medium is due to the addition of certain chemicals to the medium. For example, addition of crystal violet dye in the culture medium selectively inhibits the growth of gram-positive bacteria and permits and promotes the growth of gram-negative bacteria.
Important examples of selective media are MacConkey-Agar for E. coli, Deoxycholate-Citrate-Agar (DCA) for Samonella and Shigella, Wilson and Blair's medium for Salmonella, and Mannitol-Salt-Agar medium for pathogenic staphylococci.
Mannitol-Salt-Agar medium contains high concentration of sodium chloride which inhibits the growth of bacteria other than pathogenic staphylococci. The fermenting ability of the staphylococci colonies induced by a yellow halo can be detected by mannitol present in the medium. Mannitol is metabolized and fermented by staphylococci and the acid produced can be detected by phenol-red, a pH indicator.

The following is the composition for the preparation of Mannitol-Salt-Agar medium:

Mannitol…….10.0 g
Beef extract…..……..1.0 g
Peptone…….……10.0 g
Sodium chloride……75.0 g
Phenol red….……0.025 g
Agar……..……..15.0 g
Distilled water……..100 ml

(iii) Transport Media:

Transport media have wide applicability in medical field. It is used specifically to maintain the pathogenic microorganisms during transportation to laboratory from hospital or from a distant area.

When a patient is far from the pathological laboratory, the delicate pathogenic microorganism (e.g., Neisseria gonorrhoeae that causes gonorrhoea) may not survive or the normal microorganisms (e.g., Escherichia coli) may overgrow pathogenic microorganisms (e.g., Salmonella, Shigella, Vibrio cholerae) even

before the transportation of clinical sample to the testing laboratory. To avoid this, culture media have been devised to maintain the viability of the pathogen.

Some of the best examples of the transport media are the following:

Stuart's medium: Stuart's medium used to maintain the viability of gonococci bacteria.

Pike's medium: Pike's medium used to preserve Streptococcus pyogenes.

Glycerol-saline medium: Glycerol-saline medium used to prevent normal intestinal microflora from overgrowing the enteric fever bacilli.

Bile-Peptone medium: Bile-Peptone medium used to maintain the viability of cholera causing bacteria.

(iv) Differential (Indicator) Media:

A differential (indicator) medium is one which causes a visible change between different groups of bacteria growing in the medium and even permits tentative identification of microorganisms based on their biological characteristics. Blood-Agar medium, MacConkey-Agar medium and Christensen's medium are good examples of differential (indicator) medium.

Blood-Agar medium is used to differentiate between hemolytic and non-hemolytic bacteria. Hemolytic bacteria (e.g., many streptococci and staphylococci isolated from throats) produced clear zones around their colonies because of red blood cell destruction.

Such a clear zone is not formed around non-hemolytic bacterial colonies. MacConkey-Agar medium contains lactose and neutral red. Lactose fermenting bacteria after growth on this medium produce acid and in acidic pH the neutral red becomes red in colour.

Thus *E. coli*, which is lactose fermenter, produces red or pink colonies on the medium. Christensen's medium possesses urea and phenol red. When urease producing bacteria (e.g., Proteus, Klebsiella) grow on this medium, urea is broken into ammonia and CO2. Ammonia turns the medium alkaline and in alkaline pH the medium becomes pink in colour because phenol red becomes red in colour in alkaline medium.

However, the constituents of Blood-Agar medium are the following:
Infusion from beef heart……..500.0 g
Sodium chloride…….……5.0 g

Tryptose…….……5.0 g
Agar…….15.0 g
Distilled water……..100 ml

(v) Enriched Media:

Enriched media are prepared to meet the nutritional requirements of metabolically fastidious microorganisms by addition of specific growth substances such as blood, serum and egg to a basal medium.

Important examples of enriched media are blood agar for isolation of Streptococcus, chocolate agar for isolation of Neisseria and Haemophilus, Bordet-Gengou for isolation of *Bordetella*, and Loeffer's serum slope for the isolation of *Corynebacterium dephtheriae.*