FREQUENTLY ASKED QUESTIONS

1. What is the difference between culture medium and culture?

Ans). A culture medium is nutrient material prepared in the laboratory for the growth of microorganisms. While microorganisms that grow in size and number on a culture medium are referred to as a culture.

2. What is the function of agar in culture media?

Ans). Agar is a polysaccharide that is derived from red seaweed. Its ability to melt at 85 to 90 ^{o}C and solidify at 34 to 42 ^{o}C. The most popular function of agar in microbiology culture media is used as solidifying agent.

3. What is the optimum pH of culture media for growth of microorganisms?

Ans). The growth of organisms in a particular medium may be completely inhibited if the pH of the medium is not within certain limits. The enzymes of microorganisms are greatly affected by this factor. Since most bacteria grow best at around pH 7 or slightly lower, the pH of nutrient broth should be adjusted to pH 6.8. Pathogens on the other hand, usually prefer a more alkaline pH. Trypticase soy broth, a suitable medium for the more fastidious organisms, should be adjusted to pH 7.3.

4. Describe briefly different classes of microorganisms on the basis of energy requirements.

Ans). Organisms that have pigments that enable them to utilize solar energy are called photoautotrophs. Media for such organisms will not include components to provide energy. Autotrophs that cannot utilize solar energy but are able to oxidize simple inorganic substances for energy are called chemoautotrophs. The essential energy yielding substance for these organisms may be as elemental as nitrite, nitrate or sulfide. Most bacteria fall into the category of chemoheterotrophs that require an organic source of energy such as glucose or amino acids. The amounts of energy yielding

ingredients in media for both chemosynthetic types are on the order of 0.5%. A small number of bacteria are classified as photoheterotrophs. These organisms have photosynthetic pigments enabling them to utilize sunlight for energy, but must have an organic source of carbon, such as alcohol.

5. Who is the father of culture media?

Ans). Robert Koch (1843-1910) could be considered the father of culture media. His first success in bacteriology was in the isolation of Bacillus anthracis which at the time was causing the disease anthrax in cattle. This was the first time that any pathogenic organism had been isolated and studied outside of the host's body.

6. What are the demerits of using gelatin as solidifying agent in media?

Ans). The demerits of using gelatin as solidifying agent in media are:

a) It changed from a solid to a liquid at about 25 ^{o}C, preventing it from being incubated at 37 ^{o}C, which is optimal for most bacteria.

b) Gelatin is liquefied by the enzyme gelatinase, which is produced by most proteolytic bacteria.

7. Name different classes of media on the basis of consistency?

Ans). Culture media can be differentiated into three classes on the basis of consistency.

a. Solid media

Solid media contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

b. Semisolid media

They are prepared with agar at concentrations of 0.5% or less. They have soft

custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

c. Liquid (Broth) media

These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies and various other tests e.g., sugar fermentation tests, MR-VR broth.

8. What is the difference between basic media and enriched media?

Ans). Basic media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal media. These media are generally used for the primary isolation of microorganisms. While addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc. are few of the enriched media.

9. Name different factors used to make a media selective?

Ans). Selective media are used for the growth of only selected microorganisms. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the bacteria of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing.

10. Describe briefly the functions of differential media.

Ans). Differential media make it easy to distinguish colonies of desired organisms from non-desirable colonies growing on the same plate. Pure cultures of microorganisms have identifiable reactions with different media. Certain media are designed in such a way that different bacteria can be

recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc., so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

11.Define transport media?

Ans). 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 pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency.

12.What are the culture requirements of anaerobic bacteria?

Ans). Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation–reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to 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. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

13.Describe the functions of antibacterial agents in fungal media?

Ans). Antibacterial agents are used to kill the contaminating bacterial species. Antibacterial agents such as chloramphenicol, gentamicin and ciprofloxacin are commonly used to inhibit the growth of bacteria.

14.Enlist different laboratory media used for cultivation of molds and yeasts.

Ans). The different media used for cultivation of molds and yeasts include:

a) Brain-heart infusion (BHI) agar: It is a non-selective fungal culture medium that permits the growth of virtually all relevant fungi. It is used for the primary recovery of saprophytic and dimorphic fungi.

b) Czapek's agar: It is used for the subculture of Aspergillus species for their differential diagnosis.

c) Inhibitory mold agar (IMA): Primary recovery of dimorphic pathogenic fungi. Saprophytic fungi and dermatophytes will not be recovered.

d) Sabouraud's dextrose agar with cycloheximide and chloramphenicol added. It is used for the primary recovery of dermatophytes.

e) Potato Dextrose Agar (PDA): It is a relatively rich medium for growing a wide range of fungi.

f) Sabouraud's Heart Infusion (SABHI) agar: Primary recovery of saprophytic and dimorphic fungi, particularly fastidious strains.

g) Potato flake agar: Primary recovery of saprophytic and dimorphic fungi, particularly fastidious and slow growing strains.

ASSIGNMENTS

1. What are the different nutritional needs of microorganisms?
2. What is the difference between chemically defined and undefined media?
3. Enlist different classes of media on the basis of purpose/functional use/ application.
4. Name different ingredients used in nutrient media for cultivation of yeasts and molds?