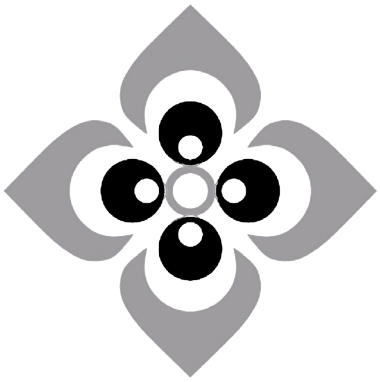

Consortium for Educational Communication

Module on

Culture media for microorganisms

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Text

History of culture media

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. Although Louis Pastuer (1822-1895) and Koch are both considered to be responsible for the establishment of the science of microbiology, Koch and his coworkers systematically identified all the agents of the major bacterial infectious diseases of the 19th century. Previously, other early scientists had experimented with various substrates for growing bacteria; among them are bread, potato, coagulated egg albumin, starch paste and meat. Koch observed that few pathogenic organisms would grow on potato and other media such as egg, prevented adequate inspection of the colonies for growth. Credit must also be given to the Italian, Bartolomeo Bizio, whose work preceded Koch's by 50 years. He was possibly the first to attempt to grow an organism on a solid medium. In 1832 there were reports of the "miraculous" appearance of "blood spots" on bread and communion wafers. Bizio was able to subculture chromogenic bacteria onto other bread surfaces in pure culture and named it Serratia marcescens. Partial success was reached when Koch discovered the use of gelatin that could be mixed with broths. In 1881 he presented this technique at the International Medical Congress in London, which was attended by Louis Pasteur and Joseph Lister. However, there were two discouraging problems associated with the use of this new "nutrient gelatin" medium:

1. 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.
2. Gelatin is liquefied by the enzyme gelatinase an exoenzyme, which is produced by most of the proteolytic bacteria that degrades gelatin.

These problems were soon overcome when agar was tried out as a solidifying agent for the first time in late 1881. Credit for this discovery

belongs to Fanny Hesse, who was born in New Jersey. Fanny married the German physician Walter Hesse, who was experimenting with airborne bacteria in Saxony. Fanny, who served as Walter's laboratory technician and illustrator, pondered the problems associated with gelatin based media and suggested using agar instead. Fanny and her mother had used agar to prepare fruit jelly, as did her Dutch friends who once lived in Java. There it had been used for generations as a jellifying agent which was useful in hot climates.

Agar is a polysaccharide that is derived from red seaweed (Rhodophyceae). Its ability to melt at 85 to 90 ^{o}C and solidify at 37 to 42 ^{o}C makes it ideal for culture media. Another benefit is that it is clear for ease of observing colonies and was found not to be toxic to bacteria. When Dr. Hesse visited Koch's lab for several weeks, he introduced him to the agar based media. Koch immediately recognized the value of this new agent and began to use it routinely in his work. Unfortunately, Fanny Hesse was not recognized for her contribution to microbiology until 1939, five years after her death. The simple creation of this sterile, transparent agar based medium revolutionized and accelerated the research of that time, and remains the basis of all practical bacteriology and mycology today. Now bacterial colonies could be easily incubated, isolated, enumerated, and consequently characterized and identified. This was the golden age of medical bacteriology as Pasteur and Koch identified the bacterial origins of one disease after another. As Koch gleefully wrote, the discoveries came "as easily as ripe apples fall from a tree!".

When agar was discovered, it was common practice at that time to pour the media onto a flat glass plate, then cover it with a bell jar while it cooled. In 1887, another associate of Koch's, by the name of Richard Petri decided to modify this method by putting the media into glass dishes, with sides that were about 15 mm tall and a diameter of about 100 mm. The original design of this "Petri Dish" has not changed up to this day, except for being made of disposable plastic. Koch and his associates continued to experiment with bacteria using meat infusions or extracts as their nutrient base. Although this is an adequate source of most growth factors

for bacteria, it lacks sufficient concentration of amino-nitrogen for optimal growth. It was Frederick Loeffler, who was investigating Corynebacterium diphtheriae who first had the idea of adding peptones to the nutrient broths. Peptones are short chains of amino acids that are prepared by digesting meat with enzymes. Loeffler also added sodium chloride to the media, which created the proper osmotic level for optimal growth. Naegeli published a study in 1880 that found that peptones were the most effective of the nitrogenous compounds tested in promoting bacterial growth. Amino acids, being too small, and protein too large to be utilized by some bacteria, the midrange size of peptones appeared to be the ideal source of nitrogen. These peptones produced by protein hydrolysis are still the most significant ingredient in modern bacteriological culture media. Emmanual Merck of Darmstadt, Germany began to manufacture these peptones in 1892, which gave European microbiologists the option of purchasing their media ingredients rather than fabricating their own.

Nutritional requirements of microorganisms

Before one can construct a medium that will achieve a desired result in the growth of microorganisms, one must understand their basic needs. The following are the minimum components required in a microbial medium for cultivation of microbes in a laboratory:

Water: Protoplasm is from 70 to 85% water. The water in a single celled organism is continuous with the water of its environment and the molecules pass freely in and out of the cell, providing a vehicle for nutrients inward and secretions or excretions outward. All the enzymatically controlled chemical reactions that occur within the cell occur only in the presence of an adequate amount of water. The quality of water used in preparing media is important. Hard tap water, high in calcium and magnesium ions should not be used. Insoluble phosphates of calcium and magnesium may precipitate in the presence of peptones and beef extract. The best policy is to always use distilled water.

Carbon: A simple carbon source, which is simple to use and easily available can be used for synthesizing cell components. Sugars such as glucose,

lactose, sucrose, and complex polysaccharides such as starch, glycogen cellulose, a mixture of various carbohydrates and other compounds such as cereal grain powders, cane molasses etc., are usually used as carbon sources in microbial culture media. The main purpose of the carbon source is to provide energy and carbon skeleton for the synthesis of various other biological compounds.

Nitrogen: Although autotrophic organisms can utilize inorganic sources of nitrogen such as nitrates or ammonium slats, the heterotrophs get their nitrogen from amino acids and intermediate protein compounds such as peptides, proteoses and peptones. The major types of nitrogen sources used in culture media are ammonium salts, urea, animal tissue extracts, amino acid mixtures and plant-tissue extracts.

Micro elements or trace elements: All living organisms require metal ions such as K^+, Ca^{2+}, Mg^{2+}. Elements required in small amounts or in traces are to be added into the medium as salts in required amounts. The elements such as copper, cobalt, iron, zinc, manganese, magnesium, etc., are the microelements, that are sufficient to support microbial growth.

Growth factors: Growth factors are certain organic compounds that are essential for the growth and multiplication of cells, but cannot be synthesized by the cells. Such compounds should be supplemented in the medium. Certain amino acids and vitamins are also included in this category.

Energy: Organisms that have pigments that enable them to utilize solar energy are called photoautotrophs e.g., Chromatium okenii. 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 e.g., Nitrosomonas spp. 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.

Hydrogen ion concentration: The growth of organisms in a particular medium may be completely inhibited if the pH of the medium is not within the 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.

Classification of bacterial culture media

Bacterial culture media can be classified on the basis of composition, consistency and purpose.

Classification based on consistency

1. 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. Silica gel is sometimes used as inorganic solidifying agent for autotrophic bacteria.

2. 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.

3. Liquid (Broth) media

These media contains specific amounts of nutrients but don’t have traces 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, Methyl Red Voges Proskauer (MR-VP) Broth, etc.

Classification based on composition

1. Synthetic or chemically defined media

A synthetic or chemically defined media is one whose exact chemical composition is known. Chemically defined media must contain growth factors that serve as a source of energy and carbon. Chemically defined media are used for the growth of autotrophic bacteria.

2. Non synthetic or chemically undefined media

Non-synthetic or chemically undefined media contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts. Non-synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms. Heterotrophic bacteria and fungi are normally grown on complex media, which are made up of nutrients, such as yeasts, meat, plants, or proteins (the exact composition is not quite known and can vary with each mixture). In complex media, the energy, carbon, nitrogen, and sulfur needed for microbial growth are provided by protein. Proteins are large molecules that some microorganisms can use directly. Partial digestion by acids and enzymes break down proteins into smaller amino acids called peptones. Peptones are soluble products of protein hydrolysis. These small peptones can be digested by bacteria. Different vitamins and organic growth factors can be provided by meat and yeast extracts. If a complex medium is in a liquid form it is called a nutrient broth. If agar is added, it is called a nutrient agar. Agar included as a non-nutritive solidifying agent, when a solid medium is desired.

Classification based on purpose/functional use/application

Many special purpose media are needed to facilitate recognition, enumeration and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1. General purpose media/ Basic media

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.

2. Enriched media (Added growth factors)

Addition of extra nutrients in the form of blood, serum, egg yolk, etc., to basal media 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. Blood agar is prepared by adding 5-10% (by volume) to a blood agar base. Chocolate agar is also known as heated blood agar or lysed blood agar.

3. Selective and enrichment media

These media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover specific bacteria from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. 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.

a. Selective media

Selective media are used for the growth of only selected microorganisms and do not enhance and may even inhibit other type of microorganisms that may be present. 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. Selective media are agar based (solid) media so that individual colonies may be isolated. Examples of selective media include:

i. Mannitol salt agar and salt milk agar used to recover Staphylococci aureus contain 10% NaCl.

ii. Potassium tellurite medium used to recover Corynebacterium diphtheriae contains 0.04% potassium tellurite.

iii. MacConkey's agar used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.

iv. Pseudosel agar (Cetrimide agar) used to recover Pseudomonas aeruginosa contains cetrimide (antiseptic agent).

v. Crystal violet blood agar (sheep blood 5%) used to recover Streptococci pyogenes contains 0.0002% crystal violet.

vi. Lowenstein Jensen medium used to recover Mycobacterium tuberculosis is made selective by incorporating malachite green, in order to inhibit unwanted bacteria.

vii. Wilson and Blair's agar for recovering S. typhi is rendered selective by the addition of dye brilliant green.

b. Enrichment culture media

Enrichment media is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective media. Unlike selective media, enrichment culture is typically used as broth media. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to recover pathogens from fecal specimens.

4. Differential/ indicator media

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 differential 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. Examples of differential media include:

a) Mannitol salt agar is differential medium where Staphylococcus aureus ferments manitol and produces acid that changes indicator red to yellow.

b) Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis)

c) Mac Conkey agar (lactose fermenters, produces pink colonies whereas non- lactose fermenter produces pale or colorless colonies.

d) Thiosulfate-citrate-bile salts-sucrose agar (TCBS) on which Vibrio cholera produces yellow colonies due to fermentation of sucrose)

5. 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 pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Moreover transport media should be free from carbon, nitrogen, organic growth factors, molecular oxygen to prevent microbial multiplication.

- Cary Blair medium and Venkatraman Ramakrishnan (VR) medium are used to transport feces from suspected Vibrio 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.

6. Anaerobic media

Anaerobic bacteria needs 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. Robertson cooked meat that is commonly used to grow Clostridium spps medium contain a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. 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.

Nutrient media for yeasts and molds

Although many selective agars exist for the cultivation and determination of mold and yeast cultures, a majority of them do not depend on strict nutritive requirements for growth. Many fungal strains will grow on Sabouraud Dextrose Emmons Agar. Alternative agars for growth include Sheep Blood Agar, Nutrient Agar, Tryptic Soy Agar (Soybean Casein Digest), Potato Dextrose Agar and Standard Methods Agar (Plate Count Agar). Antibacterial agents such as chloramphenicol, gentamicin and ciprofloxacin are commonly used to inhibit the growth of bacteria. Antibacterial agents are used to kill the contaminating bacterial species. If the sample is taken from sterile site, it is not necessary to use media containing antibacterial agents. An example of yeast and mold media includes:

1. 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

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

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

4. Mycosel/Mycobiotic agar:

a) It is generally Sabouraud's dextrose agar with cycloheximide and chloramphenicol added.

b) It is used for the primary recovery of dermatophytes.

5. Niger Seed Agar: It is used for the identification of Cryptococcus neoformans.

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

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

8. Sabouraud's dextrose agar (SDA):

a) Sabouraud's agar is sufficient for the recovery of dermatophytes from cutaneous samples and yeasts from vaginal cultures.

b) Not recommended as a primary isolation medium because it is insufficiently rich to recover certain fastidious pathogenic species, particularly most of the dimorphic fungi.

c) Sabouraud's dextrose agar (%2) is most useful as a medium for the subculture of fungi recovered on enriched medium to enhance typical sporulation and provide the more characteristic colony morphology.

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