MOOC 4, Module 16

Types of culture media

Text

Bacterial cultural media can be classified in various ways.

Classification

Bacterial culture media can be classified in at least three ways;

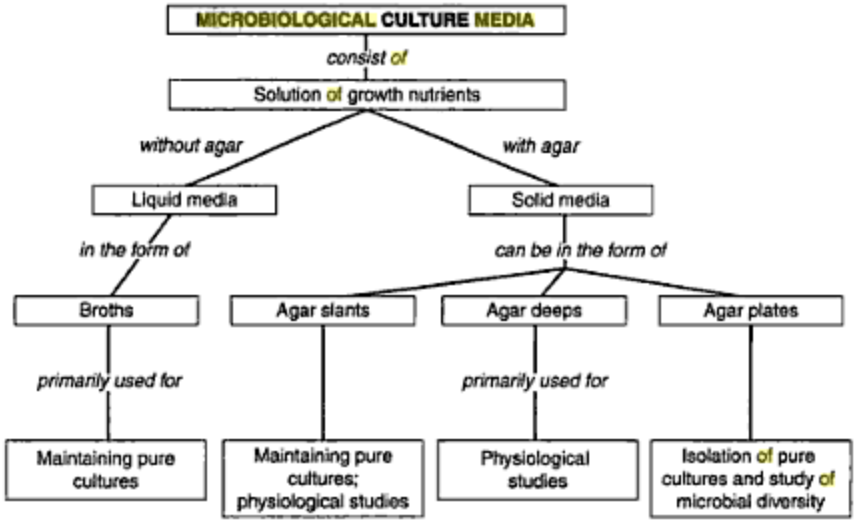
- Based on consistency
- Based on nutritional component
- Based on its functional use.

1. Culture media based on consistency:

Culture media are liquid, semisolid, or solid and biphasic.

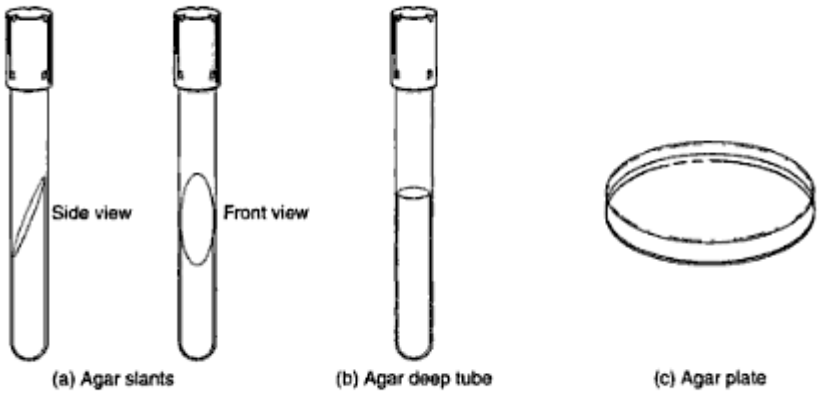
A) **Liquid media**: These are available for use in test tubes, bottles or flasks. Liquid media are referred to as broths, eg. Nutrient broth. Bacteria generally uniformly producing general turbidity. The liquid media is preferred when a particular population of bacteria is desired within a short period of time for pure cultures of bacteria.

B) **Solid Media**: Any liquid medium can be rendered 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 wall of some species of red algae such as the genera *Gelidium*. It melts at 95^{o}C (sol) and solidifies at 42^{o}C (gel). It is not easily hydrolyzed by the bacterial species and thus contributes only as a solidifying agent.The most common concentration used for bacteriological purpose is at concentration of 1-3% to make a solid agar medium.
The advantage of solid culture over liquid culture is that it immobilizes the bacterial cell as a result of which discrete colonies are formed which help us to study the bacterial morphology (Fig. 1, 2).
The solid agar medium can be various types depending upon the application.
The agar medium after it is poured into the culture tubes and after sterilization is cooled and hardened in a slanted position then it is known as agar slants. It is used mainly for physiological studies or maintenance of pure cultures.
Similar tubes when hardened in an upright position are called as agar deeps. Agar deeps are mainly used for physiological studies of the microorganisms and in the maintenance of anaerobic bacteria (Fig. 3).
When sterilized solid media is spread over large surface area such as petri plates it is known as agar plates and provides large surface area for the separation of pure cultures from mixed cultures (Fig.4).



A Concept Map for Microbiological Media. The different types of microbiological media and their uses are shown.

Figure 1. The types of liquid and solid microbiological media (Alcamo's fundamentals of microbiology-Pommerville, 2007).



The Different Types of Solid Media. Solid media can be prepared using agar to prepare (a) agar slants, (b) agar deeps, and (c) agar plates.

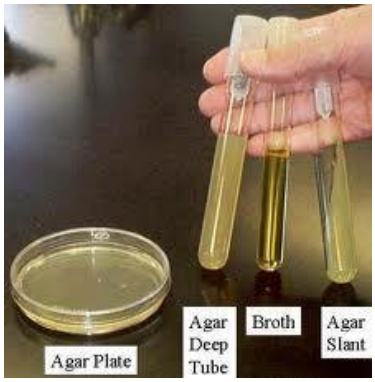


Figure 2. The different types of solid media (Alcamo's fundamentals of microbiology-Pommerville, 2007**)**

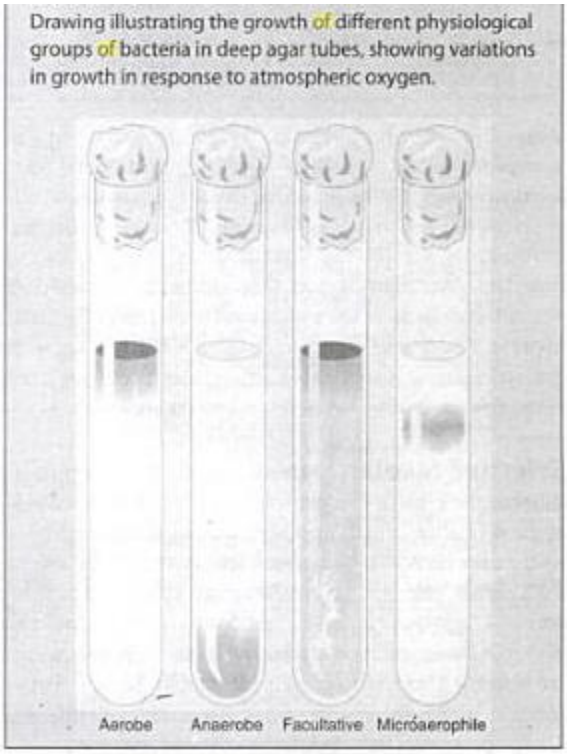


Figure 3: The function of agar deeps for isolation of anaerobes (Microbiology-Practical based approach by Pelczar, 2010).

Figure 4.Agar plate used for isolation of pure colonies of a particular strain of bacterium.

C) **Semi-solid media**:The amount of agar when reduced to 0.2-0.5% renders a medium semi-solid. The application of such media is in demonstrating bacterial motility and separatingmotile from non-motile strains (U-tube and Cragie's tube). Certain transport media such asStuart's and Amies media are semi-solid in consistency. Hugh and Leifson's oxidation fermentation test medium are also semi-solid (Fig. 5).

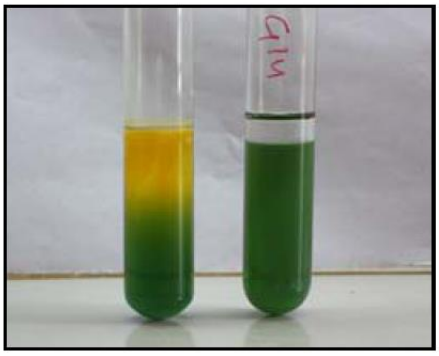


Figure 5: Semi-solid media

D) **Biphasic media:** Sometimes, a culture system comprises of both liquid and solid medium inthe same bottle. This is known as biphasic medium (Castaneda system for blood culture). Theinoculum is added to the liquid medium and when subcultures are to be made, the bottle issimply tilted to allow the liquid to flow over the solid medium. This removes the need forfrequent opening of the culture bottle to subculture (Fig. 6).

Figure 6: Biphasic medium

2. Classification of medium based on its nutritional component

Media can be classified further as complex, synthetic/defined and semi-synthetic based on the nutrients required for the growth of microorganisms. The bacteria which are able to grow with minimal requirements are said to be non-fastidious and those that require supplements or extra nutrients are said to be fastidious.

A) Synthetic or defined media

Some microorganisms as photolithotrophic autotrophs such as cyanobacteria and eukaryotic algae can be grown in this kind of media. They can be grown on relatively simple media containing CO_2 as a carbon source, nitrate or ammonia as a nitrogen source, phosphate and a variety of minerals (Table 1). The medium in which all components are known is called a defined or synthetic medium. It is generally prepared by adding precise amounts of highly purified inorganic or organic chemicals to distilled water. The exact composition of a defined medium both in the sense of qualitative and quantitative is known. Many chemoorganotrophic heterotrophs also can be grown in this kind of media with glucose as an organic carbon source and an ammonium salt as a nitrogen source.

This medium is used when an organism's specific growth requirements are being determined.

Examples of Defined Media

BG–11 Medium for Cyanobacteria	Amount (g/liter)
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na_2Mg salt)	0.001
Na_2CO_3	0.02
Trace metal solution[a]	1.0 ml/liter
Final pH 7.4	

Medium for *Escherichia coli*	Amount (g/liter)
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Sources: Data from Rippka, et al. *Journal of General Microbiology,* 111:1–61, 1979; and S. S. Cohen, and R. Arbogast, *Journal of Experimental Medicine,* 91:619, 1950.

[a]The trace metal solution contains H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2Mo_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$.

Table 1. The examples of defined medium required for the growth of selected bacteria (Taken from Microbiology-Prescott, 2002).

B) Complex media

Media that contain some ingredients whose exact chemical composition is not known are complex media. These kinds of media are very useful as a single complex medium can be sufficiently rich and can be able to meet all the nutritional requirements of a diverse group of bacteria. Sometimes the exact nutritional requirement of a particular group of microorganism is not known; hence they can be grown in complex media. Thisis especiallyapplicable for the group of fastidious bacteria; some of them may even require a medium containing serum or blood (Table 2).

Some Common Complex Media

Nutrient Broth	Amount (g/liter)
Peptone (gelatin hydrolysate)	5
Beef extract	3
Tryptic Soy Broth	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
MacConkey Agar	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Table 2: List of some complex media used in routine microbiological based works (Taken from Microbiology-Prescott, 2002).

Complex media generally contains undefined ingredients as peptones, meat extract and yeast extract. Peptones are protein hydro lysates which is prepared by incomplete proteolytic digestion of meat, casein, soya, meal, gelatin and other protein sources. They are sources of carbon, energy and nitrogen. Beef extract commonly used in complex media are sources of amino acids, peptides, nucleotides, organic acids, vitamins and minerals. Whereas yeast extract derived from brewer's yeast is a rich source of vitamin B as well as nitrogen and carbon sources. Three commonly used complex media are nutrient broth, tryptic soy broth and MacConkey agar as shown in Table 2.

Table 3: Function of ingredients used in complex media (Taken from Microbiology-Pelczar, 1993)

Beef extract	An aqueous extract of lean beef tissue concentrated to a paste	Contains the water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water-soluble vitamins, and salts
Peptone	The product resulting from the digestion of proteinaceous materials, e.g., meat, casein, and gelatin; digestion of the protein material is accomplished with acids or enzymes; many different peptones (depending upon the protein used and the method of digestion) are available for use in bacteriological media; peptones differ in their ability to support growth of bacteria	Principal source of organic nitrogen; may also contain some vitamins and sometimes carbohydrates, depending upon the kind of proteinaceous material digested
Agar	A complex carbohydrate obtained from certain marine algae; processed to remove extraneous substances	Used as a solidification agent for media; agar, dissolved in aqueous solutions, gels when the temperature is reduced below 45°C; agar not considered a source of nutrient to the bacteria
Yeast extract	An aqueous extract of yeast cells, commercially available as a powder	A very rich source of the B vitamins; also contains organic nitrogen and carbon compounds

Complex media are commonly used in the teaching laboratory as the purpose is to simply grow prokaryotes and not be concerned about their specific growth requirements are.

C) **Semi-synthetic medium**: The semi synthetic medium comprises of a mixture of ingredients whose exact composition is known and unknown. The examples of semi-synthetic medium are Potato dextrose agar medium, peptone water, etc.

Peptone water is used as a growth medium and as a component of carbohydrate fermentation media. It is generally used for the cultivation of non-fastidious organisms.

Types of media based on functional use:

A) Basal Medium: are basically simple media that supports most non-fastidious bacteria. Ex- peptone water, nutrient broth and nutrient agar. They are generally considered for growing broad spectrum of bacteria.

B) Minimal medium: Minimal media are those kinds of media that contain the minimum nutrients required for colony growth, generally without the presence of amino acids, and are often used in laboratories to isolate wild type microorganisms. Minimal media can also be used to select for or against recombinants bacteria.

Minimal medium typically contains:

- a carbon source for bacterial growth, which may be a sugar such as glucose
- various salts, which may vary among bacteria species and growing conditions; these generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the bacteria to synthesize protein and nucleic acid
- water

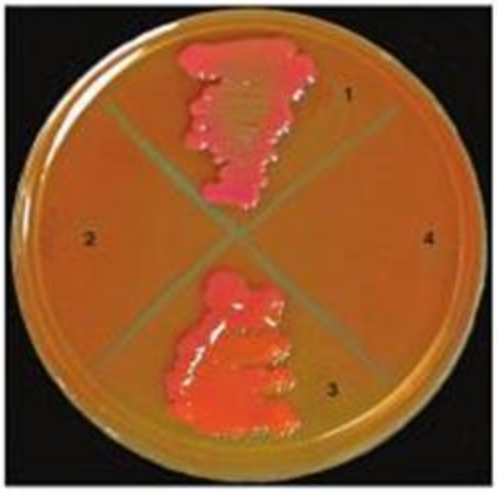
C) Selective medium: These kind of media provide nutrients that promotes the growth and predominance of a particular type of bacterium and do not increase (or even prevents) other type of organisms that may be present. For instance, a medium in which cellulose is the only carbon source utilizing organisms when it is inoculated with a soil sample containing many kinds of bacteria.

Bile salts or dyes like basic fuchsin and crystal violet favor the growth of gram negative bacteria by inhibiting the growth of gram positive bacteria without hampering the growth of gram-negative bacteria (Fig. 7A, B, D).

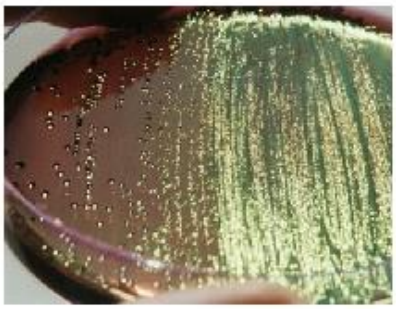
Examples of selective media:

- eosin methylene blue (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram negative bacteria
- YM (yeast and mold) which has a low pH, deterring bacterial growth
- MacConkey agar for Gram-negative bacteria
- Hektoen enteric agar (HE) which is selective for Gram-negative bacteria
- mannitol salt agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol
- Terrific Broth (TB) is used with glycerol in cultivating recombinant strains of *Escherichia coli*.
- xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria
- buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, especially *Legionella pneumophila*

These kinds of media is widely used for the detection of E. coli and related bacteria in water supplies and elsewhere, contains dyes that suppress gram-positive bacterial growth.

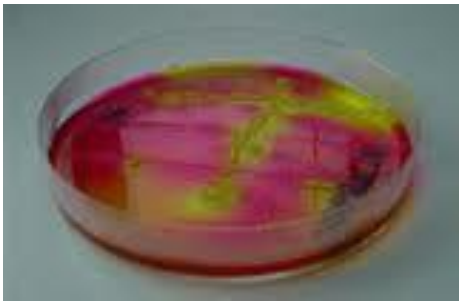


A



E. coli on EMB

B

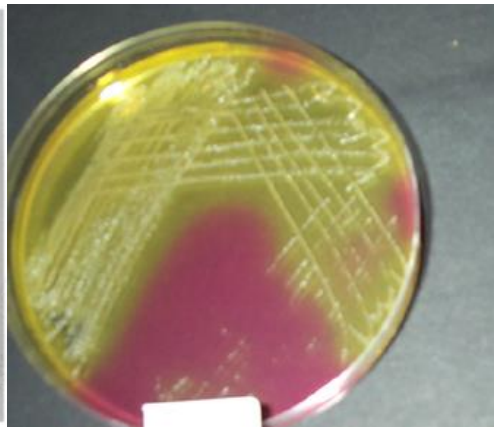


BGA for *Salmonella*sp

C

MacConkey agar for Gram negative bacteria

D



MSA for *Staphylococci*

E

Figure 7. Different types of Selective medium used for a particular group of bacteria.

The brilliant green agar, used to isolate the Gram-negative bacilli in the genus *Salmonella*. *Salmonella* cause food-borne infections in human beings. The dye when added to agar inhibits the Gram-positive bacteria, which is commonly found in the intestinal tract. Antibiotics are added to some media making them selective for microbes that are resistant to the antibiotics. For instance, if the antibiotic rifampicin is used, the bacteria spirochetes can grow in the presence of antibiotic, while the other kinds fail to grow (Fig. 5C).

Selective media as mannitol salt agar (MSA) is used in the isolation of pathogenic staphylococci. The ingredients are mannitol, a phenol red indicator, and 7.5% sodium chloride. The high salt concentration prevents the growth of other organisms. The colonies of pathogenic *Staphylococcus aureus* produce small colonies surrounded by yellow zones. They ferment the mannitol, producing an acid, which in turn changes the phenol red to yellow. The growth of other types of bacteria is generally inhibited (Fig. 5E).

D) Differential Media

Another type of media regularly used in routine microbiological work is differential media. It employs the addition of one or more substances that allowdifferentiating between very closely related species based on specific biochemical or physiological properties. The differential contains in the culture medium contains specific chemicals to indicate which species possess and which lack a particular biochemical process.

Examples of differential media include:

- blood agar (used in strep tests), which contains bovine heart blood that becomes transparent in the presence of hemolytic *Streptococcus*
- eosin methylene blue (EMB), which is differential for lactose and sucrose fermentation
- MacConkey (MCK), which is differential for lactose fermentation
- mannitol salt agar (MSA), which is differential for mannitol fermentation
- X-gal plates, which are differential for lac operon mutants

Blood Agar : Sometimes the differential media are also enriched ones. If a mixture of bacteria is inoculated into a blood-containing agar medium (blood agar), some of the bacteria may produce enzymes that break the blood cells and others may not. Depending on the pattern of lysis surrounding each bacterial colony, one can discriminate between hemolytic and nonhemolytic bacteria form the same specimen.

- **<u>Hemolysis with Blood Agar</u>**

- This medium contains agar contains 5% sheep's blood
- **differential for**: **hemolysis**...particularly in *Streptococci*, which is judged by the ability to break down hemoglobin or red blood cells, 3 groups of microorganisms can be observed
  - **alpha-hemolysis**: a green to light-brown halo is seen around the colonies; bacteria partially break down hemoglobin leaving a green pigment (biliverdin)
  - **beta-hemolysis**: a clearing is seen around the colonies; bacteria produce a "beta-hemolysin" which lyses red blood cells in the medium
  - **gamma-hemolysis (no hemolysis)**: no hemolysis is observed; bacteria do not produce a hemolysin (Fig.8)

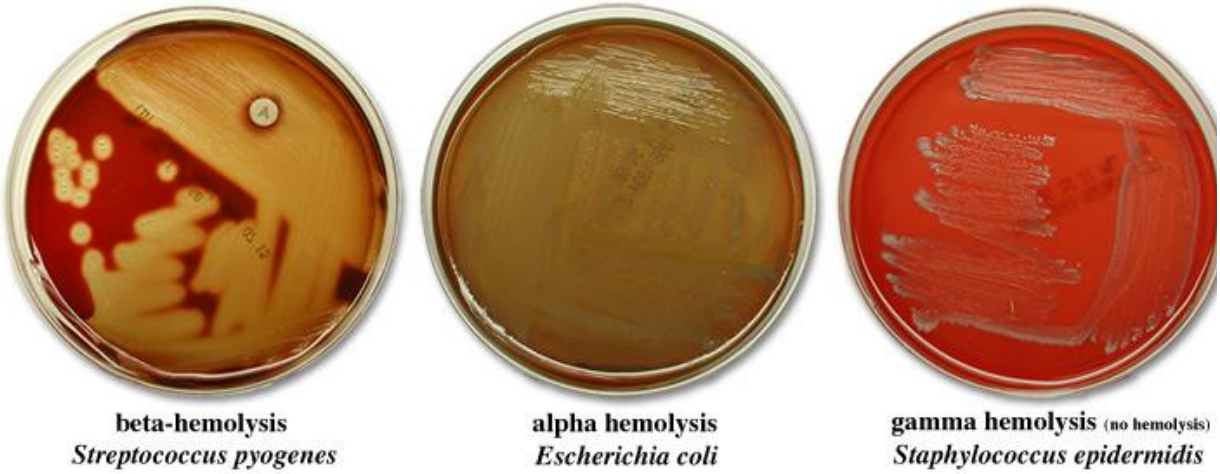


Figure 8. Different types of hemolysis seen on blood agar plate.

EMB (Eosin Methylene Blue) Agar

- **selective for**: **gram-negative bacteria**
  Eosin methylene blue agar is a differential as well as a selective medium. A combination of eosin and methylene blue is used as an indicator and allows differentiating between organisms that ferment lactose and those that do not. In addition, methylene blue acts as an inhibitor to Gram-positive organisms.

- **differential for**: **lactose fermentation**
  - gram-negative Enterobacteria-*Escherichia coli* and *Enterobacteraerogenes* ferment lactose
  - *E. coli* produces colonies with a characteristic green metallic sheen on EMB agar
  - *E. aerogenes* produces pink colonies often with a central dark purple dot (fish eye colonies) on EMB agar
  - gram-negative bacteria *Proteus vulgaris* and *Salmonella typhimurium* grow on EMB agar, but do not ferment lactose (Fig. 9)

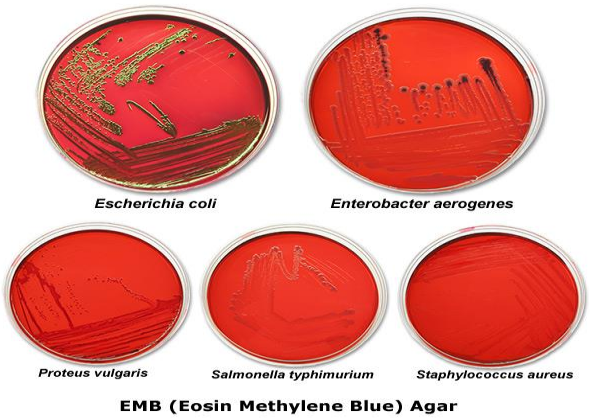


Figure 9. Different types of Enterobacteria growing on EMB plates.

Mac Conkey Agar

differential for: **lactose fermentation**

- neutral red pH indicator turns red in the presence of acid by-products of lactose fermentation
- gram-negative Enterobacteria*Escherichia coli* and *Enterobacteraerogenes* ferment lactose
- *E. coli* produces pink to red colonies often with a reddish bile precipitate surrounding colonies on MacConkey's agar
- *E. aerogenes* produces pink to red mucoid colonies on MacConkey's agar (Fig.10)
  gram-negative bacteria *Proteus vulgaris* and *Salmonella typhimurium* grow on MacConkey's agar, but do not ferment lactose (media appears yellow to light pink in color & colonies are colorless; swarming of *Proteus* is inhibited)

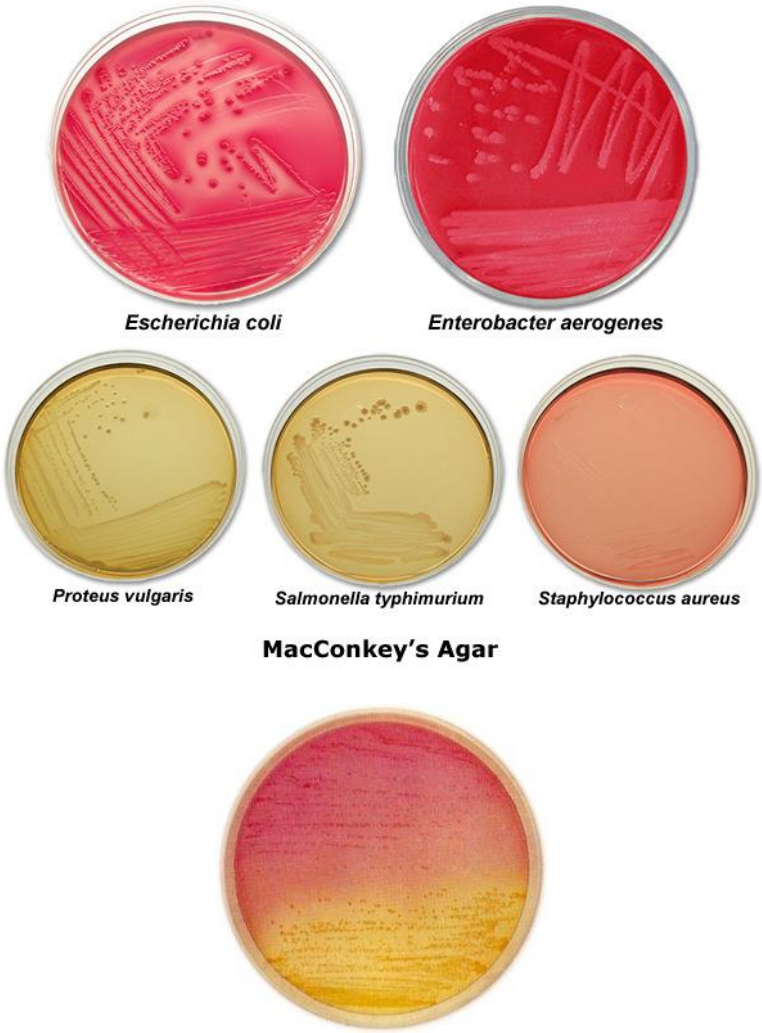


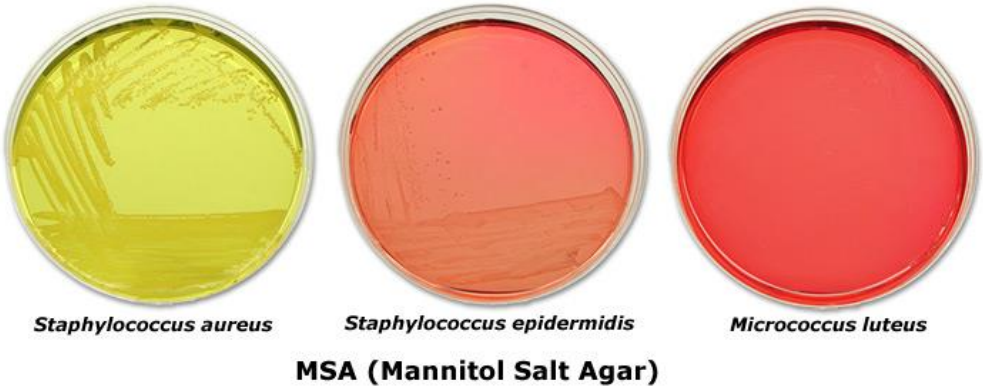
Figure 10.Groups of Enterobacter on MacConkey agar.

Mannitol Salt Agar (MSA)

differential for: **mannitol fermentation**

- phenol red pH indicator turns yellow in the presence of acid by-products of mannitol fermentation
- *Staphylococcus aureus* ferments mannitol

- *S. aureus* changes the color of the medium from pink to yellow due to acid by-products of mannitol fermentation
- *Staphylococcus epidermidis* grows on MSA, but does not ferment mannitol (media remains light pink in color & colonies are colorless)



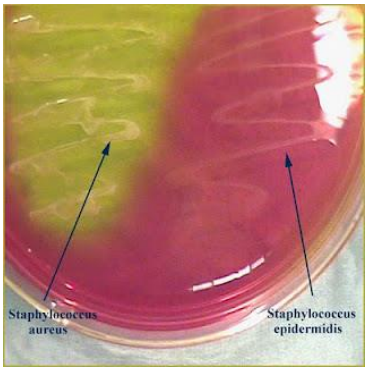


Figure 11.Mannitol Salt Agar used as a differentiating medium.

E) **Enriched medium:** Natural environments usually contain numerous kinds of bacteria or other microorganisms. Some microorganisms are very particular in their growth requirements and are cultured only when they are provided with rich in special nutrients. These kinds of media are generally used to grow fastidious organisms. It is generally done by addition of extra nutrients in the form of blood, serum, egg yolk, etc. to basal medium to make them enriched media. Blood agar, chocolate agar are examples of enriched media.

Chocolate agar (CHOC) - is a non-selective, enriched growth medium. It is a deviation of the normal blood agar plate. It contains red blood cells, which have been lysed by heating very slowly to 56 °C. Chocolate agar is used for growing fastidious (fussy) respiratory bacteria, such as *Haemophilusinfluenzae*. These bacteria reuires growth factors, like NAD and hemin, which are inside red blood cells; thus, a prerequisite to growth is complete lysis of the red blood cells. The agar is named for the color and contains no actual chocolate.

However, with the addition of antibiotics the chocolate agar media becomes selective in nature. Chocolate agar as such is non-selective, however it has been modified with the addition of bacitracin the media becomes selective, most critically, for the genus *Haemophilus*. A further variant containing an assortment of antibiotics selects for *Neisseria* (Fig. 12).

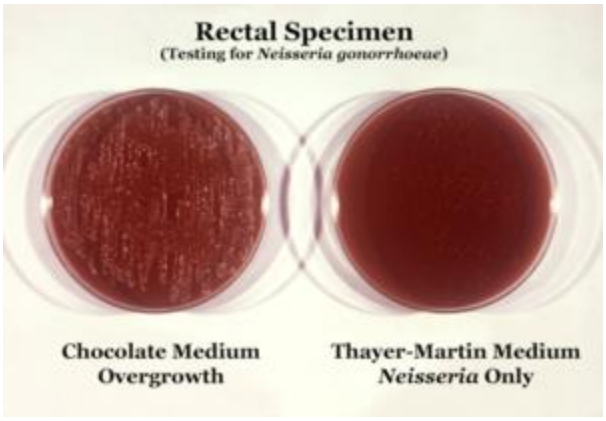


Fig 12.Two types of chocolate medium are been shown. The overgrowth medium mainly allowed for the growth of organismal colonies other than those of *Neisseria gonorrhoeae*, whereas the selective Thayer-Martin medium on the right, contained antimicrobials that inhibit the growth of organisms other than *N. gonorrhoeae*, shows no overgrowth, but is positive for *N. gonorrhoeae* bacteria (Taken from http://en.wikipedia.org/wiki/Chocolate_agar).

A comparison among the three most commonly used types of media are shown in Table 4.

Name	Components	Uses	Examples
Selective medium	Growth stimulants Growth inhibitors	Selecting certain prokaryotes out of mixture	Mannitol salt agar for staphylococci
Differential medium	Dyes Growth stimulants Growth inhibitors	Distinguishing different prokaryotes in a mixture	MacConkey agar for gram-negative bacteria
Enriched medium	Growth stimulants	Cultivating fastidious prokaryotes	Blood agar for streptococci; chocolate agar for *Neisseria* species

F) **Transport media:** This kind of media is especially important when the clinical specimens are being transported to the laboratory immediately after collection of samples to prevent overgrowth of contaminating organisms or commensals. They prevent drying (desiccation) of specimen, maintain the pathogen to commensal ration and inhibit the overgrowth of unwanted bacteria. Addition of charcoal serves to nullify the inhibitory factors.

Examples of transport media include:

- Thioglycolate broth for strict anaerobes.
- Stuart transport medium - a non-nutrient soft agar gel containing a reducing agent to prevent oxidation, and charcoal to neutralise
- Certain bacterial inhibitors- for gonococci, and buffered glycerol saline for enteric bacilli.
- Venkat-Ramakrishnan(VR) medium for*V. cholerae*

G) Maintenance media: The satisfactory maintenance of the viability and physiological characteristics of a culture with time requires a medium different from that which is required for the optimal growth of cells. The optimum growth medium produces prolific rapid growth but it is associated with the rapid death of the cells at the end of growth phase. Glucose which is generally added in a medium frequently enhances growth but the end products of bacteria are harmful to the growth of the cells. Hence, glucose is generally omitted from the maintenance medium.

Anaerobic media: Anaerobic bacteria need special media for growth as they require low oxygen content, reduced oxidation-reduction potential and extra nutrients. Media for anaerobes has to be supplemented with nutrients like hemin and vitamin K. The medium is boiled to remove the dissolved oxygen. These kind of media are also called reducing media as anaerobes might be killed by exposure to oxygen. The sodium thioglycollate chemically combines with dissolved oxygen and depletes the oxygen in culture medium. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can keep the medium reduced. Methylene blue or resazurin is an oxidation-reduction potential indicator that is added to the thioglycollate medium. Under reduced condition, methylene blue is colourless (Fig. 13).

Figure 13. Anaerobic media

Some species of anaerobic bacteria cause disease in humans. For ex. *Clostridium* species that cause tetanus and gas gangrene multiply in the dead, anaerobic tissue of a wound and produce toxins causing tissue damage.

A common way to test an organism's oxygen sensitivity is to use a thioglycollate broth, which binds free oxygen so that only fresh oxygen present at the surface of the tube would only be available (Fig. 13, 14).

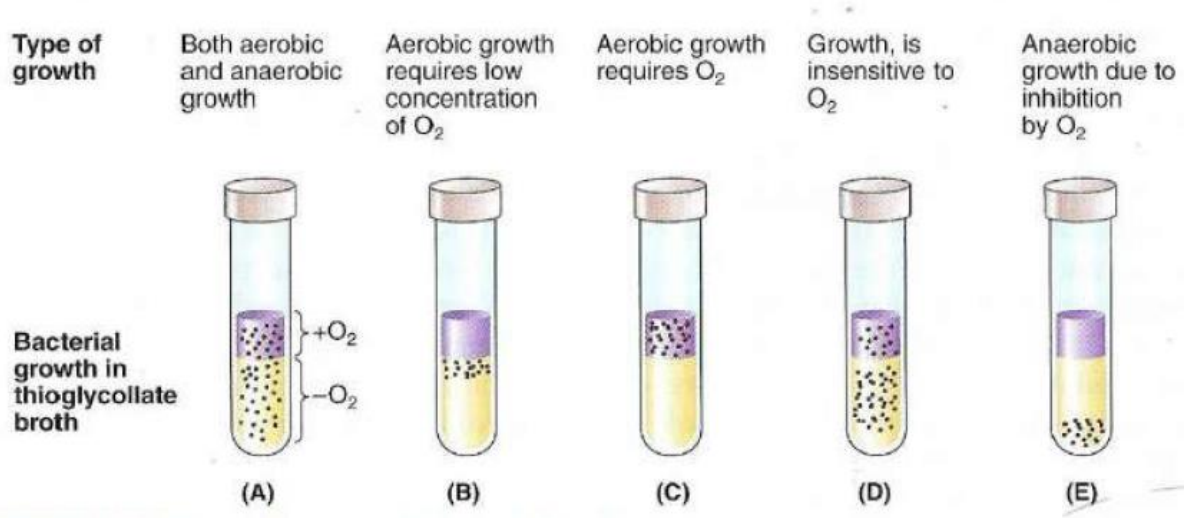


Figure 14. The thioglycollate broth is used to determine microbial-oxygen relationships (Taken from Alcamo's Principles of Microbiology-Pommerville, 2007).

1. The summary of various types of differential and selective media can be seen in Table 5 (http://amrita.vlab.co.in/?sub=3&brch=73&sim=720&cnt=1).

Media	Classification	Selective and Differential agents	Type of organisms isolated
Mannitol salt agar (MSA)	Selective and differential	7.5% NaCl and mannitol for isolation and identification of most *S.aureus* strains	Staphylococci and Micrococci
MacConkey's Agar	Selective and differential	Lactose, bile salts, neutral red, and crystal violet	Gram-negative enteric bacilli
Eosin methylene blue agar(EMB)	Selective and differential	Lactose, eosin Y, and methylene blue	Gram-negative enteric bacilli
Phenylethyl Alcohol Agar (PEA)	Selective	Phenylethyl alcohol (inhibits gram negatives)	Gram-positive bacteria
Hektoen Enteric Agar (HE)	Selective and differential	Lactose, sucrose, bile salts, ferric ammonium sulfate, sodium thiosulfate, bromthymol blue, acid fuchsin	Salmonella and Shigella species (enteric pathogens)
Blood Agar	Enriched and differential	5% defibrinated sheep blood	Almost all bacteria; differential for hemolytic organisms
Chocolate Agar	Enriched	1% hemoglobin and supplements	Most fastidious pathogens such as Neisseria and Haemophilus