Culture media

A culture media is a solid or liquid preparation used to grow, transport and store microorganisms. The medium must contain all the nutrients the microorganisms requires for growth. In general, all the media have a source of energy, carbon, nitrogen, phosphorus, sulfur and various minerals. However, the media composition can vary based on the need.

Media can be classified on the basis of several criteria as is shown in the table below:

Types of Media		
Physical Nature	**Chemical Composition**	**Functional Type**
Liquid	Defined (Synthetic)	Supportive (General purpose)
Semisolid	Complex	Enriched
Solid		Differential
		Selective

Defined medium: A medium in which all chemical components are known is called as defined or synthetic medium. It can be in the liquid form or solidified by addition of agar. These media are often used for culture of photolithotrophic autotrophs such as cyanobacteria and photosynthetic protists. One such media called as BG-11 is used for Cyanobacteria culture and is given in the table-1 below.

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	

Table-1: BG-11 medium for Cyanobacteria culture

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	

Table-2: Media for the culture of *E. coli*

The BG-11 medium has sodium carbonate as carbon source and ammonium salts act as nitrogen source and other salts act as sources of sulfur and phosphate.

The media can be slightly changed and used to grow chemoorganotrophic heterotrophs as is shown in the table-2 above. It contains glucose as carbon source and ammonium salt as nitrogen source.

Defined media are used extensively in research and their formulation is better if one knows what the experimental microbe is metabolizing.

Complex media: These are media which contain some ingredients of unknown chemical composition. These media are often used due to their high utility which is due to the fact that a single complex medium is almost sufficiently rich to completely meet the nutritional requirements of many different microbes. They are also used because nutritional requirements of a particular microbe are usually not known and a defined media can`t be constructed. E.g. Many fastidious bacteria. They have complex nutritional or cultural requirements and they may even require serum or blood in the media.

Undefined components in complex media include peptones, meat extract and yeast extract.

Peptone: They are protein hydrolysates and prepared by partial proteolytic digestion of meat, casein, soya meal, gelatin and other protein sources. These components serve as sources of energy, carbon and nitrogen.

Beef extract: It is aqueous extract of lean beef and contains amino acids, peptides, nucleotides, organic acids, vitamins and minerals etc. which serve as sources of energy, carbon and nitrogen.

Yeast extract: It is aqueous extract of Baker`s yeast. Its components serve as sources of energy, carbon and nitrogen. It is also an excellent source of B vitamins.

Three commonly used complex media are Nutrient broth, Tryptic Soy broth and MacConkey agar. Their composition is given in the table below:

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

Media can be solidified by addition of 1-2% agar. Agar is isolated from red algae and is a sulfated polymer composed of D-galactose,3,6-anhydro-L-galactose and D-glucuronic acid. It is most commonly used solidifying agent due to following reasons:

1. It melts at 90^{o}C and once melted it doesn`t solidify till 45^{o}C. So after melting, it can be cooled to a temperature which suits human hands and working is easy.
2. Microbes growing on Agar can be incubated at a wide range of temperatures facilitating flexibility in experimental design.
3. Since most microbes can`t degrade agar, it doesn`t add additional variables in the experiment.

Note: In some cases, other solidifying agents are used. E.g. Silica gel is used to grow autotrophic bacteria on solid media in the absence of organic substances. It is also used when one is trying to determine carbon sources for heterotrophic bacteria.

Functional media: They are of following types:

Supportive media: They are used to sustain the growth of the microorganisms. E.g. Tryptic Soy broth and Tryptic Soy agar.

Enriched media: These are fortified media and prepared when some specific nutrients are added to the supportive or general purpose media. Example of such media are blood agar and chocolate agar. Both have blood as specific

nutrient and chocolate agar is formed when blood is heated is heated before adding them to the medium. Blood is heated to lyse the RBCs and release haemin and NAD. In case of blood agar, blood is added without prior heating.

Generally, sheep blood is used for the purpose.

These media are used to encourage the growth of fastidious microbes such as *Haemophilus influenzae, Streptococcus pneumonia and Neisseria* species.

Selective media: These are used when we need to favor the growth of specific microbes and their media composition is adjusted accordingly.

Addition of bile salts or dyes such as basic fuchsin and crystal violet favour the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria.

A medium consisting only of cellulose as carbon and energy source is used when purpose is isolation of cellulose-digesting bacteria.

Note:

Endo agar, Eosin methylene blue agar and MacConkey agar are three media that are commonly used for the detection of *E. coli* and related bacteria in the water.

The possibilities of selection are endless and therefore there can be a large number of selective media that can be designed and prepared.

Differential media: These are media which can distinguish among different groups of microbes and sometimes are also helpful in tentative identification of microbes.

Blood agar is both a differential and enriched medium. It is used to distinguish between hemolytic and non-hemolytic bacteria as the hemolytic bacteria produce a clear zone around their colonies due to destruction of red blood cells.

MacConkey agar is differential and selective media because it contains lactose and neutral red dye and due to this it can distinguish between lactose fermenters and lactose nonfermenters.

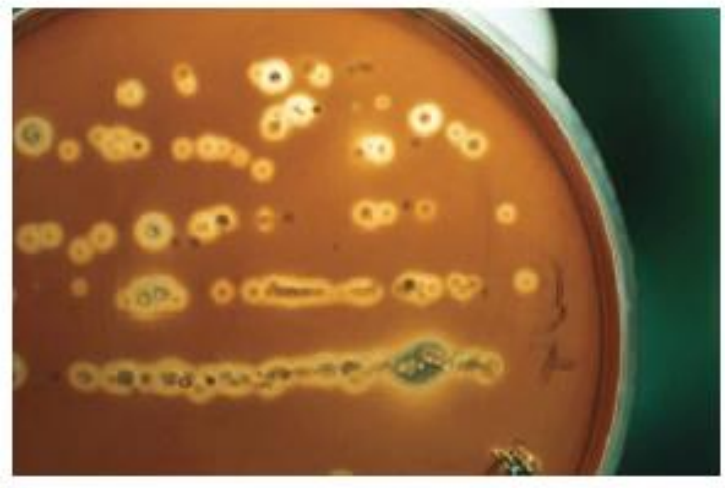
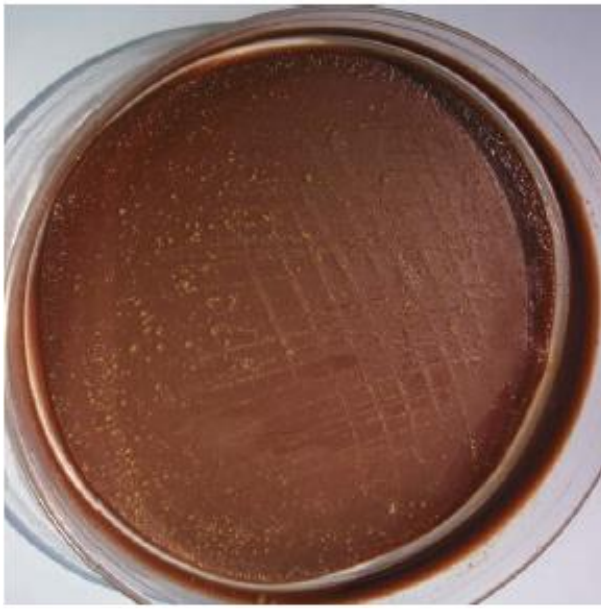


Figure: Blood agar culture of bacteria from human throat.

The shining white zone around colonies is a clear zone due to hemolysis by respective colonies.

Figure: Chocolate agar showing the colonies of *Neisseria gonorrhoeae*, a fastidious microbe.



Medium	Functional Type	Mechanism of Action
Blood agar	Enriched and differential	Blood agar supports the growth of many fastidious bacteria. These can be differentiated based on their ability to produce hemolysins—proteins that lyse red blood cells. Hemolysis appears as a clear zone around the colony (β-hemolysis) or as a greenish halo around the colony (α-hemolysis) (e.g., *Streptococcus pyogenes*, a β-hemolytic streptococcus).
Eosin methylene blue (EMB) agar	Selective and differential	Two dyes, eosin Y and methylene blue, inhibit the growth of gram-positive bacteria. They also react with acidic products released by certain gram-negative bacteria when they use lactose or sucrose as carbon and energy sources. Colonies of gram-negative bacteria that produce large amounts of acidic products have a green, metallic sheen (e.g., fecal bacteria such as *E. coli*).
MacConkey (MAC) agar	Selective and differential	The selective components in MAC are bile salts and crystal violet, which inhibit the growth of gram-positive bacteria. The presence of lactose and neutral red, a pH indicator, allows the differentiation of gram-negative bacteria based on the products released when they use lactose as a carbon and energy source. The colonies of those that release acidic products are red (e.g., *E. coli*).
Mannitol salt agar	Selective and differential	A concentration of 7.5% NaCl selects for the growth of staphylococci. Pathogenic staphylococci can be differentiated based on the release of acidic products when they use mannitol as a carbon and energy source. The acidic products cause a pH indicator (phenol red) to turn yellow (e.g., *Staphylococcus aureus*).

Table: Mechanisms of Action of Selective and Differential Media

Note: Another example of enriched media-

Milk agar: It is a non-selective solid medium and is enriched with milk solids. This media and its variants are commonly used in the dairy industry. This is most commonly used to assess the bacteria in the milk, milk products, ice cream and water. It is also used to assess the proteolytic activity in the cultured microbes because milk solids have casein and microbes producing casein hydrolase can digest casein and form clear zone around such colonies.

A variant called starch milk agar is used to detect the spores in heated milk and milk products. Another variant called milk salt agar is used for selective isolation and cultivation of *Staphylococci*.