Culture Media used in Microbiology

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.

- A microbial culture medium is a mixture of substances that promotes and supports the growth and differentiation of microorganisms. Culture media contain nutrients, energy sources, growth-promoting factors, minerals, metals, buffer salts, and gelling agents (for solid media).

- A culture medium is a solid or liquid preparation used to grow, transport, and store microorganisms. To be effective, the medium must contain all the nutrients the microorganism requires for growth.
- Specialized media are essential in the isolation and identification of microorganisms, the testing of antibiotic sensitivities, water and food analysis, industrial microbiology, and other activities.

- Although all microorganisms need sources of energy, carbon, nitrogen, phosphorus, sulfur, and various minerals, the precise composition of a satisfactory medium will depend on the species one is trying to cultivate because nutritional requirements vary so greatly. Knowledge of a microorganism's normal habitat often is useful in selecting an appropriate culture medium because its nutrient requirements reflect its natural surroundings .

- Frequently a medium is used to select and grow specific microorganisms or to help identify a particular species. In such cases the function of the medium also will determine its composition. Culture media can be classified on the basis of several parameters : the chemical constituents from which they are made, their physical nature, and their function (**table**). The types of media defined by these parameters are described here .

Physical Nature	Chemical Composition	Functional Type
Liquid	Defined (synthetic)	Supportive (general purpose)
Semisolid	Complex	Enriched
Solid		Selective
		Differential

Classification of bacterial culture media on the basis of consistency (State) .

1 - Solid medium
Solid medium 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.

2 - **Semisolid media**
Semisolid media 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) medium
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 .**

Chemical and Physical Types of Culture Media .

- A medium in which all chemical components are known is a **Defined** or **Synthetic** **medium .** It can be in a liquid form (broth) or solidified by an agent such as agar. Defined media are often used to culture photolithotrophic autotrophs such as cyanobacteria and photosynthetic protists.

- They can be grown on relatively simple media containing CO2 as a carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as a nitrogen source, sulfate, phosphate, and a variety of minerals .

- Many chemoorganotrophic heterotrophs also can be grown in defined media with glucose as a carbon source and an ammonium salt as a nitrogen source . Defined media are used widely in research, as it is often desirable to know what the experimental microorganism is metabolizing.

-Media that contain some ingredients of unknown chemical composition are **Complex** **media.** Such media are very useful, complex medium may be rich to completely meet the nutritional requirements of many different microorganisms.

- Complex media often are needed because the nutritional requirements of microorganism are unknown, and thus a defined medium cannot be constructed. This is the situation with many fastidious bacteria that have complex nutritional or cultural requirements; they may even require a medium containing blood or serum.

- Complex media contain undefined components like **peptones, meat extract, and yeast extract**. **Peptones** are protein hydrolysates prepared by partial proteolysis digestion of meat, casein, soya meal, gelatin, and other protein sources. They serve as sources of carbon, energy, and nitrogen .

- Beef extract contains amino acids, peptides, nucleotides, organic acids, vitamins, and minerals. **Yeast extract** is an excellent source of B vitamins as well as nitrogen and carbon compounds.

- **Agar** is a sulfated polymer composed usually extracted from red algae. Agar is well suited as a solidifying agent for several reasons. One is that it melts at about 90°C but once melted does not harden until it reaches about 45°C. Agar is an excellent hardening agent because most microorganisms cannot degrade it.

Functional Types of Media .

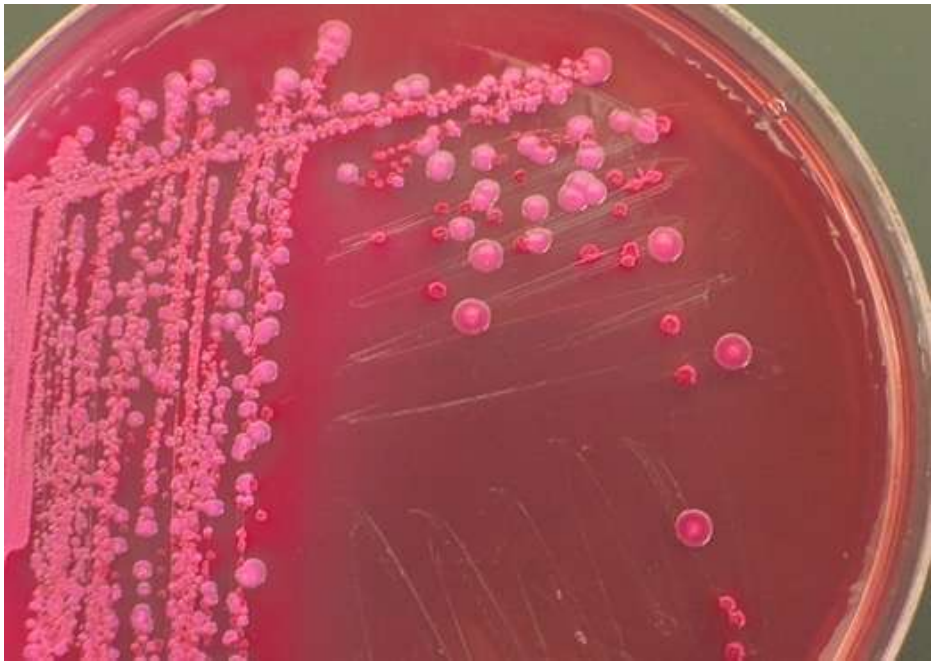
- **General purpose media** or **Supportive media** is they sustain the growth of many microorganisms . Blood and other special nutrients may be added to general purpose media to encourage the growth of fastidious microbes. These specially fortified media are called **Enriched media** .

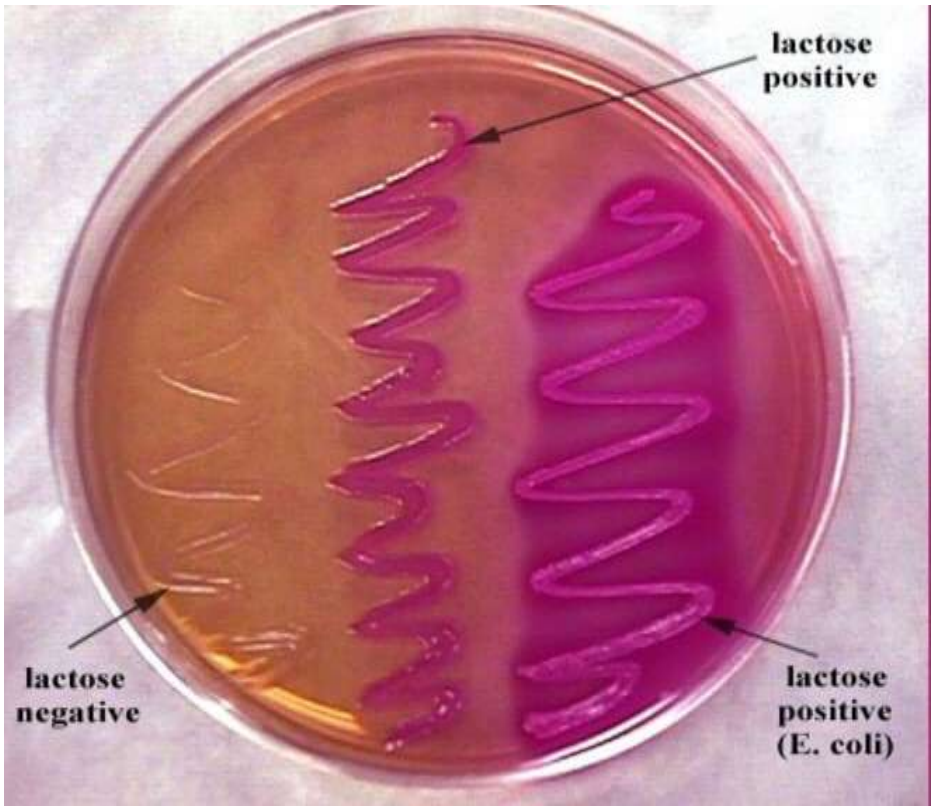
- **Selective media** favor the growth of particular microorganisms . Bile salts or dyes like basic fuchsine and crystal violet favor the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria; the dyes have no effect on gram negative organisms.

- Endo agar, Eosin methylene blue agar, and MacConkey agar are three media widely used for the detection of *E. coli* and related bacteria in water supplies. These media contain dyes that suppress gram positive bacterial growth. MacConkey agar also contains bile salts.

Examples of Selective media include :-

1. Thayer Martin Agar used to recover *Neisseria gonorrhea* contains antibiotics; vancomycin , Colistin and Nystatin .
2. **Mannitol Salt Agar** and Salt Milk Agar used to recover *S. aureus* contains 7.5% Na Cl .
3. Potassium tellurite medium used to recover *C. diphtheria* contains 0.04% potassium tellurite.
4. **MacConkey's Agar** used for **Enterobacteriaceae** members contains bile salt that inhibits most gram positive bacteria.

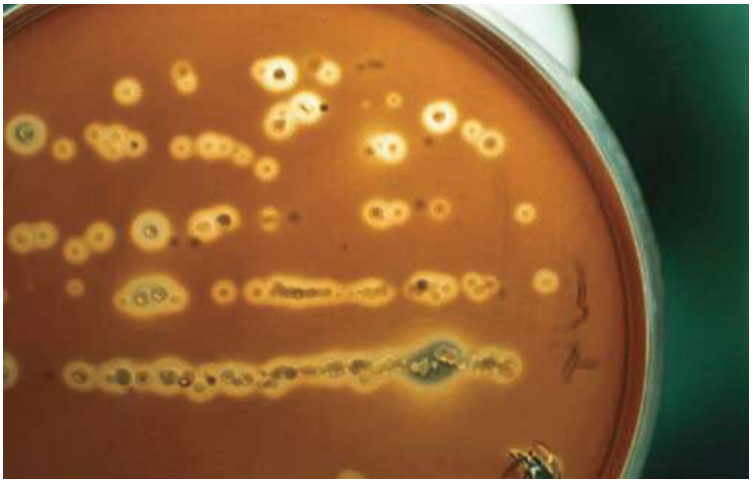




(MacConkey's Agar)

5. Pseudosel Agar (Cetrimide Agar) used to recover *P. aeruginosa* contains cetrimide (antiseptic agent).
6. Crystal Violet Blood Agar used to recover *S. pyogenes* contains 0.0002% crystal violet.

7. **Lowenstein Jensen Medium** used to recover *M. tuberculosis* is made selective by incorporating malachite green.
8. Wilson and Blair's Agar for recovering *S. typhi* is rendered selective by the addition of dye brilliant green.
9. Selective media such as **TCBS Agar** used for isolating *V. cholerae* from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.



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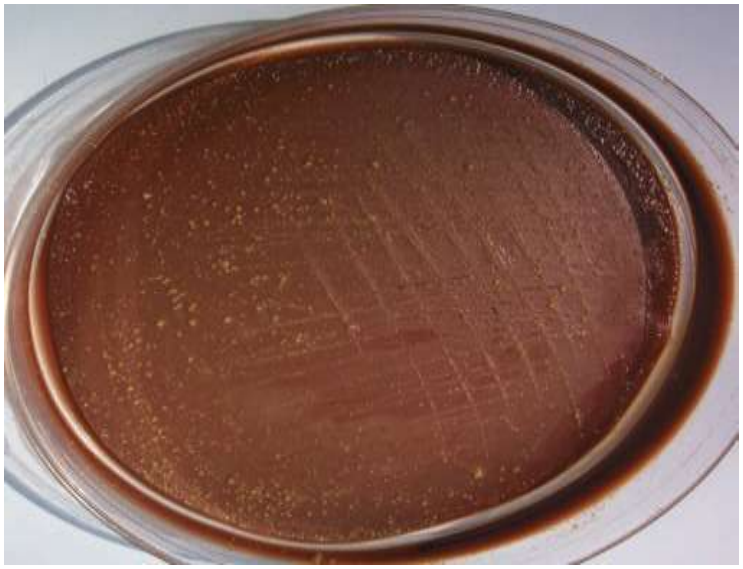
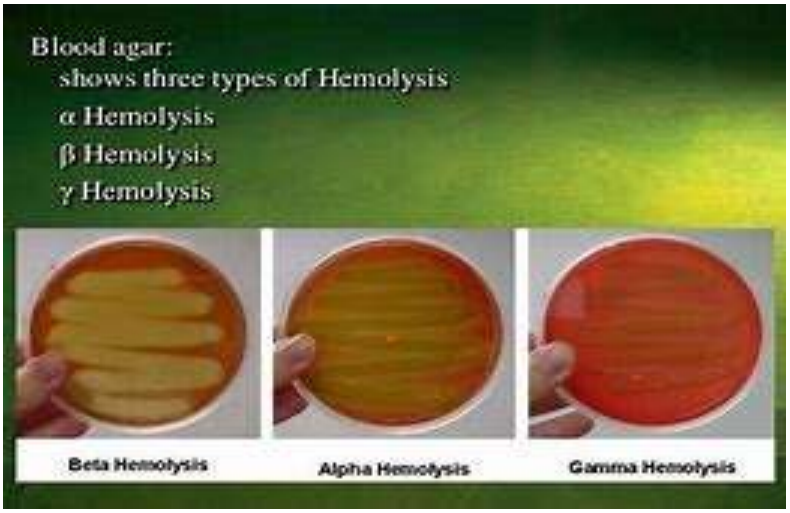


(B)

(a) Blood agar culture of bacteria from the human throat. **(b)** Chocolate agar, an enriched medium used to grow fastidious organisms such as *Neisseria gonorrhoeae*. The brown color is the result of heating red blood cells and lysing them before adding them to the medium. It is called chocolate agar because of its chocolate brown color.

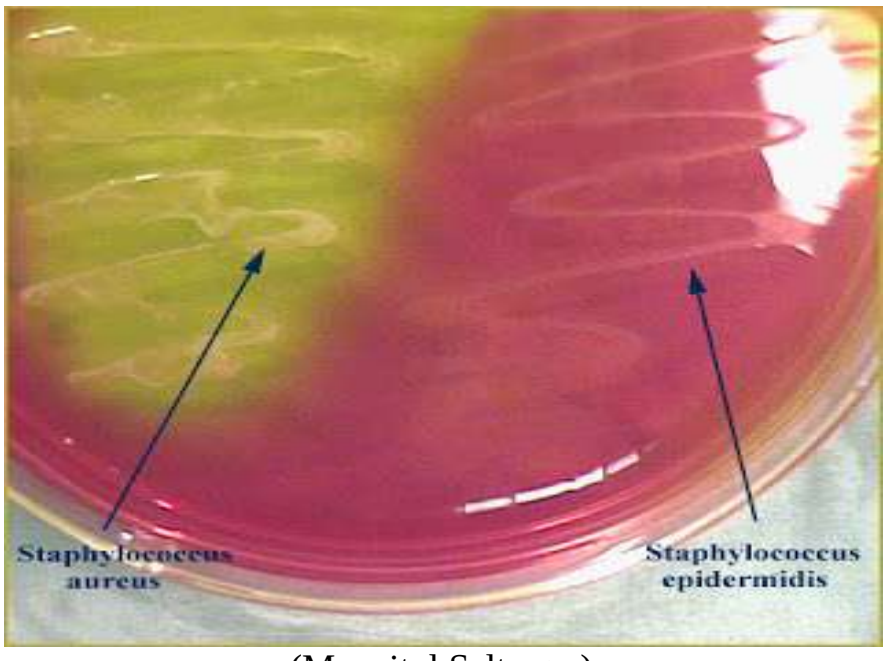


Differential media are media that distinguish among different groups of microbes based on their biological characteristics .

- Blood agar is both a differential medium and an enriched one . It distinguishes between hemolytic and non-hemolytic bacteria. Hemolytic bacteria (e.g., many streptococci and staphylococci isolated from throats) produce clear zones around their colonies because of red blood cell destruction.

- MacConkey agar is both differential and selective. Since it contains lactose and neutral red dye, lactose - fermenting colonies appear pink to red in color and are easily distinguished from colonies of non fomenters.

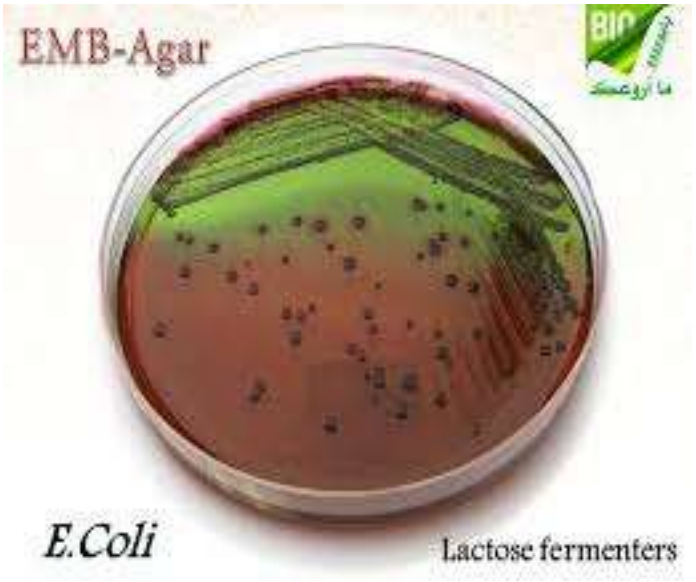
Examples of differential media include :-



(Mannitol Salt agar)

1. **Mannitol salts agar** (mannitol fermentation = yellow)
2. **Blood agar** (various kinds of hemolysis i.e. α, β and γ hemolysis)
3. **Mac Conkey agar** (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies.
4. **TCBS** (*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose) .

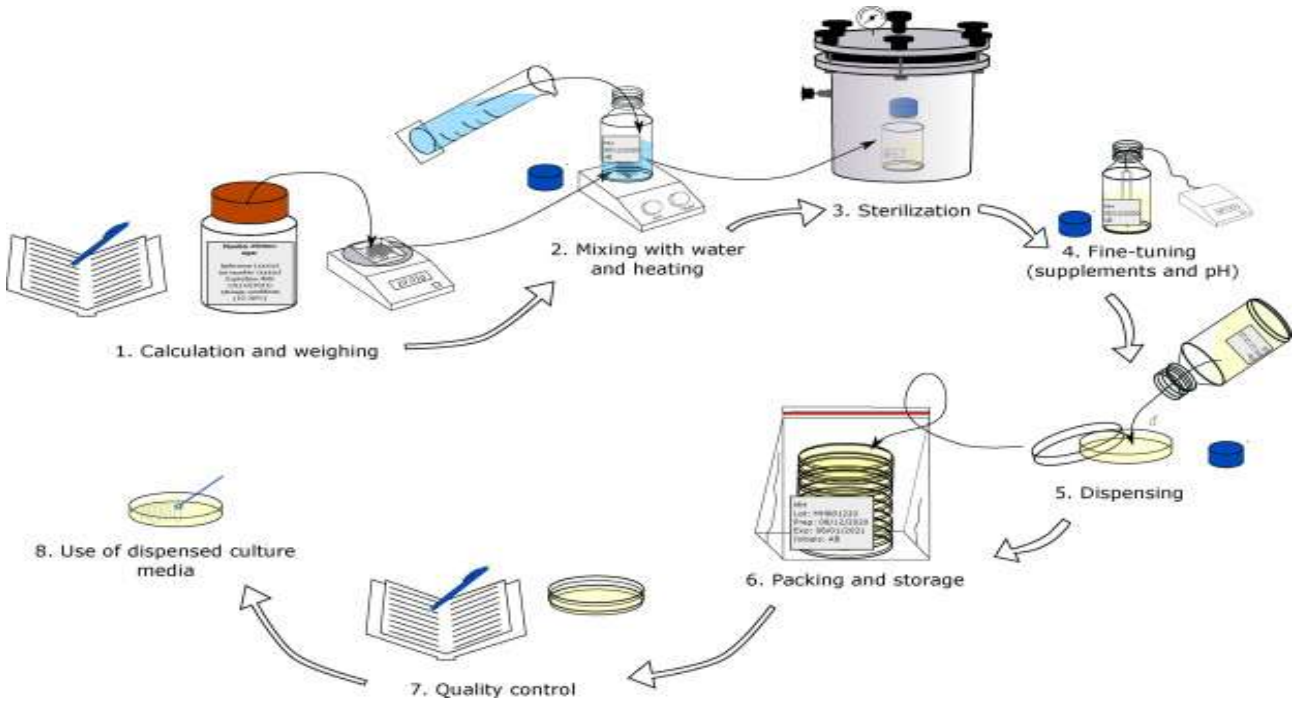
Medium	**Functional Type**	**Mechanism of Action**
Blood agar	Enriched and differential	Blood agar supports the growth of many fastidious bacteria . These can be differentiated based on their ability to produce hemolysins proteins that lyse red blood cells. Hemolysis appears as a clear zone around the colony (B-hemolysis) or as a greenish halo around the colony (a-hemolysis).
Eosin methylene blue (EMB) agar	Selective and differential	Two dyes, eosin Y and methylene blue, inhibit the growth of G+ . 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 G- that produce large amounts of acidic products have a green, metallic sheen (e.g. *E. coli*).
MacConkey (MAC) agar	Selective and differential	The selective components in MAC are bile salts and crystal violet, which inhibit the growth of G+ bacteria. The presence of lactose and neutral red, a pH indicator, allows the differentiation of G- 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*).



(Eosin Methylene Blue agar)

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 ratio and inhibit overgrowth of unwanted bacteria.



Macroscopic appearance of bacterial growth .

Once a container of medium has been inoculated, it is incubated in a temperature controlled chamber (**Incubator**) to encourage microbial growth . Although microbes have adapted to growth at temperatures ranging from freezing to boiling , the usual temperatures used in laboratory propagation fall between **20°C and 40°C**. Incubators can also control the content of atmospheric gases such as oxygen and carbon dioxide that may be required for the growth of certain microbes. During the incubation period (ranging from a few hours to several weeks) , the microbe multiplies and produces a culture with macroscopically observable growth .

1. Appearance in Petri dish .

A **Colony** is a large number of bacterial cells on solid medium, which is visible to the naked eye as a separate unit and Colony is derived from one bacterial cell . Different species of bacteria can produce very different colonies. So in the identification of bacteria and fungi much Focus is placed on how the organism grows in or on media. Colony have many feature as following :-

a) Form – The form refers to the shape of the colony.
b) Size - The diameter of a representative colony may be measured. Tiny colonies are referred to as (pin-point) Punctiform .
c) Surface - smooth, shiny, rough, dull, wrinkled.
d) Texture – Several terms that may be appropriate for describing the texture or consistency of bacterial growth are : dry, moist, Mucoid , hard, viscous .
e) Color – It is important to describe the color or pigment of the colony. Such as : opaque, dull, translucent, iridescent (rainbow-like) .
f) Elevation – This describes the side view‖ of a colony. These are the most common.
g) Margin – The margin or edge of a colony (or any growth) may be an important characteristic in identifying an organism .

2. Appearance in test tube .

- **Broth:** growth occurs throughout the container and can then present a dispersed, cloudy, or flaky appearance .
- **Semisolid** media the medium is stabbed carefully in the center with an inoculating needle and later observed for the pattern of growth around the stab line.