

CULTURAL CHARACTERISTICS OF MICROORGANISMS

Aim

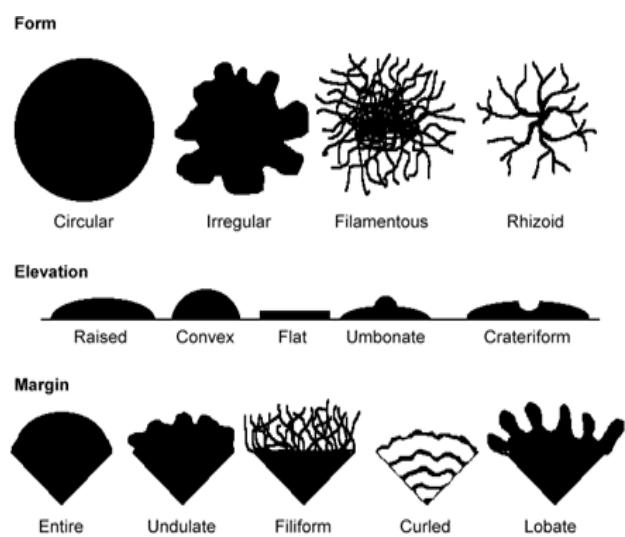
To determine the cultural characteristics of microorganisms in identifying and classifying organisms into taxonomic groups

Principle

When grown on a variety of media, microorganisms will exhibit the difference in the microscopic appearance of their broth. These differences are called cultural characteristics and are used as a basis for separating microorganism into taxonomic groups. The cultural characteristics for all taxon microorganisms are contained in Bergey's Manual of systematic bacteriology.

The following characters of colony are noted:

1. Size: in millimeter
2. Shape: circular / irregular
3. Surface : smooth, rough, granular
4. Elevation : flat, low convex, high convex, raised, umbonate, umbulate
5. Edge: entire, undulate, lobate, crenated, fimbriate, ciliate
6. Opacity : opaque, translucent, transparent
7. Colour of colony
8. Consistency : mucoid, friable
9. Other properties : hemolysis, pigmentation, swarming

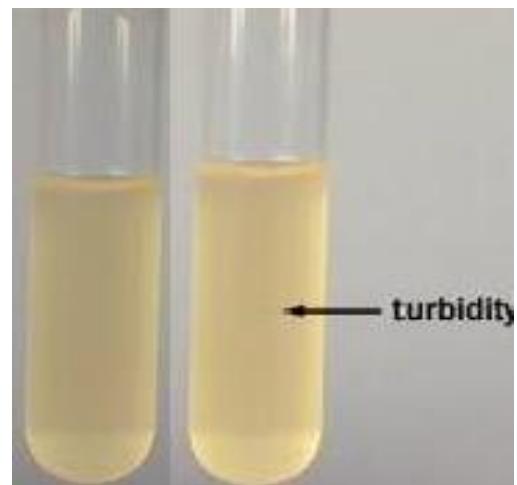


Morphology on nutrient agar slants

Fig. 5. Growth on Solid Media

The isolated bacteria can be identified based on their colony characteristics in the following manner.

1. Degree of growth : scanty, moderate, abundant
2. Surface : smooth, rough, granular
3. Elevation : convex, flat, raised
4. Edge : entire, undulate, crenate
5. Opacity : opaque, translucent, transparent
6. Consistency : firm, butyrosis, powdery, mucoid, membranous
7. Colour of Colony : creamy white, lemon yellow, bluish green
8. Form : filiform, echinulated, beaded, effuse, rhizoid
9. Changes in Medium : changes in colour, pitting of agar



Morphology on Nutrient Agar Plates

These demonstrate well isolated colonies and are evaluated in the following manner.

Fig. 6. Growth on Liquid Media

1. *Size*: pinpoint, moderate, small or large
2. *Colour of the colony*
 - a) Form: the shape of the colony:
 - b) circular: unbroken, peripheral edge
 - c) irregular: intended, peripheral edge
 - d) rhizoid: root like, spreading growth
3. *Margin*: The appearance of the outer edge of the colony is described as follows
 - a) entire: sharp
 - b) lobate: marked indentations
 - c) undulate: wavy indentations
 - d) serrate: tooth like appearance
 - e) filamentous: thread like, spreading edge
4. *Elevation*: the degree to which the colony growth is raised on the surface is described as follows:
 - a) flat: elevation not discernible
 - b) raised: slightly elevated
 - c) convex: dome shaped elevation
 - d) umbonate : raised with elevated convex central region

Growth in Liquid Media

The liquid medium (nutrient broth, peptone water and other liquid media) the following characteristics are noted:

- 1) the degree of growth : scanty, moderate, abundant
- 2) presence of turbidity and its nature (uniform turbidity)
- 3) presence of deposits, pellicle formation on surface & its quality

Morphology on Nutrient Broth Cultures

These are evaluated for the distribution & appearance of the growth as follows:

- 1) uniform time turbidity : finely dispersed throughout
- 2) flocculent: flank aggregates, dispersed throughout
- 3) pellicle: thick, pad like growth on the surface
- 4) sediment: concentration of growth at the bottom of broth cultures may be granules

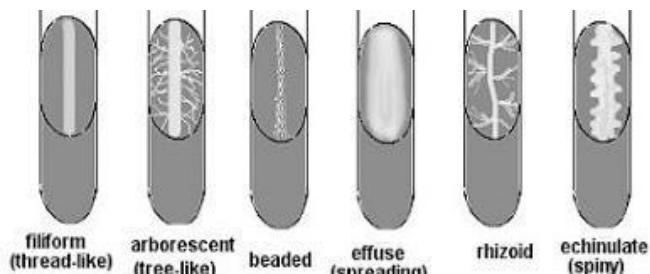


Fig. 7. Growth on Nutrient Agar Slant



CULTURE MEDIUM

The food materials on which the organism is grown is known as culture medium and the growth of organism is known as culture. Different microorganisms require different nutrient materials. Thus, culture media vary in form and composition, depending upon the species to be cultivated. It must contain all the ingredients required by the organism and in certain proportions. Basically there should be an energy source, various macro and micronutrients, vitamins etc. it must have a suitable pH. Moreover, it must be sterile so that the organism cultivated may form a pure culture.

A culture is an *in vitro* technique of growing or cultivating microorganisms or only other cells in a suitable nutrients medium called culture medium in the laboratory. The primary aim of constructing a culture medium for any microorganism is to provide a balanced mixture of required nutrients, at concentrations that will permit good growth. Culture media give artificial environment simulating natural conditions necessary for growth.

Characteristics of an Ideal Culture Medium

- Satisfactory growth for small inoculum – even for single cell.
- Rapid growth
- Easy to prepare
- Cheap
- Easily producible
- Demonstrate all the characteristics in which we are interested

Basic Requirements of Culture Medium

- Energy source
- Carbon source
- Nitrogen source
- Salts
- pH
- Adequate oxidation
- Growth factors

Common Ingredients of Culture Media

1. Water

It is essential for existence of living cells. They act as source of hydrogen and oxygen.

2. Peptone

Golden granular hygroscopic powder which are obtained from meat, casein fibrin or soya bean flour.

Function: nitrogen source, carbon source, buffers

3. Meat Extract

It contains protein degradation products, carbohydrates, inorganic salts, enzymes, excites and growth factors that are rich in vitamin B complex.

Function: Source of growth factors, inorganic salts etc.

4. Yeast Extract

It contains proteins, amino acids, growth factors (Vitamin B), Carbohydrates and inorganic salts like potassium and phosphates.

Functions: Source of growth factors and hence excellent stimulators of growth. It can be used as suitable for meat extract.

5) Electrolyte

Mainly used are sodium chloride or other electrolytes.

Functions: Essential to maintain the osmotic pressure

6) Agar

Dried mucilaginous substance obtained from gelidium species and other algae available as long shield or in powder form; contains mainly long chain polysaccharides, protein like material and inorganic salts.

Functions: it melts at 98°C and solidifies at 42°C, hence used as solidifying agent.

7) Fermentable Compounds

Mainly used are sugars, alcohols etc.

Function: Act as source of energy, fermentation reactions are helpful in the identification and classification of organisms.

8) Buffers

Carbonates and phosphates are used as buffer.

Function: To resist change in pH of the medium.

Types of Media

- Liquid Media
- Semi Solid Media
- Solid Media

a) *Liquid Media or Broth*

No solidifying agents (eg: agar) is added while preparing the medium. The most commonly used non-synthetic liquid media are nutrient broth, peptone solution, milk, blood, serum etc. Broth is a clear transparent straw coloured fluid prepared from meat extract or peptone. Beef infusions are rich in minerals, organic micronutrients, protein, protein derivatives and carbohydrates. They are often supplemented with 1% peptone or yeast extract culture fluids made from beef infusion are commonly called infusion broth, where as those made from beef extract are called extract broth.

Advantages of Liquid Media

- For obtaining bacterial growth from blood or water when large volumes have to be tested.
- For preparing bulk cultures for preparation of antigens or vaccines.
- It's used to study growth rate and the sedimentation rate of bacterial cells.

Disadvantages of Liquid Media

- It's difficult to isolate different types of bacteria from mixed population.
- It's difficult to study colony characteristics.

b) *Semi-Solid Media*

The semi-solid medium remains in the semi-solid condition. It is prepared by adding small amount of agar (0.5%) or gelatin. Agar is a complex carbohydrates prepared from algae like gelidium and gracillaria. Agar forms a colloidal solution in hot water and sets in the form of a jelly when cool. Gelatin is an animal extract prepared by hydrolysis of collagen with boiling water. When dissolved in water, it's in the form of a liquid when warm and sets in the form as it cools. Many bacteria, when grown on a gelatin medium, produce a digestive enzyme gelatinase, which liquefies gelatin. This feature is important in the identification and classification of bacteria.

The semi-solid medium may be selective which promotes the growth of one organism and retards the growth of another organism. This type of medium can be used to study bacterial motility (semisolid media are useful for cultivation of microaerophilic bacteria).

c) *Solid Media*

The solid medium is solid in consistency. It is prepared by adding 2% or 1% gelatin; agar or silica gel is sometimes an inorganic solidifying agent for autotrophic bacteria. It's used for colony characterization, colony identification, etc.

Based on composition, culture media can be classified into:

- Simple Media
- Complex Media
- Synthetic or defined Media
- Semi Solid Media
- Special Media

a) *Simple Media*

It's also called basal media. eg: Nutrient Broth. It consists of meat extract, peptone, Sodium Chloride and water. Nutrient agar made by adding 2% agar to nutrient broth is the simplest and commonest medium in routine diagnostic laboratories.

b) *Complex Media*

These have added ingredients for special purpose or for bringing out certain characteristics or providing special nutrient required for the growth of certain organisms.

c) *Synthetic or defined Media*

These media are prepared from pure chemical substances and the exact composition of the medium is known. They are used for research purpose.

d) *Semi Solid Media*

The nutritional requirements of some microorganisms include some additional ingredients of unknown chemical composition such as peptone, meat extract, yeast extract, etc. Chemical composition is not fully known. They are called semi solid media.

e) *Special Media*

No single medium or set of conditions can support the growth of all the different types of organisms that occur in nature. To cultivate, recognize, enumerate and isolate certain types of microorganisms many special purpose media are needed on the basis of their application or function, these special media can be classified into different types.

- Enriched Media
- Enrichment Media
- Selective Media
- Indicator Media
- Differential Media
- Selective Media
- Sugar Media
- Transport Media

i. *Enriched Media*

In these media, substances like blood, serum or egg are added to a basal medium
eg. Blood Agar, Chocolate Agar, Egg Media etc

ii. *Enrichment Media*

Some substances are added to liquid media with the result that wanted organism grows more in number than unwanted organism. Such media are used in mixture cultures
eg. Tetrathionate broth (inhibit coliforms and allow typhoid paratyphoid bacilli to grow freely)

iii. *Selective Media*

It favors the growth of particular microorganism. This is like enrichment media with the difference that inhibiting substance is added to solid medium.

eg. Desoxycholate citrate medium for dysentery bacilli

iv. *Indicator Media*

These media contain an indicator which changes colour when a bacterium grows in them.

eg. Incorporation of sulphite in Wilson and Blair medium *Salmonella typhi* reduces sulphite to sulphide in Wilson and Blair medium and the colonies of *S. typhi* have a black metallic sheen.

v. *Differential Media*

Media that distinguish between different groups of bacteria and even permit to identification of microorganisms based on their biological characteristics. A medium which has substances incorporated into it, enabling it to bring out differing characteristics of bacteria and thus helping to distinguish between them. Such media are called differential media eg. Mac conkey medium consists of peptone, lactose, agar, neutral red and taurocholate. It shows lactose fermenters, are colourless or pale (this may also be termed indicator medium).

Blood agar is an enriched medium, but also differentiates between hemolytic organisms and non- hemolytic organisms. So it also acts as a differential medium.

vi. *Sugar Media*

The usual sugar media consist of 1% sugar concerned. In peptone water along with appropriate indicator, a small tube (Durham's tube) is kept inverted in sugar tube to detect gas production.

vii. *Transport Media*

Delicate organisms like gonococci which may not survive the time taken which may not survive the time taken for transporting the specimen to the laboratory or may be overgrown by non-pathogens (dysentery or cholera organisms) require a special medium called transport medium.

eg. Stuart Medium for gonococci.

viii. *Anaerobic Media*

These media are used to grow anaerobic organisms.

eg. Robertson's Cooked Meat Media

MEDIA PREPARATION

1. Peptone Broth

Peptone	: 10g
NaCl	: 5g
Distilled water	: 1000ml

2. Nutrient Agar

Peptone	: 5g
NaCl	: 5g
Beef extracts	: 3g
Agar	: 20g
Distilled water	: 1000ml

The ingredients are dissolved in warm water and pH adjusted to 7.2-7.6. Autoclaved at 121°C, 15 lbs for 15 minutes.

3. Nutrient Broth

Peptone	: 5g
NaCl	: 5g
Beef extracts	: 3g
Distilled water	: 1000ml

The ingredients are dissolved in warm water and pH adjusted to 7.2-7.6. Autoclaved at 121°C, 15 lbs for 15 minutes.

4. Mac Conkey Agar

Peptone	: 20g
NaCl	: 5g
Bile salt	: 1.5g
Lactose	: 10g
Neutral red solution	: 10ml
Crystal violet	: 0.001g
Agar	: 13.5g
Distilled water	: 1000ml

5. Sabouraud's Dextrose Agar (SDA)

Peptone	: 10g
Dextrose	: 40g
Chloramphenicol	: 0.05g
Agar	: 15g
Distilled water	: 1000ml

6. Sabouraud's Dextrose Broth

Peptone	: 10g
Dextrose	: 40g
Chloramphenicol	: 0.05g
Distilled water	: 1000ml

7. Mueller – Hinton Agar

Beef infusion form	: 300g
Acid hydrolysate of casein	: 17.5g
Agar	: 17g
Starch	: 1.5g

8. Lactose Broth

Peptone	: 5g
Beef extract	: 3g
Lactose	: 5g
Distilled water	: 1000ml

9. EMB (Eosin Methylene Blue) Agar

Peptone	: 10g
Lactose	: 5g
Sucrose	: 5g
Dipotassium hydrogen phosphate	: 2g
Eosin Y	: 0.40g
Methylene blue	: 0.065g
Agar	: 13.50g
Distilled water	: 1000ml

10. Methylene Blue Solution (1:25,000)

Methylene blue dye	: 1mg
Distilled water	: 25ml

Dissolved the methylene blue in distilled water and was dispensed into regular staining bottles.

11. Carbohydrate Fermentation

Peptone	: 1g
Carbohydrates	: 10g
NaCl	: 5g
Phenol red indicator	: 10ml (0.1g in 10ml ethanol)
Distilled water	: 1000ml

Mix all the ingredients, except phenol red indicator. Adjust pH to 7. Then add phenol red indicator. Dispense the medium in 8ml test tubes containing the Durham's tubes. Sterilize the medium at 10lbs for 20 minutes.

12. Oxidation – Fermentation

Peptone	: 20g
Dipotassium hydrogen phosphate	: 2g
NaCl	: 5g
Bromothymol blue	: 3ml (1% aqueous solution)
Agar	: 13.50g
Distilled water	: 1000ml

Mix all the ingredients, except Bromothymol blue indicator. Adjust pH to 7.1. Then add Bromothymol blue indicator. The medium is poured into the tube to a depth of about 4cm. sterilized by autoclaving at 121°C for 20 minutes at 10 lbs. it was then allowed to set.

13. Voges – Proskauer

Reagents: Barrett's A

α – naphthol	: 5g
Ethanol	: 100ml

Dissolve α – naphthol in small amount of alcohol and then add remaining alcohol to 100ml. Store in brown bottle at 4°C.

Barrett's B

Potassium hydroxide	: 40g
Distilled water	: 100ml

Cool the volumetric flask in cold water with 80ml water, add KOH crystals, dissolve and make up to 100ml. Store in polyethene bottles at 4°C.

14. Citrate Utilization

MgSo ₄	: 0.2g
Ammonium dihydrogen phosphate	: 1g
Dipotassium phosphate	: 1g
Sodium citrate	: 2g
Sodium chloride	: 0.5g
Bromothymol blue	: 0.08g
Agar	: 15g
Distilled water	: 1000ml

Dissolve the ingredients in 1000ml distilled water. Dispense in tubes and sterilize by autoclaving at 121°C for 20 minutes at 10 lbs.

15. Nitrate Broth

Beef extract	: 3g
Peptone	: 5g
Potassium Nitrate	: 1g
Distilled water	: 1000ml

Dissolve all the ingredients and sterilize by autoclaving at 121°C for 20 minutes at 15 lbs.

Reagents: Sulphanic acid

Dissolve 8g of sulphanic acid in 1 l of acetic acid.

α- Naphthol amines

Dissolve 5g of α-Naphthol amines in 1 l of acetic acid. Immediately before use, mix equal volumes of Sulphanic acid and α- Naphthol amines to give the test reagent.

16. Urease Test

Peptone	: 1g
Phenol red	: 0.012g
Dextrose	: 1g
NaCl	: 5g
Disodium phosphate	: 1.2g
Mono potassium phosphate	: 0.8g
Agar	: 15g
Distilled water	: 1000ml

Dissolve ingredients in 950ml distilled water. Sterilize by autoclaving at 10lbs for 20 minutes. Cool to 58°C and aseptically add 50ml of 40% urea. Sterilize the urea solution by autoclaving at 10lbs for 15 minutes, mix well and add the Phenol red indicator. Dispense into sterilized test tubes and allow to set in a slanting position.

17. Mannitol Motility Test

Peptone	: 20g
NaCl	: 5g
Potassium Nitrate	: 2g
Mannitol	: 64g
Agar	: 6g
Distilled water	: 1000ml
Phenol red	: 4ml (1g in 100ml ethanol)

Mix all the ingredients, except phenol red indicator. Adjust pH to 7. Then add phenol red indicator.

Dispense in tubes. Sterilize the medium at 10lbs for 20 minutes.

18. Triple Sugar Iron Agar Test

Peptone	: 20g
Yeast extract	: 3g

Beef extract	: 3g
Lactose	: 10g
Sucrose	: 10g
Glucose	: 10g
Sodium chloride	: 5g
Ferrous sulphates	: 0.2g
Sodium thiosulphate	: 0.3g
Agar	: 12g
Distilled water	: 1000ml
Phenol red	: 0.024g

Mix all the ingredients, expect phenol red indicator. Adjust pH to 7. Then add phenol red indicator. Sterilize by autoclaving at 121°C for 20 minutes. Allow the medium to set in slopped form with a butt about 1 inch long. The medium is used in the form a butt and slant.

