General Microbiology

Practical 7 : CULTIVATION

Cultivation

- Multiplication of bacteria and fungi in laboratory conditions - artificial media
- Usually lost some properties of metabolic activities, virulence, change genetic information

Biological material - streaked on the plate or innoculated to the cultivation medium (solid or liquid) - for multiplication - thick, dense growth

- - for isolation - isolated colonies on solid plate media

Practical 7 - Physiology of bacteria

- Knowledges of physiology - enable to prepare required conditions for laboratory detection:
- oxygen - O2, CO2, without O2, defined athmosphere - (Campylobacter, Helicobacter)
- temperature - usually 37*C, 42*C - Campylobacter, 22*C - Pasteurella, different look according to the temperature - motility, fungi - molds/yeast
- blood - annimal blood - sheep, horse - blood agar, denaturated blood - chocolatised agar
- nutrition factors - vitamins, aminoacids, detoxification materials……...

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment. The biochemical (nutritional) environment is made available as a **culture medium**, and depending upon the special needs of particular bacteria (as well as particular investigators) a large variety and types of culture media have been developed with different purposes and uses. Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties.

pH

Microorganisms which grow at an optimum pH well below neutrality (7.0) are called **acidophiles**. Those which grow best at neutral pH are called **neutrophiles** and those that grow best under alkaline conditions are called **alkaliphiles**.

TEMPERATURE

organisms with an optimum temperature near 37 degrees (the body temperature of warm-blooded animals) are called **mesophiles**. Organisms with an optimum T between about 45 degrees and 70 degrees are **thermophiles**. Some Archaea with an optimum T of 80 degrees or higher and a maximum T as high as 115 degrees, are now referred to as **extreme thermophiles** or **hyperthermophiles**. The cold-loving organisms are **psychrophiles** defined by their ability to grow at 0 degrees. A variant of a psychrophile (which usually has an optimum T of 10-15 degrees) is a **psychrotroph**, which grows at 0 degrees but displays an optimum T in the mesophile range, nearer room temperature.

Cultivation media

- Basical (multiplication, transport)
  - solid: blood agar, Muller Hinton agar,
  - liquid: bouillon, Muller Hinton bouillon,
- Diagnostic - visualise certain kind of bacteria - the color of colonies of different kind of bacteria is different
- Selective - contain substances that enable growth of one kind of bacteria and inhibits other bacteria * ATB (broad spectrum) - eliminate susceptible bacteria , concentration of NaCl,…..
- Special - defined - detection of some properties
- Combination

- Diagnostic - visualise certain kind of bacteria - the color of colonies of different kind of bacteria is different - (biochemical properties) - DC agar, Endo, Hajn agar, urea agar, esculin agar, SC agar
- Selective - contain substances that enable growth of one kind of bacteria and inhibits other bacteria ATB (broad spectrum) - eliminate susceptible bacteria , concentration of NaCl,…..
  - manit salt (combination)
  - chocolatised agar
  - McConckey
  - Karmali
  - for M. tbc - Lowenstein, Ogawa,

Types of Culture Media

Liquid media are used for growth of pure batch cultures, while solidified media are used widely for the isolation of pure cultures, for estimating viable bacterial populations, and a variety of other purposes.

The usual gelling agent for solid or **semisolid medium** is **agar**, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100°C and remains liquid until cooled to 40°C, the temperature at which it gels) and because it cannot be metabolized by most bacteria.

Culture media may be classified into several categories depending on their composition or use. A **chemically-defined (synthetic) medium** is one in which the exact chemical composition is known.

A **complex (undefined) medium** is one in which the exact chemical constitution of the medium is not known.

Defined media are usually composed of pure biochemicals off the shelf; complex media usually contain complex materials of biological origin such as blood or milk or yeast extract or beef extract, the exact chemical composition of which is obviously undetermined. Chemically-defined media are of value in studying the minimal nutritional requirements of microorganisms, for enrichment cultures, and for a wide variety of physiological studies. Complex media usually provide the full range of growth factors that may be required by an organism so they may be more handily used to cultivate unknown bacteria or bacteria whose nutritional requirement are complex (i.e., organisms that require a lot of growth factors, known or unknown). Complex media are usually used for cultivation of bacterial pathogens and other fastidious bacteria.

A **selective medium** is one which has a component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/or promote the growth of desired species. One can also adjust the physical conditions of a culture medium, such as pH and temperature, to render it selective for organisms that are able to grow under these certain conditions.

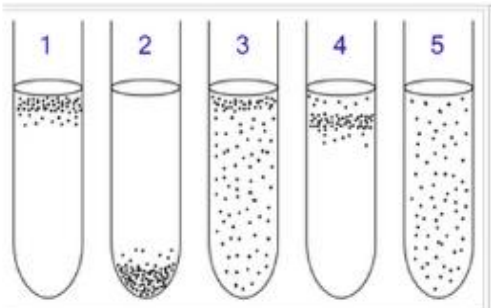
A culture medium may also be a **differential medium** if allows the investigator to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the medium. Thus a **selective, differential medium** for the isolation of *Staphylococcus aureus*, the most common bacterial pathogen of humans, contains a very high concentration of salt (which the staph will tolerate) that inhibits most other bacteria, mannitol as a source of

fermentable sugar, and a pH indicator dye. From clinical specimens, only staph will grow. *S. aureus* is differentiated from *S. epidermidis* (a nonpathogenic component of the normal flora) on the basis of its ability to ferment mannitol. Mannitol-fermenting colonies *(S. aureus) produce* acid which reacts with the indicator dye forming a colored halo around the colonies; mannitol non-fermenters (*S. epidermidis*) use other non-fermentative substrates in the medium for growth and do not form a halo around their colonies.

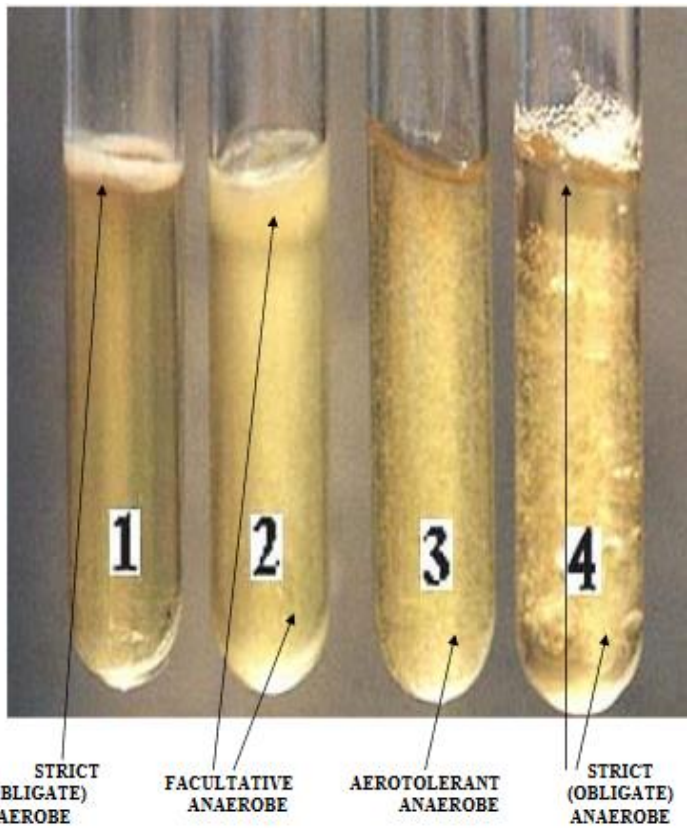
An **enrichment medium** contains some component that permits the growth of specific types or species of bacteria, usually because they alone can utilize the component from their environment.

Identification of colonies

- **Description of growth on liquid medium:**
- turbidity, density
- sediment
- surface membrane



Aerobic and anaerobic bacteria can be identified by growing them in a liquid culture:
1: Obligate aerobic bacteria gather at the top of the test tube in order to absorb maximal amount of oxygen.
2: Obligate anaerobic bacteria gather at the bottom to avoid oxygen.
3: Facultative bacteria gather mostly at the top, since aerobic respiration is the most beneficial one; but as lack of oxygen does not hurt them, they can be found all along the test tube.
4: Microaerophiles gather at the upper part of the test tube but not at the top. They require oxygen but at a low concentration.
5: Aerotolerant bacteria are not affected at all by oxygen, and they are evenly spread along the test tube.



Oxygen relationship designation

The Effect of Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. However, procaryotes display a wide range of responses to molecular oxygen O_2 (Table 6).

Obligate aerobes require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration.

Obligate anaerobes (occasionally called **aerophobes**) do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth. Obligate anaerobic procaryotes may live by fermentation, anaerobic respiration, bacterial photosynthesis, or the novel process of methanogenesis.

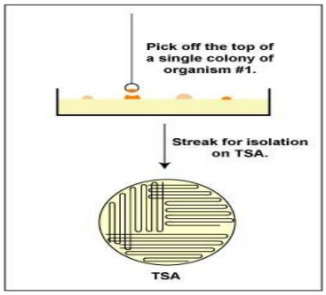
Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration.

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in their environment.

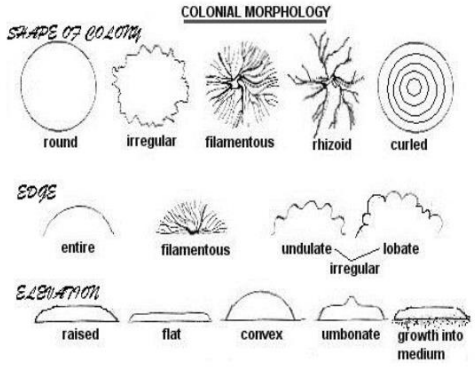
Colony

- Colony arises from a CFU - colony forming unit - a piece of biological material from which a certain quantity grow on the same place: 1 colony = thousands of bacteria (microscopy)

CULTIVATION
Colony —Pickingl
- Sterile needle or loop is touched to surface of colony and transferred to fresh, sterile media
- Incubation for another 24 hours



- **Description of growth on solid media**
- shape, look (dry, mucous), color- pigment, smell, colony edges, spread of colonies, localisation of colonies, change of environment, consistence,



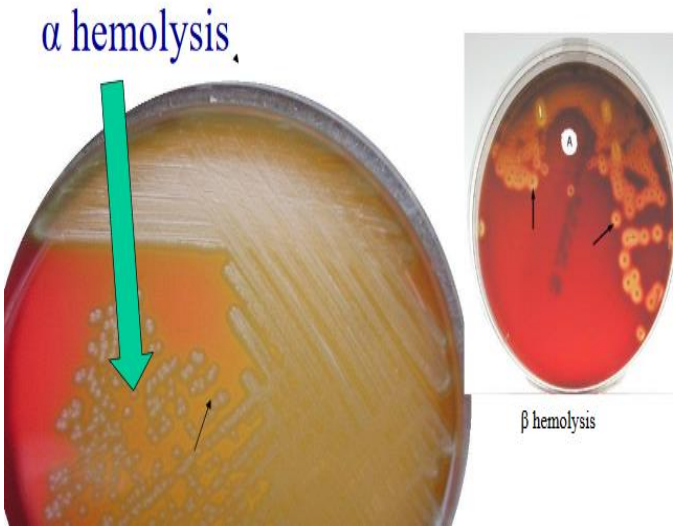
Blood agar

- Contains mammalian blood (usually sheep or horse),
- to isolate fastidious organisms
- detect hemolytic activity

Hemolytic activity

- β-hemolytic - *Streptococcus pyogenes, Str. haemolyticus, Str. agalactiae*
- α-hemolysis - *Streptococcus pneumoniae, Streptococcus viridans*
- γ-hemolysis (or *non-hemolytic)- Staphylococcus epidermidis*

alfa hemolysis - green - viridation
beta hemolysis - transparent - real hemolysis
gama hemolysis - no change



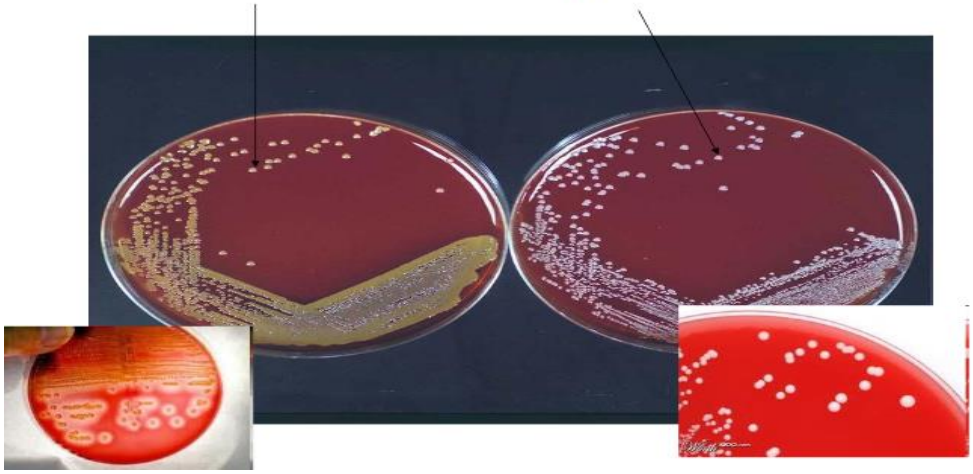
Demostration

- Hemolysis - alfa - Streptococcus pneumoniae
  - beta - Streptococcus agalactiae,
  - gama - Staphylococcus epidermidis
- Pigment . Staphylococcus aureus - yellow, Staphylococcus epidermidis - white
- Look - dry - Staphylococcus epidermidis
  - mucous - Klebsiella pneumoniae

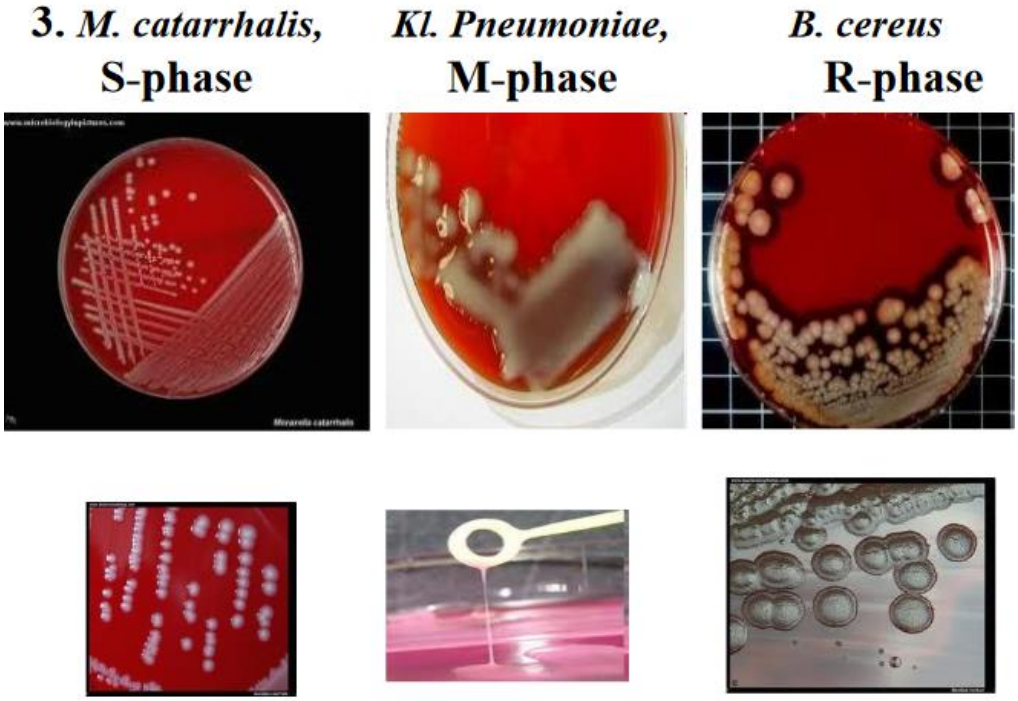
Sabouraud agar

- Sabouraud agar is used to culture fungi and has a low pH that inhibits the growth of most bacteria; it also contains the antibiotic gentamicin to specifically inhibit the growth of Gram-negative bacteria.

1. *St. aureus, St. epidermidis* – blood agar



Compare **Staphylococcus aureus** and *Staphylococcus epidermidis*



S – smooth surface of colonies

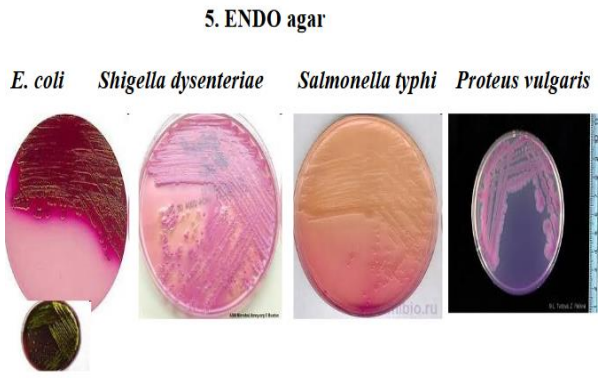
M – mucous surface of colonies

R – rough surface of colonies

EXAMPLES OF DIFFERENTIAL MEDIA:

ENDO agar

- differential
- selective
- detection of coliform and other enteric microorganisms

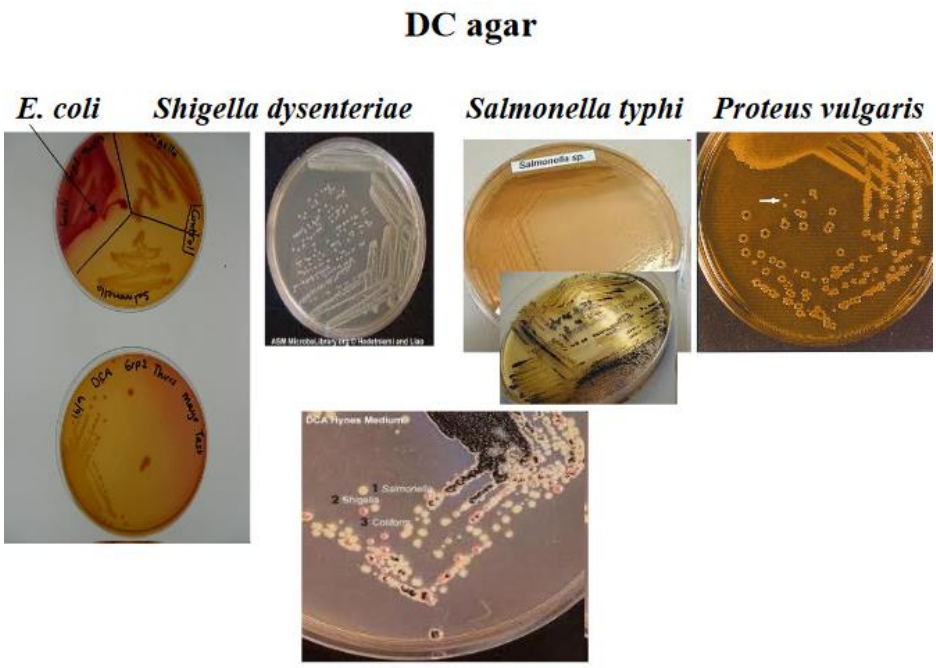


- Endo Agar was developed by Endo to **differentiate gram-negative bacteria on the basis of lactose fermentation**

- **lactose-fermenters** produce pink (red pink) colonies on fermentation of lactose (*Escherichia coli*)

- **lactose non-fermenters** produce colourless colonies on the medium (Shigella, Salmonella, Proteus)

Deoxycholate Citrate Agar (DC agar)



- This medium is used for the isolation andmaximum recovery of intestinal pathogens belonging

  To *Salmonella* and *Shigella* groups

- Lactose helps in differentiating enteric bacilli:

  lactose fermenters produce red colonies

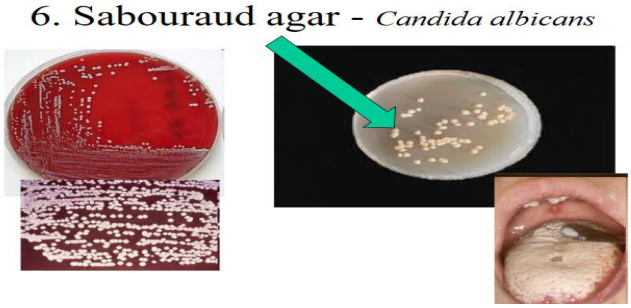
  lactose non-fermenters produce colourless colonies

- The reduction of ferric ammonium citrate (in medium) to iron sulfide is indicated by the formation of **black iron sulfide.**

- *Salmonella* and *Shigella* species do not ferment lactose but:

  Salmonella may produce H_2S, forming colorless colonies **with black centers.**

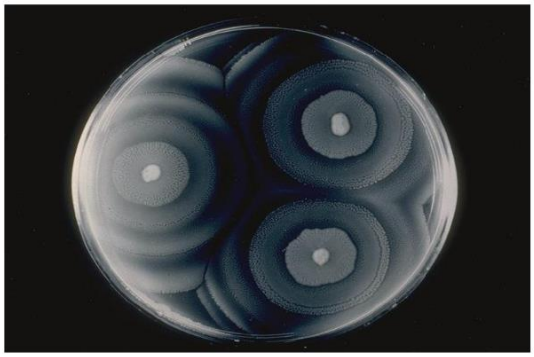
SABOURAUD AGAR



Sabouraud agar or **Sabouraud dextrose agar** (**SDA**) is a type of agar growth medium containing peptones.[1] It is used to cultivate dermatophytes and other types of fungi and yeasts – ***Candida albicans***.

Raus phenomenon:

Proteus mirabilison blood agar -for most strains of P. mirabilisand P. vulgarisis typical their ability to swarm (RAUSS phenomenon) over the surfaces of solid cultivation media



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http://textbookofbacteriology.net/growth_4.html