Growth of bacteria lect. No. 3

Glossary:

Pathogen: A microorganism capable (has the ability) of causing disease.

Non-pathogen: A microorganism that does not cause disease. May be part of the normal flora.

Pathogenicity: The ability of an infectious agent to cause disease.

Opportunistic pathogen: An agent capable of causing disease only When the host's resistance is impaired.

Infection: Multiplication of an infectious agent within the body. multiplication of normal flora is generally not considered an infection.

Adherence: (adhesion, attachment): The process by which bacteria Stick to the surface of host cell. Once bacteria have Entered the body, adherence is a major initial step in The infection process.

Invasion: The process whereby bacteria & other microorganisms Enter host cells or tissues and spread in the body.

Toxigenicity: The ability of a micro-organism to produce a toxin that Contribute to the development of disease.

Virulence: The quantitative ability of an agent to cause disease.

Virulent agents cause disease when introduced into the host in small numbers.

Carrier: A person or animal with asymptomatic infection that can be transmitted to another person or animal.

Bacterial Growth"

Most of what we know about bacteria derives from their growth.

Bacteria growth involves both an increase in the size of organisms and an increase in their number.

The net effect is an increase in the total mass (biomass) of the culture.

When placed in a suitable environment and conditions (nutrient temperature) a bacterial cell begins to grow; when it has made about twice the amounts of component materials that it started with, it divides.

Growth is a central technique in bacteriology as it is used for:-

1) Detection and identification of bacteria.

2) The assessment of antibiotic effects.

3) Produce the desirable products in biotechnology industries

Types of growth:-

In the laboratory, bacterial growth can be seen in three main forms:-

1- By the development of colonies, the macroscopic product of 20-30 cell divisions of a single cell.

2- By the transformation of a clear broth medium to a turbid suspension of 107- 109cell/ ml.

3- In biofilm formation, in which growth spread thinly (300-400μm) over the surface of the broth.

The Growth Curve: (Growth Phases in broth culture).

The Growth phase of pure culture of a single organism can be placed in 4 main phases, and those are:-

1- The lag phase:

Represents a period during which the number of cells in the broth culture appears to remain constant as cells are thought to be preparing for growth in the new environment, by forming and accumulating enzymes and intermediates to concentrations that permit growth to resume.

2- The exponential Stage:

During this phase, increase in cell number becomes detectable, its rates accelerates rapidly showing a linear increase in log cell number with time. This log- linear relationship is constant for a given bacterial strain

under certain conditions & is referred to as "**Doubling time**" for that organism; it is between 13min for ***Vibrio cholera***e and 24h for ***Mycobacterium tuberculosis.*** On this basis cholera is a disease that can kill within 12hr, whereas tuberculosis takes months to develop.

Detection of the organism by culture takes one day for ***V. cholerae*** whereas several weeks are required for ***M. tuberculosis***.

The biomass increase in an exponential manner until one of two happens:-

1) Nutrients in the medium become exhausted.

2) Toxic metabolic products accumulate and inhibit growth.7

3- Stationary phase:

Exponential growth cannot be sustained in a close system with limited nutrients. Eventually growth slows down and the total bacterial cell number reaches a maximum and stabilizes, this known as **stationary phase** in which there is a slow loss of cells through death, balanced the formation of new cells through growth and division, the count stays constant.

4- Decline phase:

After a period of time in the stationary phase, the death rate increases until it reaches a steady level. After the majority of cells have died, the death rate decreases, so that a small number of survivors may persist for months as a few cells growing at the expense of nutrients released from cells that die & lyse.

Measurement of Cell Mass:

It involved both direct and indirect methods:

1- **Direct physical measurement:**

Measurement of dry weight, wet weight and volume of cells after centrifugation, used with very dense cultures for research and industrial purposes.

2- Direct chemical measurement:

It measures some chemical components of the cell, such as total nitrogen, total protein and total DNA.

3- Indirect measurement of chemical activity:

Such as rate of O2 production or consumption, CO2 production and consumption.

4- Turbidity measurement

Determines the amount of light scattered by a suspension of cells using spectrophotometer with calibration of a standard curve.

Bacteria scatter light in proportion to their numbers. Turbidity or Optical density of the cell suspension is directly related to cell mass or number. This method is sensitive to about 10^7 cell/ml

Media for Bacterial Growth:

In order to study the properties of a given organism, it is necessary to cultivate it in pure culture on suitable growth media contain all the nutrients required by the organism, and these are:

1- A source of protein (Nitrogen source) derived from casein or infusion of brain, heart or liver.

2- Carbon source.

3- Minerals (Sulfur and phosphorus).

4- Growth factors e.g.: amino acids, vitamins.

5- Control of PH in the final product (after sterilization). There are two main types of media **Liquid & Solid.**

Liquid media are of limited use in identification of bacterial species because:-

1- Growth usually does not exhibit characteristic appearance.

2-Organism cannot be separated from mixed growth in liquid media. Solid media are useful in identification and isolation of pure culture for different bacteria.

Gelatin was used by the early bacteriologists to make the first solid media, now agar is used for gelling media, It is an acidic polysaccharide extracted from certain red algae. Agar is uniquely suitable for microbial cultivation because it is resistant to microbial action.

Culture media are of many kinds according to their ingredients such as:

1- Basal media: (Simple media) as Nutrient broth, peptone water, it is the basis of most media.

2- Enriched media: (Blood agar) with nutritional requirements.

3- Selective media: (Gentamicin blood agar) contain substances that inhibit all but a few types of bacteria.

4- Indicator media: (MacConkey agar) incorporate substance that is changing visibly as a result of the metabolic activity of organisms.

5- Transport media: Maintain the viability of a pathogen and avoid over growth of other contaminations during transit from the patient .

Staining:

Bacteria in nature are colorless, and are difficult to be recognized and studied by the light microscope, unless they are stained.

Stains react chemically with cellular material, and enhance the contrast between the cell and the background.

A stain is a dye consists of:-

1- A colored ion (chromophore), either (+ve) or (-ve) charged.

2- A counter ion to balance the final charge. Bacteria carry a net (-ve) charge at PH: 7, therefore:

a) Positive dye (cation) such as: Methylene blue, crystal violet & basic fuchsine, are useful for direct staining of cells.

b) Negative dye (anion) such as: Eosin & nigrosin will not directly stain bacterial cells, but they stain the background leaving the cells clear and bright.

There are two main types of staining:

I) Simple staining:

It means that one dye and a one step procedure are used to stain microbial cells, to reveal a microbial morphology feature like: size, shape and arrangements of cells.

The most common dyes used in simple staining are cationic (or basic) dyes, such as: Crystal violet, Methylene blue and basic fuchsine.

Staining of microbes requires a suitable smear spread in a thin film over a small area of a microscopic slide, then fixed by heating to make the cells adhere to the slide.

A good smear preparation should:

1- Be of an appropriate thickness to view individual cells.

2- Withstand repeated washing during staining.

3- The cell will retain the original morphology after fixation and staining.

II) Differential Staining:-

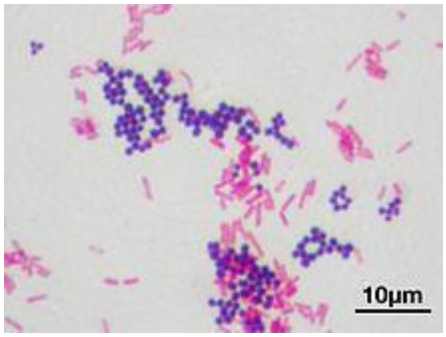
Using stains that react differently with different cell types, thus these stains are important in identification of bacteria; this staining ,mostly requires more than one dye and more than one step.

1- Gram stain:

It is the most commonly used stain; it divides bacteria into two large groups:

a) Gram- positive bacteria (blue-purple in color)

b) Gram- negative bacteria (pink in color)



The different responses and coloring of bacteria is based on fundamental differences in cell structure and composition of cell wall. Staining with Gram stain is of four steps:-

1- Application of a primary stain (crystal violet).

2- Adding of mordent (Gram iodine) for better complex formation between a dye and its target compound.

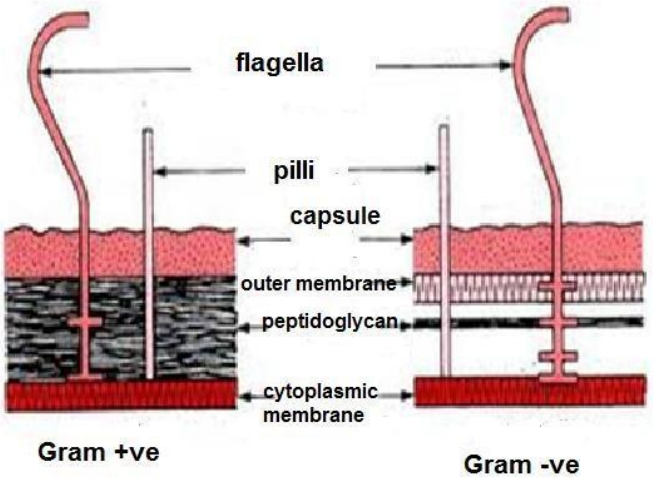
3- Decolorization by an organic solvent (acetone- alcohol) to remove the primary stain from the cell.

4- Adding a counter stain (safranin) to recolor cells that have lost primary stain after Decolorization; it should contrast in color with the primary stain.

The cell wall of a Gram +ve bacterium is composed of a heteropolymer of protein and sugar (peptidoglycan) called **murein**

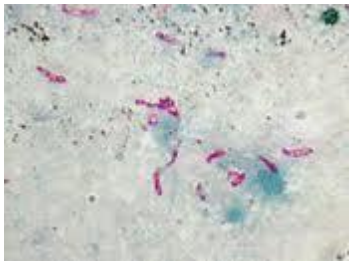
(25nm thickness). This murein provides a barrier through which the crystal violet- iodine complex cannot pass during Decolorization, and the cell appears purple in color after the staining procedure.

A Gram -ve bacterium contains less murein and more lipid than a Gram +ve one; this allows a rapid and effective removal of the dye- complex during Decolorization. The Gram –ve cells appear pink after staining with counter stain safranin.



2- Acid- Fast Stain:

In a manner quite similar to the Gram- stain, the acid- fast stain differentiates an important group of bacteria**, the Mycobacteria**, on the basis of lipid content (mycolic acid) at the surface of the cell, giving it waxy properties. Once these cells are stained by using heat to allow the stain to penetrate, they resist Decolorization with acid- alcohol, hence the name acid- fast.



3- Bacterial Endo spore stain:

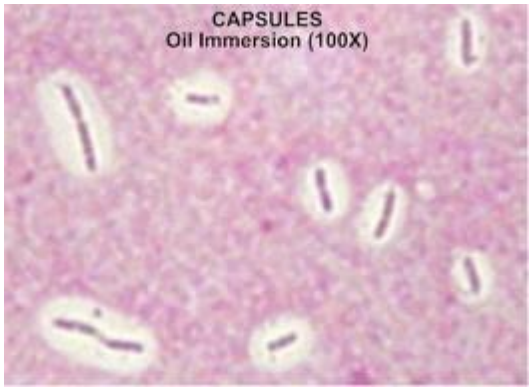
Bacterial Endospores present special problems for food industry, because of their resistance to high temperatures used to sterilize products.

Endospores form inside vegetative cells in genera like ***Bacillus*** and ***Clostridium***; they do not stain readily and require a heating step to drive the dye (e.g. Methylene blue or crystal violet) into the spore body. Once stained. Spores retain the dye. Whereas washing with water removes the stain from vegetative cells.

4-Negative staining- Capsule stain:

It is the staining of everything in the back ground but not the cells .a themselves. It is useful to demonstrate the mucoid capsule that surrounds the cells of many bacterial spp. The presence of a capsule is a major factor in determining the Pathogenicity of a bacterium.

A suspension of cells is mixed with a drop of India ink on a glass .b slide and spread thinly for viewing with phase contrast optics. The unstained capsule is visible against a grey background with the cell appears as a darker area in the center of the capsule.



5- Flagella stain:

Many bacterial spp. Are motile, most by means of flagella. Their positions are of taxonomic significance. It is critical to use young actively

culture (exponential phase). Staining flagella usually requires several attempts before success.

Normal Flora in Human

Normal flora means: - The population of microbes that inhibit regularly the skin and mucous membranes of healthy normal person and do not interfere with normal body functions (commensals).

The majority of N.F. is bacteria. Viruses, fungi and protozoa are also found in healthy person, but form only minority of the total population of N.F. Most investigators do not consider viruses and

parasites members of N.F., because they are not commensals and do not aid the host.

Why must we know N.F.?

Knowledge of N.F. of the human body is important in diagnostic Microbiology, especially for determining the clinical importance of microorganisms that are isolated from patient specimens.

Normal flora frequently are found in clinical specimens as a result of contamination during collection or because the colonizing organism is involved in the infection.

The normal flora is acquired rapidly during and shortly after birth. The new born human is exposed to microbes from the mother and the environment, skin is colonized first, followed by the oropharynx, gastrointestinal tract and other mucosal surfaces.

Factors influence normal flora

The normal flora in and on the human body is determined by many factors such as: Age, diet, environment (PH, temperature, O2, moisture..), hormonal state, health, personal hygiene.

Normal flora can be arranged into two groups:

1- The resident: relatively fixed types of microbes regularly found in a given area at a given age.

2- The transient: Non- pathogenic or potentially pathogenic microbes from the environment that inhibit the skin or mucous membrane for hours, days or weeks.

If the resident flora is disturbed, transient microbes may colonize, proliferate and produce disease.

Significance of the Normal flora to the host:

Normal flora influences the well- being of the host and plays a critical role in his health as it:

1- Provides essential growth factors and vitamins e.g. vit. K.

2- Aids absorption of nutrients in gastrointestinal tract.

3- Prevents colonization of pathogens and protects against infections with highly virulent microbes.

4- Stimulate the immune responses of the host.

Infection of the host by normal flora:

-Normal flora may act as opportunistic pathogens, especially in host with impaired immunity and may produce disease under certain circumstances. For example:

- Flora of gingival crevice causes dental caries in 80% of the population.

- Bacteria ***Streptococcus viridans*** (N.F of the upper respiratory tract), if they are introduced into blood stream in large numbers (after tooth extraction or tonsillectomy), they may produce **infective endocarditis.**

- Bacteria ***Escherichia coli*** are part of normal flora of the large intestine and are harmless in that location, if introduced into urinary tract, they cause painful **urinary tract infection (UTI).**

Normal flora of different parts of the human body:

Skin:

Acinetobacter, Bacillus, Staphylococcus, Streptococcus, Corynebacterium, Candida.

Upper Respiratory Tract:

Acinetobacter, Actinomyces, Corynebacterium, Haemophilus, Moraxella, Neisseria, Staphylococcus, Streptococcus, Candida, Entamoeba.

Gastrointestinal tract:

Bacteroides, Campylobacter, Clostridium, Enterobacteriaceae, Helicobacter, Staphylococcus, Streptococcus, Lactobacillus, Pseudomonas, Propionibacterium, Candida, Entamoeba, Trichomonas.

Genitourinary System:

Bacteroides, Clostridium, Corynebacterium, Enterobacteriaceae, Gardnerella, Haemophilus, Lactobacillus, Mycoplasma, Staphylococcus, Streptococcus, Treponema.

Bacterial Pathogenicity

Pathogenicity: Ability to cause disease.

Virulence: Degree of Pathogenicity.

Many properties that determine a virulence are unclear or unknown, but when a microbe overpowers the host defenses, disease results.

Types of bacterial pathogen:

Bacterial pathogens can be classified into two broad groups:

1- Opportunistic pathogens:

These cause disease only when the host defenses are impaired or compromised (e.g.: acquired disease, immunosuppressive therapy,

These pathogens are part of the normal flora, and when they

introduced into anatomic sites, where they are not normally found, they may develop disease.

2- Primary pathogens:-

These pathogens are capable of causing disease in individuals with intact immunological defenses, as they cause disease in individuals with impaired defenses.

Virulence Factors:

The possession of virulence factors differentiates pathogens from non-pathogens, and their number and potency separate opportunist from primary pathogens.

Some of the virulence factors in bacteria:

Bacteria have many types of virulence factors that provide microbes with the capacity to avoid host defenses and damage host cells, tissues and organs, such as:-

1) Adherence factors:-

Many pathogens colonize mucosal sites by using **Pili (fimbriae)** to adhere to cells. Fimbriae are numerous thin, rigid and rod- like

structures present on the surface of G –ve and some G+ve bacteria, they are much thinner than flagella and involved in attachment of some bacteria to the host cell surfaces. Their antigenic composition is complex. They consist of aggregates of a structural protein subunit called **Fimbrillin (Pilin).** Fimbriae are found in many bacteria like: ***E. coli***, ***Pseudomonas***, **Neisseria**, and **Vibrio**.

2) Invasive Factors: Surface components that allow the bacteria to invade host cells, these factors can be encoded to plasmid, but often are on chromosome.

3) Capsule:

Many bacteria are surrounded by capsules that protect them from opsonization and phagocytosis.

Most capsules are polysaccharides composed of Sugar monomers that vary among different bacteria. Capsule reduces the efficiency of phagocytosis and prevents efficient opsonization of bacterium by complement or specific antibody. All pathogens associated with **Meningitis** & **Pneumonia** have capsules, such as: ***H influenzae,*** *N.* ***meningitidis*** and ***Strep***. ***pneumoniae***.

4- Enzymes:-

Many pathogens secrete enzymes that contribute to their Pathogenicity:

A) Leukocidins: Prevent phagocytosis by killing WBC

B) Hemolysins: Cause the lysis of RBCs (Streptococci).

C) Coagulase: Cause blood to coagulate to protect bacteria from phagocytosis and other host defenses.

D) Kinase:

Enzymes that dissolve blood clots which the host form to isolate the pathogen and helps them escape from host defenses.

E)Collagenase: Break down collagen found in many connective tissues e.g.: **Clostridium perfringens** causes **Gas- gangrene**, uses this enzyme to spread through muscle tissues.

F) Hyluronidase:

Enzymes that hydrolyze hyluronic acid which is a constituent of the ground substance of connective tissue, and aid bacterial spread through tissue e.g.: **Staphylococci**, **Streptococci.**

G) Streptokinase: (Fibrinolysin)

An enzyme produced by hemolytic **Strep**. It activates a proteolytic enzyme of plasma and aid the spread of **Strep**. Through tissues.

Streptokinase is used in treatment of acute myocardial infarction to dissolve fibrin clots.

H) Proteases:

Enzyme hydrolyze immunoglobulin and allow pathogens (e.g.: ***N. meningitidis***, ***Strep. pneumoniae***) to inactivate the primary antibodies found on mucosal surfaces and eliminate protection of the host by antibodies.

Bacterial Toxins:

Toxins are biochemically active substances that are released by microorganisms and have a particular effect on host cells. Microorganisms use toxins to help them establish infections and multiply within the host. 40% of the toxins cause disease by damaging the cell wall.

Toxins also can cause human disease in the absence of the pathogens that produce them, and this is the common mechanism of **Food poisoning** that involve the ingestion of pre- formed bacterial toxins, it is referred to as **Intoxication**, e.g.: **Botulism.**

Bacterial toxins are generally classified into two groups.

1- Exotoxins

2- Endotoxins

Exotoxins:-

Exotoxins produced mostly inside some G+ve bacteria and less G-ve bacteria, as part of their growth and metabolism, and released into the surrounding media.

They are proteins in nature and many are enzymes.

They are soluble in body fluids, so can easily diffuse into the blood and are rapidly transport throughout the body.

Exotoxins cause damage to the host cell by:-26

1- Destroying particular parts of the host cells.

2- Inhibiting certain metabolism functions.

They cause Extreme pyrogenic response

1- Cytotoxins:-

Kill host cells by affect their function e.g.: ***Corynebacterium diphtheriae***

2- Neurotoxins:-

Target the nervous system and can interfere with normal nerve impulse transmission. e.g.: ***Clostridium tetani***, ***Clost. botulinum***.

3- Enterotoxins:-

Affect cells lining the gastrointestinal tract e.g.: ***Vibrio cholerae***

Exotoxins are among the most lethal substances known; only 1mg of the botulinum exotoxin is enough to kill 1 million guinea pigs.

Exotoxin is inactivated by heat and no longer cause disease, but stimulates the body to produce antitoxin (antibodies), that provide immunity to exotoxins.

Toxoid:-

Is altered exotoxin injected to stimulate the production of antitoxins and provide immunity (by formaldehyde.

Endotoxins:-

They are complex lipopolysacchrides in the outer envelope of the cell wall of G-ve bacteria. The outer envelope of these bacteria consists of lipoprotein, phospholipids and lipopolysacchrides (LPs).

Lipoprotein of (LPs) called (Lipid A) is the endotoxin.

The endotoxin liberates when G-ve bacteria die and the cell wall lysed. The substance is heat stable. Administration of endotoxin to animal or human results in a series of events:

Fever, leucopenia, hypoglycemia, hypotension, shock, impaired perfusion of essential organs (brain, heart, Kidney), intravascular coagulation and death.

e.g.:- ***Salmonella typhi, Proteus spp. Neisseria***

Characteristics of exotoxins & endotoxins:

Exotoxin Endotoxin

1- Excreted by living cell Released on bacterial death

2- Produced by both G+ve & Found only in G-ve bacteria

G-ve bacteria

3- Unstable, toxicity often destr- Stable, withstand heating at oyed by heating at above 60oc above 60oc for hours.

4- Highly antigenic, stimulate weakly immunogenic formation of antitoxin.

5- Converted to antigenic, non- not converted to toxoids

Toxic toxoids by formalin, heat

6- Highly toxic, fatal to animals in moderately toxic, fatal in tens μg or less. Or hundreds of micrograms

7- Usually bind to specific receptors Specific receptors not found on cells. on cells.

8- Usually do not produce fever in usually produce fever in host

Host. By release of interleukin-1

9- Frequently controlled by extra- Synthesis directed by chrom- Chromosomal genes (plasmid)

10 osomal genes

The infection process"

I) Entry into the Human Body:

Bacteria must first gain entry into the body to establish an infection. The mouth, nose, respiratory tract, ears, eyes, urogenital tract and anus, are sites through which bacteria can enter the body. Natural defense mechanisms and barriers, such as: skin,

mucus, ciliated epithelium and antibacterial secretions (e.g. lysozyme), make it difficult for bacteria to gain entry into the body.

These barriers are sometimes broken (e.g.: a tear in the skin, a tumor or ulcer) providing a portal of entry for the bacteria, or the bacteria may compromise the barriers and invade the body.

Sites of Entry:

A . Skin: Skin has a thick, horny layer of dead cells that protects the body from infection by many means such as:

1- Inactivate microorganisms by fatty acids (skin PH is about 5.5).

2- Substances secreted by sebaceous and other glands.

3- Certain peptides formed locally by keratinocytes.

4- Materials produced by the Normal flora of the skin.

Cuts in the skin provide a means for bacteria to gain access to the tissue underneath, or may enter hair follicles or sebaceous glands to cause styes & boils.

Fungi (the dermatophytes) infect the non-living keratinous structures (hair, nails) and if the parasites rate of growth into keratin exceeds the rate of shedding of keratinous product, the infection may become chronic.

B. The conjunctiva:

It's a specialized area of skin, kept clean by tears aided every few seconds by the wiper action of the eyelids contaminated fingers, flies or towel carry infection to the eye. Antimicrobial substances in tears (Lysozyme) and certain.

C. Respiratory tract:

In the upper & lower R-T, inhaled micro org., like other particles (dust, smoke) will be entrapped in mucus, carried to the back of throat by ciliary action, and swallowed; some micro org. have developed specific

mechanisms to avoid this fate. e-g: inhibiting ciliary activity or avoiding destruction by alveolar macrophages (Tubercle bacilli).

D. Gastrointestinal tract:

The acid, bile & the general flow of intestinal contents, are the natural defenses in gastrointestinal tract, however, many bacteria are unaffected or have means to evade these defenses e-g: the outer membrane of G-ve bacteria makes them more resistant to acid and bile.

E- Urinogenital tract:

Urinogenital tract is a continuum, so micro organisms can spread easily from one part to another. It has natural defenses, e-g:

1) Certain lactobacilli colonize vagina & produce lactic acid (vaginal PH=5) which inhibit other micro org.

2) The protective layer of mucus in bladder & the the production of antibodies & immune cells.

Culture media of bacteria

Classification of Culture Media

I. According to composition:

Chemically Defined Media (synthetic): Exact chemical composition is known e.g. glucose inorganic salt phosphate for E. coli Complex Media

(non-synthetic): chemical composition is not specifically defined; Extracts and digests of yeasts, meat, or plants e.g. Nutrient broth, Nutrient agar, McConkey, EMB Usually, bacteria are grown in complex media.

II. According to Consistency:

- solid- with 1.5 to 3.0% agar e.g. NA (Nutrient Agar)
- liquid- no solidifying agent e.g. NB (Nutrient Broth)
- semi solid- with less than 1.5% agar e.g. SIM (Sulfide Indole Motility Medium).

III. According to Function/Application:

- Basal/ordinary/general
- Enriched: with enrichment substances Enrichment Selective media: with inhibitory substances
- Differential media: with indicators/dyes
- Special Media for Biochemical Testing Media for Antimicrobial Susceptibility Testing

SELECTIVE MEDIA

- With inhibitors to prevent growth of unwanted organisms and favor desired organisms e.g.
- **A. to inhibit growth of Gram positive organisms**

1. Gentian violet
2. bile salts
3. Na desoxycholate

- **B. to inhibit growth of Gram negative organisms**

1. K tellurite
2. Na azide

C. to inhibit swarming growth of Proteus

1. Chloral hydrate
2. alcohol