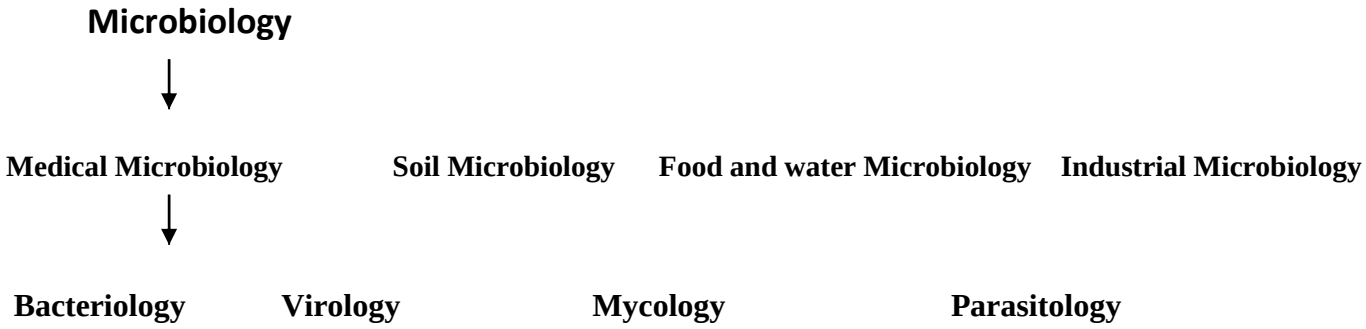
Microbiology

Microbiology :from Greek Micro (*mīkros)*, "small"; bio *(bios*), "life"; and logy(*logia*) is the study of microorganisms, those being unicellular (single cell), multicellular (cell colony), or acellular.



Medical Microbiology

Medical microbiology is large subset of microbiology that is applied to medicine, is a branch of medical science concerned with the prevention, diagnosis and treatment of infectious diseases. In addition, this field of science studies various clinical applications of microbes for the improvement of health. There are four kinds of microorganisms that cause infectious disease: bacteria, fungi, parasites and viruses.

History of Medical Microbiology

1- In 1676, Anton van Leeuwenhoek observed bacteria and other microorganisms, using a single-lens microscope of his own design.

2- In 1796, Edward Jenner developed a method using cowpox to successfully immunize a child against smallpox. The same principles are used for developing vaccines today. In 1867 Joseph Lister is considered to be the father of antiseptic surgery. By sterilizing the instruments with diluted carbolic acid and using it to clean wounds,

post-operative infections were reduced, making surgery safer for patients.

3- In the years between 1876 and 1884 Robert Koch provided much insight into infectious diseases. He was one of the first scientists to focus on the isolation of bacteria in pure culture. This gave rise to the germ theory, a certain microorganism being responsible for a certain disease. He developed a series of criteria around this that have become known as the Koch's postulates.

4- A major milestone in medical microbiology is the Gram stain. In 1884 Hans Christian Gram developed the method of staining bacteria to make them more visible and differentiable under a microscope. This technique is widely used today.

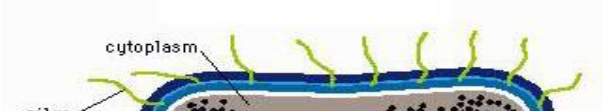
5- In 1929 Alexander Fleming developed the most commonly used antibiotic substance both at the time and now: penicillin.

6- DNA sequencing, a method developed by Walter Gilbert and Frederick Sanger in 1977,caused a rapid change the development of vaccines, medical treatments and diagnostic methods. Some of these include synthetic insulin which was produced in 1979 using recombinant DNA and the first genetically engineered vaccine was created in 1986 for hepatitis B.

7- In 1995 a team at The Institute for Genomic Research sequenced the first bacterial genome; Haemophilus influenzae. A few months later, the first eukaryotic genome was completed. This would prove invaluable for diagnostic techniques.

Bacteria

Bacteria are unicellular free living organisms without chlorophyll having both DNA and RNA .They are capable of performing all essential processes of life e.g. Grwoth , Metabolism and Reproduction. This being



unsatisfactory a third kingdom "Protrsta" was formed for them . Protista is again divided into 2 groups .1

a-Prokaryotes

b-Eukaryotes

Bacteria and green algee are prokaryotic while fungi , algae , slim molds and protozoa are eukaryotic cells. Differentiated between Prokarytic and Eukarytic cell :

Character	prokaryotic	Eukaryotic
1-Nucleus		
Nuclear membrane	Absent	Present
Nucleolous	Absent	Present
Deoxyribonuceuprotein	Absent	Present
Chromosome	One	More
Mitotic division	Absent	Present
2-Cytoplasi		
Mitochondria	Absent	Present
Lysosomes	Absent	Present
Golgi apparatus	Absent	Present
Endoplasmic reticulum	Absent	Present
3-chemical composition	Absent	Present
Steroil	Present	Absent
Muramic acid		
4- Example	Bactria	Protozoa

Nomenchulated of bacteria

Bacteria have two named system

1- **Genus**

2- **Species**

e.g :- Salmonella typhi.

Salomnella paratyphi.

Staphylococcus aureus.

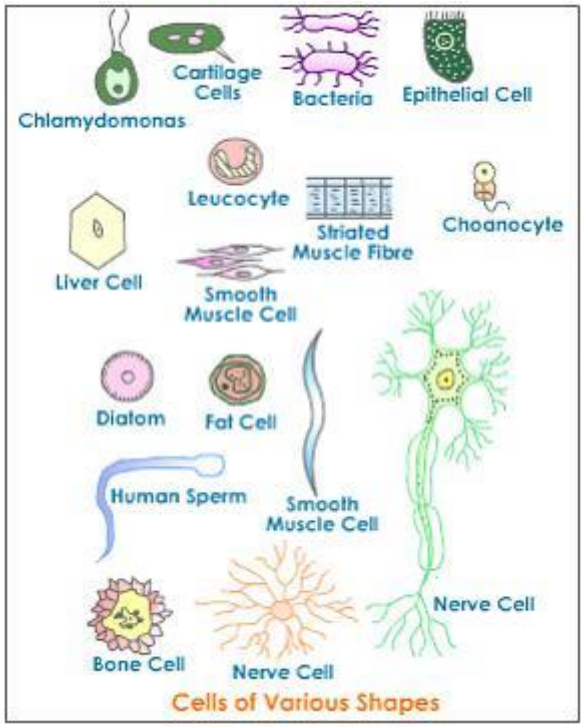
Staphylococcus albus.

An animal cell

1- Shape.

2- Size.

3- Structure.



1- Shape:-

1- Circular cell . e.g. - Red blood cell .

2- Cubic cell . e.g. - Epithelial cell .

3- Star cell . e.g. - Nerve cell .

4- Spindle cell . e.g. - Muscle .

5- Unorganized cell .e.g. - White blood cell .

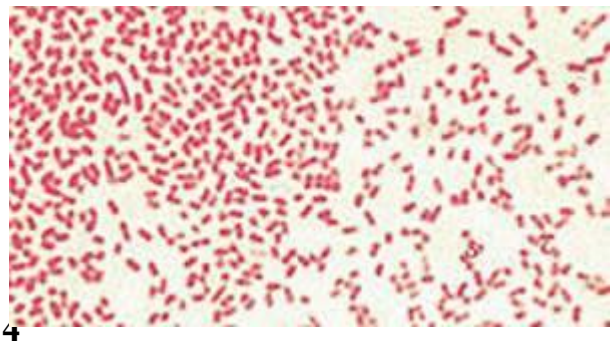
2- Size :-

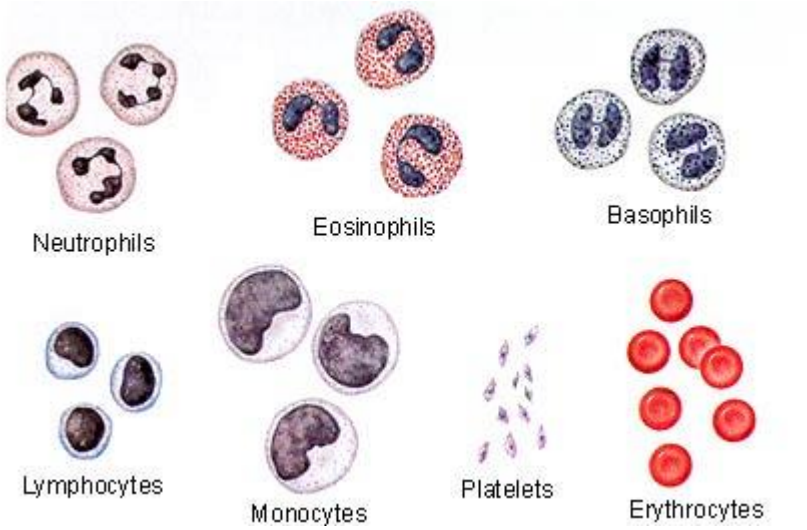
1- Micro cell :- is so small to see by naked eye just by the microscope .

e.g. – Blood cells , Bacteria cells .

2- Macro cell :- is large sowe can see it by naked eye.

e.g. – The chiken egg .





3- Strueture

1- Cell wall :- it surrounds the contents of the cell .

It consist of two layers of phosphoric lipids .

We can see it by the electronic microscope only .

The cell wall organizing water transport between inside and outsid the cell .

2- Cytoplasm :- it is aliving part of the cell , it lies out of the nucleus and coverd with cytoplasmic membrane it cosists of .

Water 80%

Proteins 15%

Lipids , Sugars and saltes 5%

Cytoplasme has many contents :-

A- **Nucleus** :- It surrounds by the Nuclear envelop (Nuclear membrane) and it contains of :

1- Nucleoplasme.

2- Nucleolus :- Which contains of RiboNucleic Acid (RNA) and protein.

Nucleolus function is to built Ribosomal RNA (rRNA) to

making Ribosomes .

3- Chromatin network .

4- Chromosomes : To carry Deoxyribo Nucleic Acid (DNA).

B- Endoplasmic reticulum:

It has two types :

1-Rough Endoplasmic reticulum : It named that because it containes of Ribosomes which creates protein for the cell .

2-Smooth Endoplasmic reticulum which its job to remove toxins from the system like liver.

C- Mitochondria :-

It is the center of providing the energy to the cell . It stores the energy as Adinosine Triphosphate (ATP), for that reason the main function of Mitochondria is Cellular respiration.

D- Golgi apparatus :-

It is an important benefit:

1- Built the complex sugars.

2- Secretion the protein that will be leave the cell .

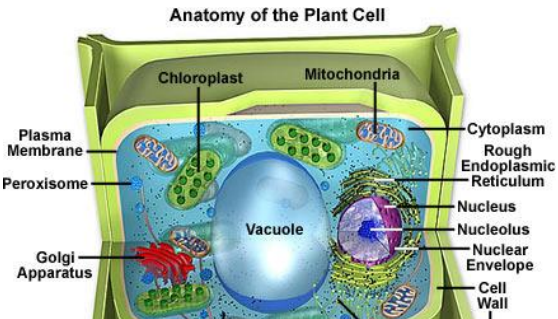
3- Secretion complex sugirs , proteins , several hormones and enzymes.

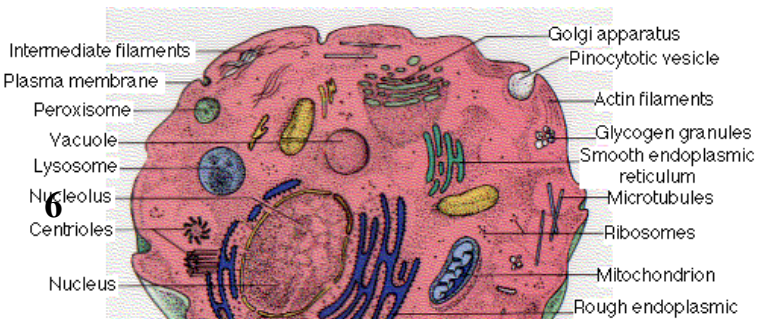
E- Lysosomes :

It can digest liarge particles like proteins and nucleic acids to smaller units.

F- Cilia and Flagella :

There are moving fingers in Monocellular individuals that are living in water area.





Differentiated between plant and animal cell :-

BASIS	PLANT CELLS	*ANIMAL* CELLS
1-CELL WALL.	Cell wall made of cellulose is present	Cellulose in any form including cell wall is absent
2-Cytoplasim	It is pushed to the periphery and forms a thin line within the cell wall	It denser and occupies the maximum space in the cell
3-Centrosome	Centro some is absent ,polar caps are present instead	Centrosome is present.
4-Vacuole	One or more large and prominent vacuoles are present	Mostly absent , if present they are small and temporary
5-Plastid	Plastids are present	Plastids are absent
6-Size	Comparatively larger in size	Usually small in size

Morphology of bacteria

1 - Size of Bacteria :-

Most of bacteria are so small that their size is measured in micron .

1 micron (µ) or micrometer (µm) = 1000 of millimeter .

1 millimicron (m µ) or nanometer (nm) = 1000 of micron .

1 angstrom units (Å) 0.1 of nanometer .

Generally cocci are about 1 micron in dimeter and bacilli are (2-10)m in length and (0.2- 0.5) m in width . Obviously bacteria can be visualized only under magnification .

2 - Shape of Bacteria :-

On the bases of shape , bacteria are calssified as under :-

1. Cocci :-

a. Staphylococci : They are spherical .On the basis of arrangement of individual of arrangement of individual organisms , they are described as staphylococci (clusters like bunches of grapes) .

b. Streptococcus :- Arranged in chain .

c. Diplococci :- Forming pairs .

d. Tetrads :- Arranged in groups of four packets .

e. Sarcina :- Arranged in groups of eight cells .

2. Bacilli :- The cylindrical or rod shaped organisms are called bacilli .Their length may approximate the width of the organisms .

e.g. :Brucella .

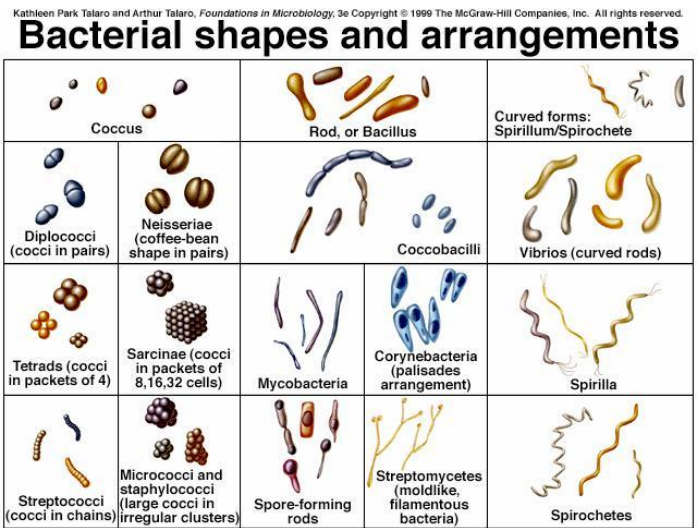
3. Chinese letter :- This arrangement is seen in corynebacteria **.**

4.Vibrio :- They are curved rods and derive the name from their characteristic vibratory .

5. Spirochaetes : They are relatively longer , thin , flexible organisms having several coils .

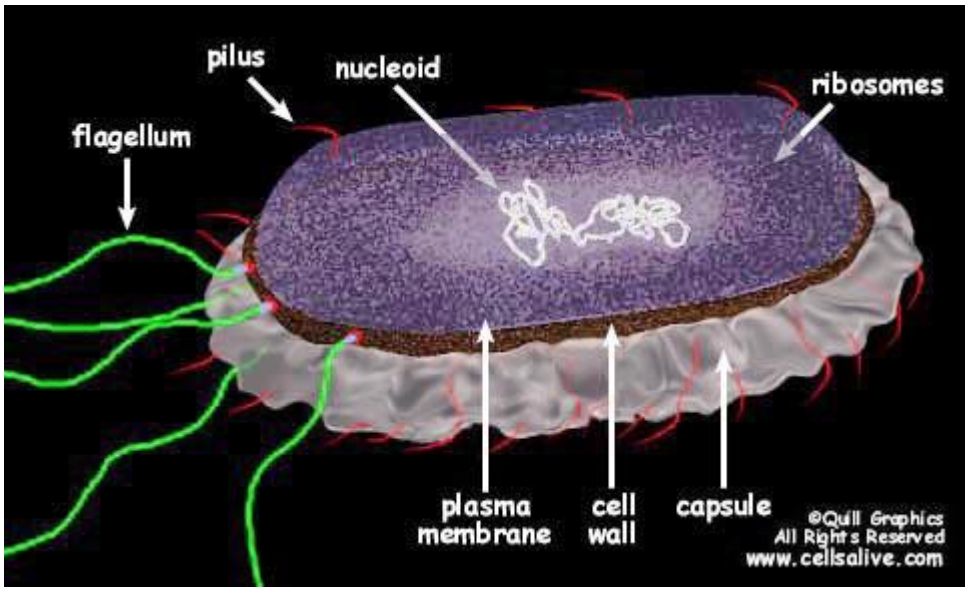
6. Actinomycetes : They are branching bacteria .

7. Mycoplasma :They are organisme which lack cell wall .They are round or oval bodies with interlacing filaments .



3 – Structure of Bacteria

The important structure of bacterial cell is found under electronic microscope and described below :



Slim Layer :

Some bacteria secrete viscid substance which may diffuse out into surrounding media or remain out side cell wall .This viscid carbohydrate material is called slime layer .Bacteria secreting large amount of slime produce mucoid growth on agar with stringy consistency . Slime has little affinity for basic dye and so not visible in Gram stain .

Capsule :

It is gelatinous secretion of bacteria which gets organized as a coat around cell, it is known as capsule .It may be composed of complex polysaccharides .Capsule have no affinity for dyes and so they are not seen in stained preparations .

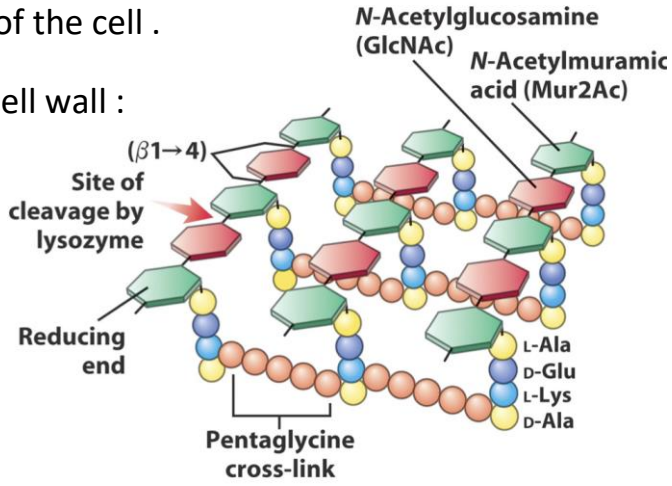
Function of capsule :

1. Protection against deleterious agents . e.g. : Lytic enzymes .
2. Contribute to the virulence of pathogenic bacteria by inhibiting phagocytosis .

Cell Wall :

The cell wall is the outer most supporting layer which protect the internal structure . It is about (10 – 25) nm. In thickness and shares (20 - 30)percent of dry weight of the cell .

Chemical structure of cell wall :



Cell wall is composed of muc peptide , scaffolding formed by N-acetyle glucosamine and N- acetyl muramic acid molecules alternating in chain cross linked by peptide chain .

Cell wall antigens of Gram negative organismsact as endotoxin . A comparision of cell walls of Gram positive and Gram negative bacteria is as following :

Contents	Gram positive	Gram negative
1. Thickness	15 -23 millimicron	10 -15 millimicron
2. Variety of amino acids .	Few	several
3. Aromatic and sulfur	Absent	present

4. Lipids	Low (2-4) percent	High (15-20) percent
5. Techoic acids	Present	absent

Cell wall synthesis may be inhibited or interfered by many factors . Lysozyme enzyme present in many tissue fluid cause lysis of bacteria . They act by splitting cell wall mucopeptide linkages .When lysozyme acts on Gram positive organism in hypertonic solution a **protoplast** is formed consisting of cytoplasmic membrane and contents .With Gram negative bacteria the results is **spheroplast** . It differs from protoplast in that some cell wall material in retained . Protoplast and spheroplast are spherical shape .

Function of cell wall :

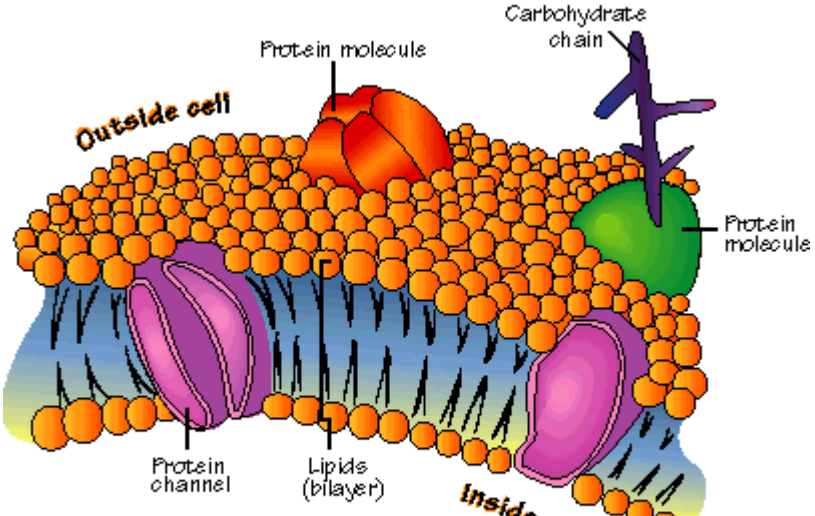
1. Protection of internal structure (supporting layer).
2. Gives shape to the cell .
3. Confers rigidity and ductility (mucopeptide) .
4. Role in division of bacteria .
5. Offers resistance to harmful effect of environment

Cytoplasmic Membrane :

It is thin semipermeable membrane which lies just beneath the cell wall . It is (5-10) nm in width .Electron microscope shows the presence of three layers consistuting a unit membrane structure .

Chemically the membrane consists of lipoprotein with small amount of carbohydrate .

Sterol are absent except in mycoplasma .



Function of cytoplasmic membrane

1. It controls inflow and outflow of metabolites to and from protoplast .
2. Presence in the membrane of specific enzyme plays important role in passage through membrane .

Cytoplasm :

The bacterial cytoplasm is suspension of organic and inorganic solution in viscous watery solution .

It doesnt exhibit protoplasmic streaming (internal mobility) and it lacks endoplasmic reticulum or mitochondria .It contains ribosomes , mesosomes , inclusions and vacuoles .

Ribosomes :

These are ribonucleoprotein granules measuring (100 -200) A units in diameter and their sedimentation coefficient is 70 units . The 70 units ribosome is composed of two smaller units of 50 and 30 units .

Function of Ribosomes :

They are the sites of protein synthesis .

Mesosomes :

They are vesicular , convoluted or multilaminated structures formed as invaginations of plasma membrane in to the cytoplasm .They are more prominent in Gram positive bacteria .

Functions of Mesosomes :

1. They are the sites of repiretory enzymes in bacteria .

2. Coordinate nuclear and cytoplasmic division .

Inttracytoplasmic inclusion :

a. **Volutin granules** : - They are highly refractive , basophilic bodies consisting of polymetaphosphate .

Function of Volutin granules :

To represent a reserve of energy and phosphate for cell metabolism.

b. **Polysaccharide granules** : -
May be demonstrated by staining with iodine . They appear to be storage product .

c. **Lipid inclusion** :
Also for storage product and demonstrated with fat soluble dyes such as sudan black .

d. Vacuoles :
They are fluid containing cavities separated from cytoplasm by a membrane . Their function and significance is uncertain .

Nucleus :

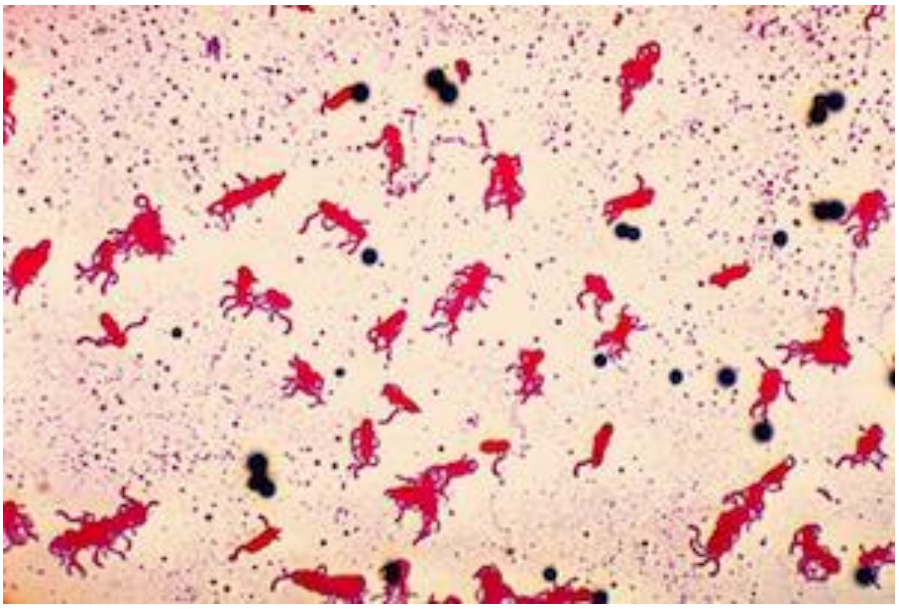
It is a long filament of DNA tightly coiled inside the cytoplasm . The bacterial nucleus is not surrounded by nuclear membrane . They don't have nucleolus . Nucleus cant be demonstrated under direct light microscope . They appear as oval or elongated bodies , generally one per cell .

The genome cnsists of a single molecule of double stranded DNA arranged in the form of circle . It may open under certain conditions to form long chain about 1000 micron in length . Genes are arranged along the length of chromosome in fixed order and bear hereditary characters .

Flagella :

These are long , contractile filamentous appendages known as flagella . They are organ of locomotion . e .g. : E. coli , Salmonella , Vibrio , Pseudomonus. They are extremely thin (0.05 micron or less), helical shape .structure of uniform diameter throughout their length . Each flagellum originate in a spherical body (basal granule) located just inside cell wall . They are antigenic and are composed of protein called flagellin which has properties of fibrous protein , kerasin and myosin .

The number and arrangement of flagella are characteristic of each bacteria . Flagella may be arranged on bacterial body in following manner .



Arrangement of flagella in bacteria

Monotrichate : -

One flagellum at one end of the organism .e.g. : vibrio , pseudomonas , spirillum .

Amphitrichate :-

One flagellum at both the poles . e.g. : alcaligenes faecales .

Lophotrichate : -

A tuft of flagella at the end . e. g. : pseudomonas .

Peritrichate :-

Several flagella present all over the surface of bacteria e. g. : Salmonella.

Fimbriae : -

They are filamentous short , thin , straight hear like appendage . They are also

called **Pilli** . They project from cell surface .as straight filaments . They tend to

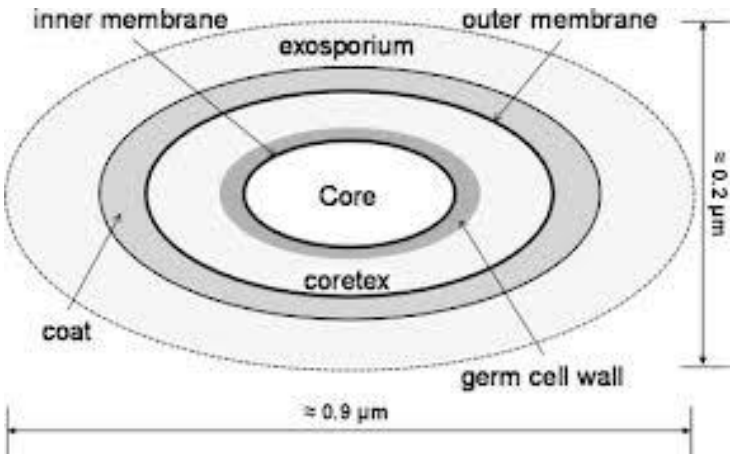
disappear following subcultures on solid media .

Function of Fimbriae :-

1. Organ of adhesion .
2. Conjugation tube through which genetic material is transferred from donor to recipient cell .
3. They are antigenic .

Spores :

They are highly resistant dormant state of bacteria found in certain genera .e.g.:- Bacilli and clostridium .They are not destroyed by ordinary methods of boiling for several hours . They are killed when autoclaved at 15 pressure at 121c for 20 min.



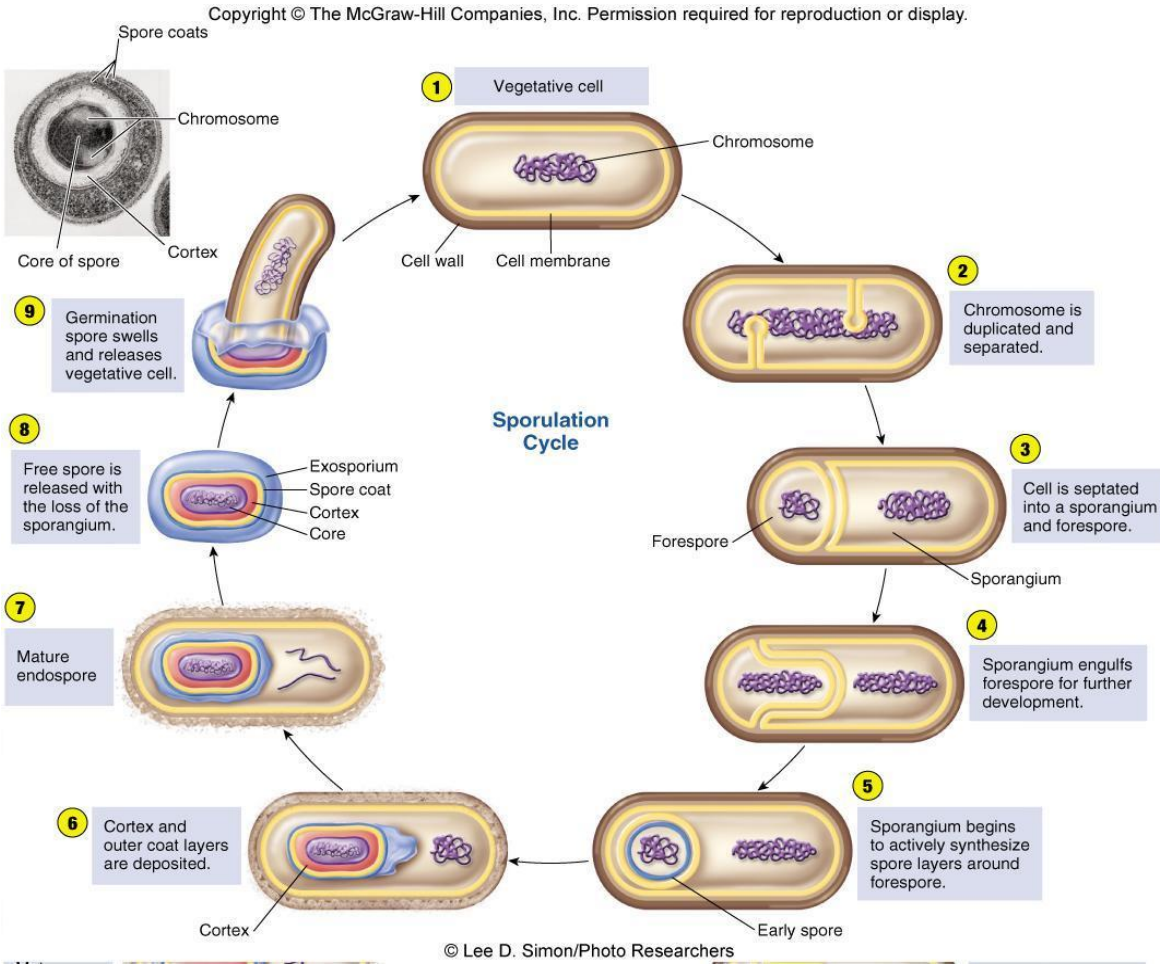
Spore structure

Function of Spores : -

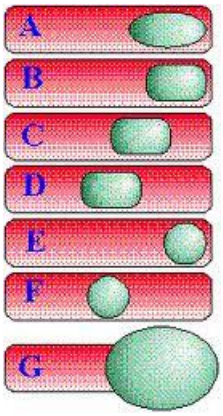
They make survival of organism possible under unfavorable conditions like dry state .Spores are resistant to heat , drying , freezing and toxic chemicals .

Formation of spores :-

Sporolation is initiated by appearance of clear area near one end of cell which gradually becomes more opaque to form forespore . The fully developed spore has at its cor nuclear body surrounded by spore wall , a delicate membrane (future cell wall) .Out side this spore cortex which in turn is inclosed by multilayered spore coat .



Some spores have an additional outer covering called exosporium having ridges and grooves .



Types of bacterial spores : -

1. Central bulging .
2. Central not bulging .
3. Sub terminal bulging .
4. Sub terminal not bulging .
5. Terminal spherical.
6. Terminal oval .

Nutritional Requirement of Bacteria

Bacteria may require adequate nutrition of optimum pH , temperature and oxygen for multiolication and growth .Bacteria can be classified into following types on the basis of nutritional requirement .

1- On the basis of energy sources .

A- Photrophics which get energy from photochemical reactions .

B- Chemotrophic gets energy from chemical reactions .

2- On the basis of their ability to synthesize essential metabolites .

A- Autotrophic :- These are the organisms in which all essemtial metabolites are

synthesized from inorganic sourses . They use carbon dioxid as the main source of

carbon and simple inorganic salts . e.g. : nitrates , niters , ammonium sulphate , phosphates .

B-Heterotrophic :- Here some of the essential metapolites are not synthesized . Orqanic

compounds e.g. protein , peptones , amino acids , vitamins and growth factor are supplied from outside . Most of the bacteria producing disease in man are heterophic.

The other nutritional requirement are as under :

1-Minerals :- These are sodium , potassium ,magnesium , calcium , iron , clorine

, zinc , copper , iodine and traces . These are essential for physiological activities of

bacteria.

2-Gas requirements :

A-Oxyyen :- The capacity of bacteria to grow in the presence of oxygen and to

utilize it depends on possession of a cytocherome oxidase systems .

1. **Aerobes** :- The aerobe organisms grow only in the presence of oxygen .e.g.

Pseudomodaceae , bacillus , nitrobacter sarcina etc.

2. Facultative anaerobes :- They are the organisms that can live with or without Oxygen .e.g. vibrio , E.coli , salmonella and staphylococcus. The micro – Aerophilic organism grow well with relatively small quantites of oxygen .e.g. Haemophilus .

3. Obligate anaerobes :- The strict anaerobes multiply only in the absence of oxygen .e.g. bacteroides ,clostridium.

B. **Carbon dioxide :** The metabolic activiter of some organisms like neisseria , Brucella abortus are greatly enhanced by the presence of extra amount of carbondioxide in atmospheric air .

3- Moisture :- Bacteria require water for their growth . Desiccation may kill most of bacteria like Neisseria gonorrhoeae .

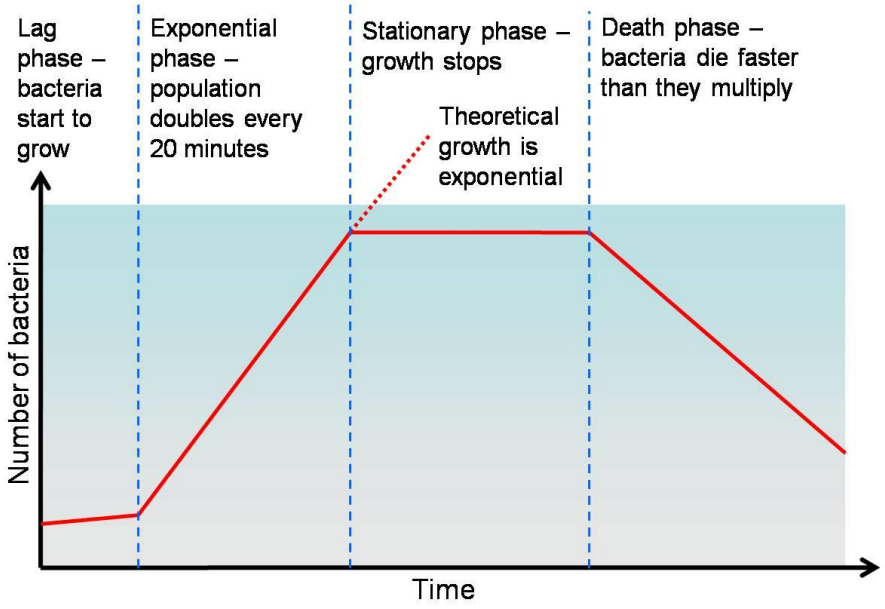
4- Necessary nutritional requirement :- Most ofen the necessary growth factors are vitamins . The requirement of growth factors differ widely in various bacteria .

e.g. : Neisseria gonorrhoeae …… Glutathione .

Staphylococcus aureus ……However 0.5% sodiuom .

Growth Curve

When organism are cultured in appropriate fluid medi there would be increase in the size of bacteria without any multiplication for some time . This is followed by multiplication and increase in numbers of bacteria to the extent that media look turbid to the naked eye (log phase) . After some time growth rate becomes stationary and later on declines . Counting of bacteria at different periods after inoculation and then events of sequences are represented on a graph which is called growth curve .



Growth curve

A-Lag phase :- During this phase there occurs

1- Increase in size of cell .

2-Increase in metabolic rate .

3-Adaptation to new environment and necessary enzymes and intermediate metabolites are built up for multiplication proceed.

The length of lag phase depends upon :

a-Tupe of bacteria .

b-Better the medium , shorter the lag phase.

c-The phase of culture from which inoculation in taken

d-Size of inoclum.

e-Environmental factors like temperature .

B-Log phase :- Following lag phase . The cells start dividing and their number increase by geometric prpgress on with time . Lagarithms of viable count plotting against time gives straight line , during this periods .

i. Bacteria have high rate of metabolism .

ii. Bacteria are more sensitive to antibiotics .Control of log phase is brought about by :

a-Nature of bacteria .

b-Temperature .

c-Rate of penetration of the medium . It depends on the concentration of material in the medium.

C-Stationary phase :-After some time a stage comes when rate of multiplication and death becomes almost equal. It may be due to :

a-Depletion of nutrient .

b-Accumulation of toxic products . Sporulation may occur during this stage .

D- Decline phase :-During this phase population decrease due to death of cells .

Factors responsible for the phase are :

a-Nutritional exhaustion

b-Toxic accumulation .

c-Autolytic enzymes . Inrolution is common in phase of decline.

Survival phase :-When most organisms have died , afew survive for several months or years .

Factors influencing growth:

1/ Temperature :The temperature range at which an organism grows best is called optimum temperature . It human , parasitic organisms , optimum temperature ranges between 30c and 37c .

There are three groups of bacteria as regards the temperature of growth:

a-Psychrophilic:- These are the organisms growing between 0c to 25c . They are mostly soil and water bacteria.

b-Mesophilic:- They grow between 20c and 45c . This group includes bacteria producing disease .

c-Thermophilic:- Some organisms grow between 50c and 60c . e.g. bacillus and algae.

2/Hydrogen ion concentration :- Most of pathogenic bacteria grow best at pH(7.2-7.6) .
However lactobacilli grow at acidic ph while Vibrio cholera grows at alkaline pH .

3/Moisture:- Water in quite essential for the growth of bacteria . Organism like Neisseria gonorrhoe and treponema pallidum dia almost at once on drying .

However my cobacterium tuberulosis and staphy lococcus aureus survive for quite along time even on drying .

4/Osmotic pressure :-

Bacteria are usually resistant to changes of osmotic pressure. However 0.5% sodium chloride is added to almost all culture media to make invironment isotonic.

5/ Light :-

Darkness usually farourable for the growth and viability of all the organisms . Direct light exposure shorthen the survival of bacteria

Photochromogenic mycobacteria form pigment on exposure to light . Organism are sensitive to ultraviolet and other radiations

6/ Mechanical and sonic stress :-

Bacetria tough cell wall . Vigrous shaking and exposure to ultra sonic vibration may cause rupture or disintegration of cell wall .

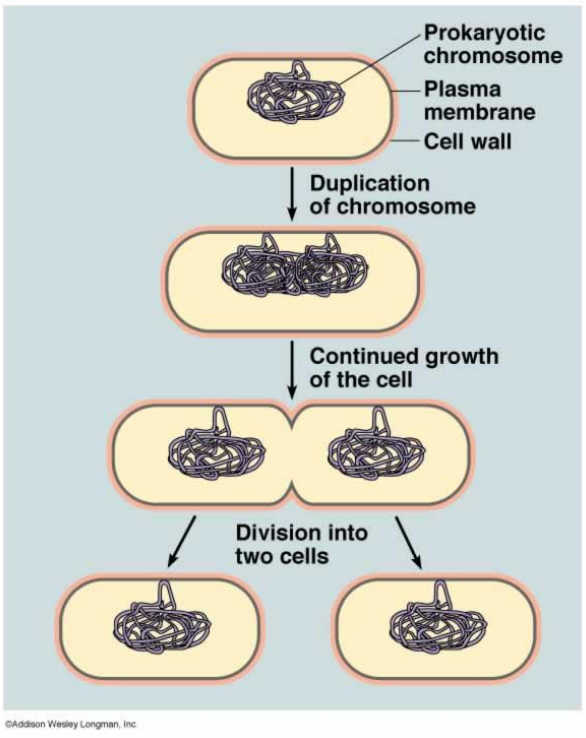
Reproduction in bacteria

Bacteria divide by simple binary fission .The cll grows in size , almost double its size . the process of cell division is initiale .

The sequence of cell division include :-

1. Formation of initial of chromosome replication .

2. Chromosome duplication .
3. Separation of chromosomes .
4. Formation of septa and cell divition .



Reproduction in bacteria

Counting of microorganisms

A- Total bacteria count .

1. Direct count :-

a. Direct staning method using stained smears prepared by spreading known volium of culture over measured area of slide .
b. Using chamber :-
Direct count using a haematocytometer chamber .

2.Indirect count:-

By estimating the turbid of the suspension against standard turbidities .

B. Viable Count :-

Appropriate dilutions are inoculated on solid media either on the surface of plate or as pour plates .

The number of colonies that develops after incubation gives an estimate of viable count .

Sterilization and disinfection

Sterilization :- It is a process by which articles are freed of all microorganisms either in vegetative or spore state .

Disinfection :- It is a process of destruction of pathogenic organisms capable of giving rise to infection .

Antiseptic :- It means prevention of infection by inhibiting growth of bacteria .

Bacteriocidal agents :- They are those which are able to kill bacteria .

Bacteriostatic agents :- Only prevent multiplication of bacteria and they may remain alive .

Factors which effect sterilization

1- The number of microorganism which to be sterilized.
2- The temperature degree which used.
3- Time of sterilization , if the temperature increase the time is reduce and vice versa.
4- Types of microorganism.
e.g.:- a- Syphilis bacteria need 43c for 10min.

b- Hepatitis virus need 60c for 10min.

c- Clostridium need 100c for 10min.

5- The nature of the compound which have to be sterilized .

Sterilization methods

A- Physical methods **B- Chemical methods**

1- Sun light 2- Drying 3- Heat 4- Radiation 5- Filtration

A- Dry heat.	B- Moist heat
1- Red heat. below…..100c °	1- Temperature
2- Flaming. at…….100c	2- Temperature
3- Incineration. above…..100c	3- Temperature
4- Hot air oven .	4- Tendalization.

A) Physical methods :-

1) Sun light :- It is the bacteriocidal activity . The action is due to ultraviolet ray e.g. :- water in tanks , river and lacks .

2) Drying :- Drying in air has deleterious effect on bacteria .Spores are unaffecting by drying .

3) Heat :-

A. Dry heat :-

1. Red heat :- It used to sterilize metallic objectives by holding them in flam till they become red hot . e.g. :- inoculating wire loop , needl , forceps .

2. Flaming :- The article passed over flam without allowing it to be red hot . e.g. :- Mouth of culture tubes , cotton and wool plugs , glass slide and media in Petri- dish .

3.Incineration :- This is an excellent method for rapidly destroying infected material .

4. Hot air oven :- Sterilization by hot air oven requires temperature of 160c for one hour . We can sterilize all glass syringes , Petri-dishes , test tubes , flasks , pipettes , cotton swabs , scalpel , scissors , liquid paraffin , dusting powder, etc.

B. Moist heat :-

The lethal effect of moist heat is by denaturation and coagulation of protein .

1. **Temperature below 100c :-**
    a. Pasteurization of milk :- Temperature employed is either 65c for 30minutes (Holder method) or 72c for(15 – 20) seconds (Flash method) . Organism like Mycobacterium , Salmonella and Brucella are killed .
    b. Inspissations :- The slow solidification of serum or egg is carried out at 80c in an inspissator. e.g.:- serum slopes, Lowenstein jensen's medium .
2. **Temperature at 100c :-**

Boiling :- most of vegetation form of bacteria , fungi viruses are killed between(50 – 70)c in short time , therefore needle and instruments boiling in water at 100c between (10 – 20)min.

3. Temperature above 100c :- (by autoclave)

In this apparatus material used for sterilization are exposed to 121c for (15 – 20)m. at 15 pressure per square inch . This method can sterilized materials like culture media , cotton , surgical instruments , dressing ,syringes by steam under pressure , it is the best method for sterilizing , so vegetative bacteria and spore can be destroyed .

4.Tendalization :-

Some culture media which contain sugar and gelatin media cann't stand above high level of temperature , so the material is exposed to steam atmospheric pressure for 30min. on 3 successive days

1.First day : ……Steaming kill all vegetative form of bacteria

2. Second day ……..Steaming kill the germinated spore .

3. Thired day ……..Steaming kill all bacteria if it remained .

4) Radiation :-

A. Non ionizing radiation :-

1. Ultraviolet radiation :- It is chief bacteriocidal factor present in sun light. It causes following changes in cell .

a. Denaturation of protein .

b. Damage to DNA .

c. Inhibition of DNA replication .

d. Making bacterial and viral vaccines .

2. Infra red radiation :- Sterilizing large number of syringes in a short time

B. Ionizing radiation and x- ray :-

Ionizing radiation have grater capacity to induce lethal changes in DNA cell . They are useful for the sterilization of disposable material catgut , disposable syringes ,adhesive dressing etc.

5) Ultrasonic and sonic vibrations :- They are bacteriocidal causing mechanical agitation and rupture of bacteria .

6) Filtration :- It is a method of sterilization useful for sterilizing liquids which damage by heat such as antibiotic solutions, sera, carbohydrate solution . e.g. :- Asbestos disc .

B) Chemical methods :- antiseptic

1. Acids and alkalines :- They are inhibit the bacterial growth . Boric acid is weak antiseptic

2.Halogens :- Iodine is used chiefly for skin .Chlorine combines with water to form hypochloric acid which is bactericidal effects .

3. **Oxidizing agents :-** They are weak antiseptic e.g. :- potassium permanganate .

4. **Formaldehyde :-** It is useful in sterilizing bacterial vaccine . 5 -10% solution in water kills many bacteria . bacteriocidal , sporicidal and lethal to viruses also .
5. **Phenol :-** It is used for sterilizing surgical instruments . It is generally used in 3% solution .
6. **Alcohol :-** Ethyl alcohol is most effective in 70% solution but it doesn't kill spores .
7. **Dyes :-** Gentian violet and malachite green etc. are active against Gram positive bacteria .
8. **Soap and detergents :-** They are bacteriocidal and bacteristatic for Gram positive and some acid fast organism .

Culture media

Culture media gives artificial environment simulating natural conditions necessary for growth of bacteria .

The basic requirement of culture media are :-

1. Energy source .

2. Carbon source .

3. Nitrogen source .

4. Salts like sulphates , phosphates , chlorides carbonates of sodiume , potassume , magnesiume , calisum and copper .

5. Satisfactory pH(7.2 – 7.6) .

6. Adequate oxidation –reduction potential .

7. Growth factor like tryptophan for Staphylococcus typhi , glutathione for gonococci X and V factors for haemophilus .

Media used for obtaining the growth of bacteria are :-

A. **Fluid media :-**They are used as enrichment media before plating on solid media

They are not suitable for the isolation of organism in pure culture . We can't study colony character as well .

Example of fluid media are nutrient broth , peptone water .

Types of liquid media :-

1. Broth . 2. Infusion broth . 3. Digest broth . 4. Meat extract broth . 5. Peptone . 6. Yeast extract .

B. **Solid media :-** They are used to study colonies of individual bacteria . They are essential for isolation of organism in pure form .

Agar :-

It is important constituent of solid media . It is complex polysaccharide obtained from sea plants (marine algae) . It melt at(80-100)c and solidifies at (35-42)c . It doesn't provided any nutrition to the bacteria . It acts only as solidifying agent . It is not metabolized by any pathogenic bacteria . Agar used in form of Slopes , Plates and deep agar tubes .

C. Semi – solid media :- It is between solid media and liquid media , the concentration of agar (0.2- 0.5)% .It is used for study the motility of bacteria .e.g. :- Gelatin media .

Gelatin :-

It is protein prepared by hydrolysis of collagen with boiling water. It melts at 37c. It forms transparent gel below 25c . The main use of gelatin is to test the ability of bacteria to liquefy it. This feature is important for the identification and classification of bacteria .

Classification of media

A. Classify on the base of physical proparties :-

1. Solid media .
2. Liquid media .
3. Semi – solid media .

B. Classify on the base of consisting :-

1. **Simple media (basal media) :-**
    a. Nutrient broth :- This medium is uses for the study of the common pathogenic bacteria . It is composed of peptone water , meat extract and sodium chloride .

b. Nutrient agar :- This media is prepared from nutrient broth + agar , also support the growth of many common pathogenic bacteria .

2. **Enrich media :-** Many substances if added to basal media for the Fulfillment of the growth of some microorganisms . Blood serum
is addid to basal media , so it known as enrich media.

a,g,:- Blood agar .

3. Selective media :- It is the media which contain chemical substances which inhibits growth of most of microorganism.

e.g. :- Lowen stain Jensen media .

malachite green …….inhibits the growth of bacteria

other than Mycobacteria tuberculosus .

e.g. :- (S.S.A.) Salmonella – shigella agar .

4. Differential media :- Certain reagents or indicator substances when added to culture media may allow differentiation of various kinds of bacteria .

e.g. 1. Blood agar , it make differential between haemolytic and

non – haemolytic bacteria (10% of animal or human body blood)

e.g. 2. MacConkey agar :- Lactose fermenter colonies , rose pink

(E.coli) ,and non lactose fermenter colonies , paie yellow

(Salmonella) .

e.g. 3. Chocolate agar :- Heated blood agar for isolation

many bacteria like Neisseria .

Pure Culture Technique

Pure culture :-

A single kind of microorganism grow in protected environment .

In order to study any kind of bacteria we must be isolated as pure calture .

Methods of isolating pure culture

1. **Streak – plate method :-**
   This method is used for isolation culture from urine ,stoole ,and pus ,by means of transfer loop , a part of the mixed culture is placed on the surface of agar media and streaked across the surface . This technique thin out the bacteria on the agar so ,colonies of bacteria are separated from each other (isolated colonies) after incubated at 37c about 24 hours.

   A colony from a single streak plate does not sure pure , so it is important to streak two or more plate .

2- Pure – plate method :-

In this method , the mixed culture is first diluted to provide only few cells per milliliter before being used to inoculate media .

Culture is diluted in tubes of liquid agar media , this media is mentained in liquid state in 45c to allow distribution of inoculum .

The inoculum's media is dispensed in to Petri – dish allow to solidify and then incubated . Both surface and subsurface colonies develop .

Staining methods

Bacteria are so transparent in living condition , therefore it is necessary to

Stain with dyes to be visible inorder to identify and classify them .

Bacterial stain dividing in to two groups :-

1. **Positive stain . (+ ve) .**
2. **Negative. (- ve) .**

(1) Positive stain

A) Simple stain B) Differential stain C) Special stain

Basic Acidic

Stain stain

(1) Gram's stain (2) Ziehl –Neelsen

Gram's (- ve) Gram's (+ve) Asid fast stain

A) **/Simple stain :-** stain method which used one stain only to stain smear uniformly .
e.g. :- Methylen blue .

Safranine .

Crystal violat .

Simple stain divided in to :-

1. Basic stains .
2. Acidic stains .

Basic stain :- It is important and more active to stain bacteria because the nuclic acid of bacteria contains negative charged phosphate group (H3Po4) which combine with positive basic dye .

1. **Acidic stain :-** It is important when it used alone because it will stain the back ground of the smear of bacteria and left bacteria without stain .

B) **/Differential stain :-** It is the staining procedure that make visible the differences between bacterial cell . This stain method contains more than one stain which at last differential the bacteria cell .

1//Gram's stain :-

Is one of the most important and widely used differential staining technique in microbiology . This technique was introduced by Christian Gram in 1889 using two dyes in sequence , each of them different colour. He found bacteria in two different categories :-

A) **Gram – positive bacteria :-**
Deep violet in colour , that retained the first dye (crystal violat) throughout the staining procedure .

B) **Gram negative bacteria :-**
Res in colour , that lost the first dye (crystal violet) after washing with a decolorizing solution and staind with the second dye (safranin) .

Principle theory of gram stain method –

1. Differences in the thickness of cell wall between the groups of bacteria .Gram negative (G-ve) generally thinner than those of Gram positive (G+) bacteria .
2. G-ve bacteria contain a higher% of lipid than G+ve bacteria .
3. G+ve bacteria a higher% of mucopeptide than G-ve .
4. Permeability differences between the two groups of bacteria .
5. G+ve bacteria are usually more susceptible to penicillin .

2// Ziehl – Neelsen stain or (Acid fast stain)

This method divided bacteria in to :-

- Acid fast bacteria (red colour)
- Non acid fast bacteria (blue)

Acid fast bacteria are difficult to be stained by ordinary stain , because the high % of wax in cell wall so it require a strong basic stain (carbol fuchsin) which resist decolorization by strong acid solution so Mycobacterium tuberculosis which staind by this method known as acid fast bacilli .

Ziehl – Neelsen stain cosist of :-

a. Basic stain……Carbol fuchsin .
b. Decolorize……Acidic alcohol .
c. Counter stain ……Methylene blue .

C)/ Special stains :-

There are many kind of special stain for bacteria and parasites such as malaria parasite , spirocheate , bacteria , also ther are special stain for :-

1. Capsule stain ……Giemsa stain .
2. Spores stain ……...Fontana stain .
3. Flaggela stain……..Moller .

(2) Negative stain

Stain the back-ground and leave bacteria without stain (unstained)