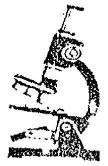
BACTERIA

> *"Tell me no tales*
> *Read me no rhymes*
> *Don't waste my time*
> *Show me"*
>
> \- MY FAIR LADY



INTRODUCTION

1)Bacteria are unicellular microscopic prokaryotic organism lacking chlorophyll.

2)Habitat - Universal occurrence i.e the bacteria live in all possible environment such as water, air, soil, inside plants and animals, on dead organic matters.

3)Size

0.5 micron to 3 microns

CLASSIFICATION OF BACTERIA

a. On the basis of morphology

According to morphology the bacteria can be classified into six groups.

I. True bacteria
II. Actinomyces
III. Spirochetes
IV. Mycoplasma
V. Rickettsia
VI. Chlamydia

I. True bacteria

These microorganisms are free living in nature. They have rigid cell wall. They are unbranched. Based on their shape they are further classified into

- Cocci
- Bacilli

• Cocci

These are spherical or oval shaped bacteria. Many are pathogenic, causing such diseases as sore throat, scarlet fever, rheumatic fever, pneumonia, gonorrhea, meningitis etc.

On the basis of arrangement they are classified into

1. Diplococci
2. Streptococci
3. Staphylococci
4. Sarcina

1 Diplococci

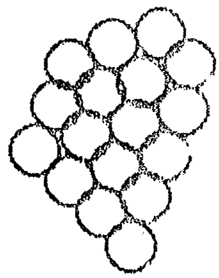
A pair of bacterial cells is facing each other.
e.g Neisseria gonorrhoeae.

2 Streptococci

Bacteria are arranged in a chain form.
e.g Streptococcus pyogens, Streptococcus viridans.

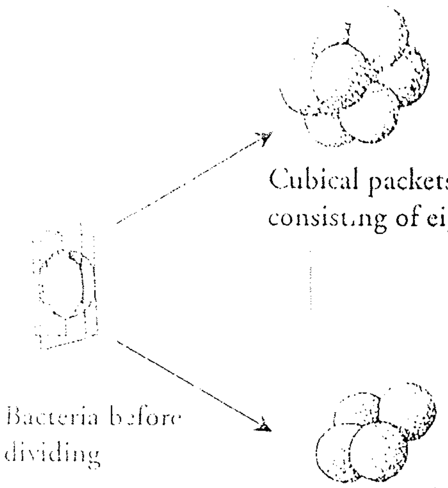
3 Staphylococci

Bacteria are arranged in clusters.
e.g Staphylococcus aureus, Staphylococcus albus.



4 Sarcina

A genus of spherical saprophytic bacteria of the family Micrococcaceae. The individual organisms remain adherent to each other after splitting in two or three perpendicular directions. This process yields square tetrads or cubical packets consisting of eight cells
e.g Micrococcus tetragena.



Bacilli

Bacilli are cylindrical or rod shaped bacteria, sometimes occurring in chains. They are spore bearing, aerobic, motile or non motile; most of them are Gram positive.

On the basis of arrangement they are further divided into

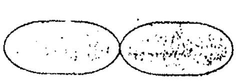
1. Monobacillius
2. Diplobacillus
3. Streptobacillus
4. Coccobacillus
5. V shaped pattern
6. Coma shaped bacilli
7. Spirilla

1. Monobacillus

It is a single rod shaped cell freely present in nature.

2. Diplobacillus

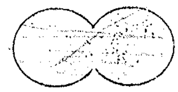
A pair of rod shaped cells are adhered and facing each other.



3. Streptobacillus

The rod shaped cells are attached in chain form.

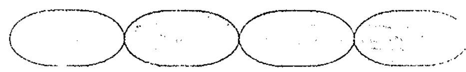
e.g Streptococcus bacillus moniliformis.



4. Coccobacillus

The rod shaped cells are looking like cocci and are called coccobacilli.

e.g Brucella.



5. 'V' shaped pattern

The rod shaped cells are arranged at an angles to one another.

e.g Corney bacterium.

6. Coma shaped bacilli

The rod shaped cells become coma shaped, curved appearance is due to the presence of a single flagellum at one end.

e.g Vibrio cholera.

7. Spirilla

The rod shaped bacteria have several spirals.

e.g Spirillum minus.

II. Actinomyces

A genus of bacteria of the family actinomycetaceae that contains Gram positive filaments. These bacteria resembles fungi.

III. Spirochetes

These are non branched, longer, slender and spiral shape bacteria having several coils.

IV. Mycoplasmas

These bacteria do not possess a stable morphology due to lack of rigid cell wall. They are very small in size i.e 50 to 300 nm in diameter.

V. Rickettsiae

These microorganisms are the intermediate between viruses and bacteria. They differ from bacteria in that they are obligate parasites requiring living cells for growth and differ from viruses in that they are filterable.

VI. Chlamydia

Chlamydia are small, non motile, Gram negative obligate parasites. They were previously considered as viruses. But they differ from viruses in several properties such as

- Multiplication by binary fission.
- They have rigid cell wall.
- They have both DNA and RNA.
- They contain metabolically active bacterial enzymes.
- They are more susceptible to antibiotics.

So that now it is accepted as small bacteria that have evolved as obligate intra cellular parasites.

b. On the basis of staining

❶ Gram positive organisms

It shows violet colour after staining. e.g Actinomyces and all cocci except Neisseria.

❷ Gram negative organisms

It shows pink colour after staining. e.g All bacilli are Gram negative except corynebacterium, mycobacterium, clostridium.

❸ Acid fast organisms

It shows red colour after staining. e.g mycobacterium tubercle and mycobacterium leprae.

STRUCTURE OF A BACTERIAL CELL

The bacteria cell consists of

1. Capsule
2. Cell wall
3. Cytoplasmic membrane
4. Cytoplasm
5. Ribosomes
6. Mesosomes
7. Nucleoid
8. Flagella
9. Fimbriae or pili

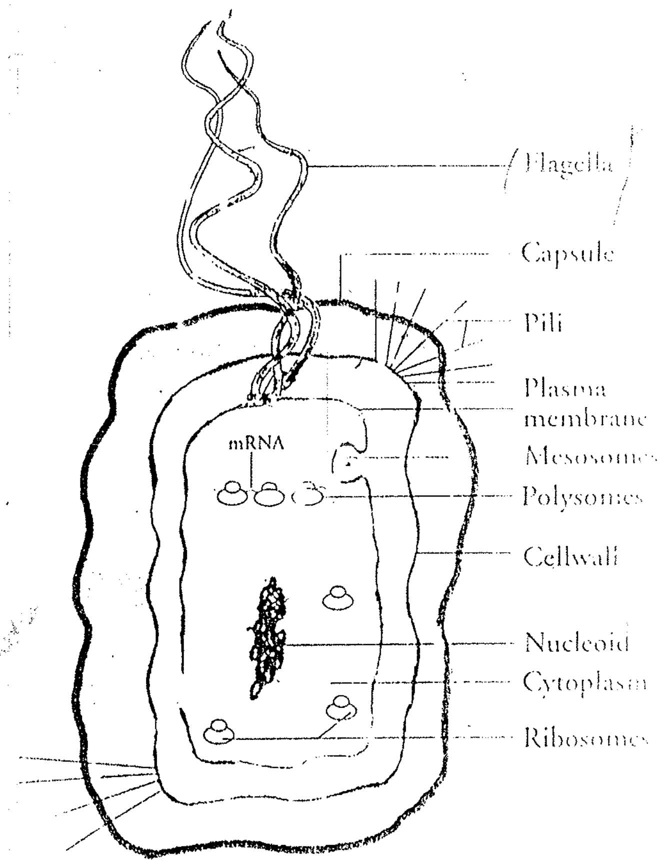


Fig 2.1 Structure of a Bacteria

Spirilla Spirilla CMC

CAPSULE

a. Bacteria synthesize loose amorphous organic exopolymer which is deposited outside and tightly attached to the cellwall called capsules.

b. Generally the cellwall is made up of exopolysaccharides and exopolypeptides.

For e.g.

Bacillus anthracis and Bacillusmegaterium contain exopolypeptides whereas Acetobacter xylinum, Agarobacterium tumefaciens, Streptococcus species and Pseudomonas species contain exopolysaccharides. The composition of exopolymer varies with bacteria.

c. Some times the capsular material is loosely associated with the bacterium, it can be easily washed away. The loose layer is called a **slime** layer.

d. Capsulated bacteria produces **smooth** colonies and non capsulated bacteria produces rough colonies. But in some Gram negative bacteria does not having capsule. But produces smooth colonies. This is due to the presence of outer membrane in the organisms.

Functions

1. They protect the cell from drying.
2. They protect the bacterial cell against antibacterial agents and phages.

CELL WALL

a. It is rough inelastic membrane which maintains the shape of bacteria.

b. The cell wall is formed of mucopeptides called peptidoglycan.

c. The peptidoglycans are present more in Gram positive bacteria when compared to Gram negative bacteria.

Cell wall nature of Gram positive bacteria

The cell wall of Gram positive bacteria is mainly composed of several layers of peptido glycans. The thickness of peptido glycan layer is more in Gram positive bacteria (16-80 nm) when compared to Gram negative bacteria (2nm).

The peptidoglycan layer consist alternating units of N - acetyl glucosamine and N-acetylmuramic acid. The two units are linked by peptide chains.

In addition to peptidoglycan layer theicholic acids are found on cellwall and plasma membranes.

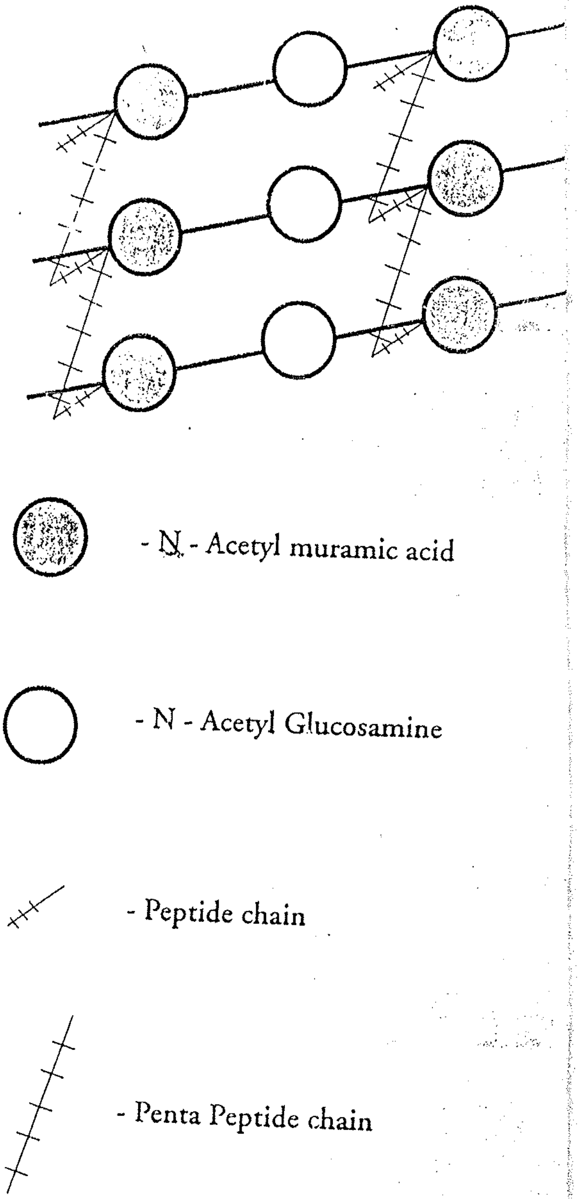
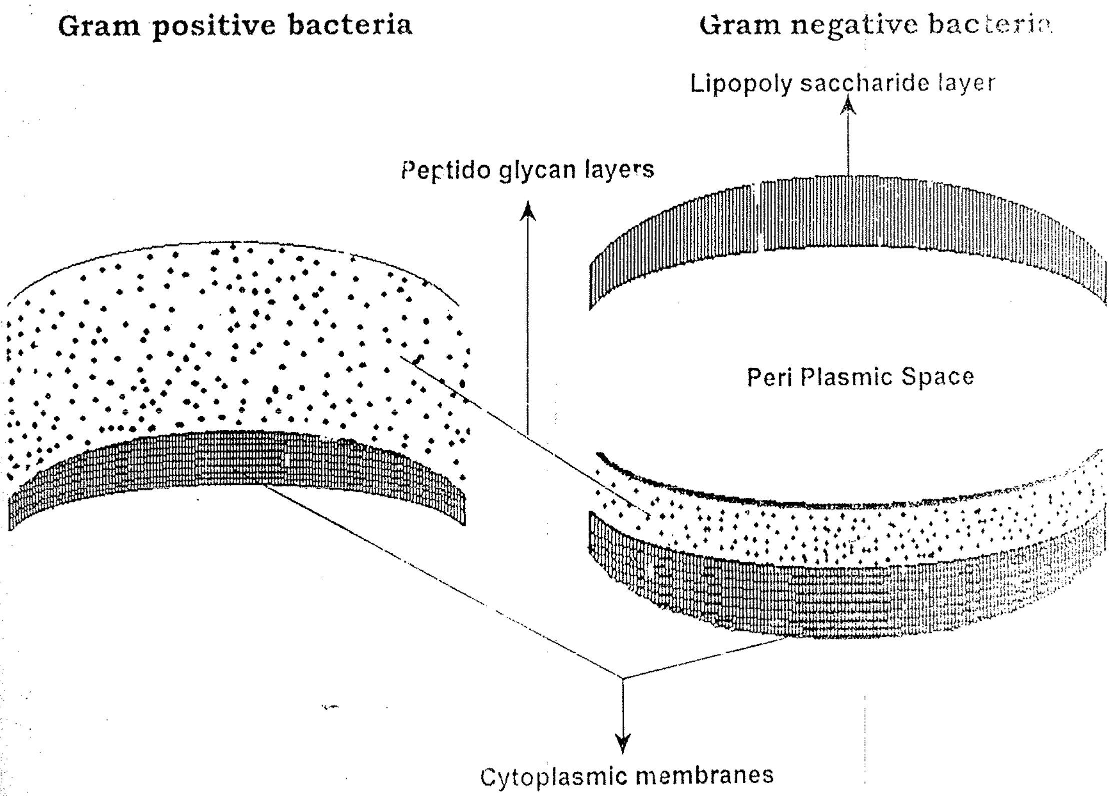


Fig 2.2 Cross section of Gram positive cellwall

COMPARISON OF GRAM POSITIVE AND GRAM NEGATIVE CELL WALL



Component	Gram positive	Gram negative	
		Inner layer	Outer layer
Peptidoglycan	+	+	–
Teichoic acid	+	–	-
Polysacaccharides	+	–	–
Proteins	+	–	+
Lipoproteins	–	+ or -	+
Lipopolysaccharides	–	–	+
Thickness	Thicker	Thinner	
Aromatic sulphur containing amino acid	Absent	Present	
Periplasmic space	Absent	Present	

Table 2.1 Gram positive and Gram negative cellwalls are compared

There are two types of teichoic acid.

- Ribitol teichoic acid.
- Glycerol teichoic acid.

The cell wall associated protein protects the bacteria from phagocytosis.

For e.g. Streptococcal M Protein, Staphylococcal A Protein.

Teichoic acids and cell wall associated proteins are the major surface antigens of Gram positive organisms.

Cell wall nature of Gram negative bacteria

In Gram negative bacteria the cell wall is made up of two distinct layers namely

- Inner layer
- Outer layer

The outer layer contains proteins, lipoproteins and lipopolysaccharides.

The lipopolysaccharides (LPS) forms the major component of the Gram negative bacteria.

The lipopolysaccharides contain subunits and each subunit consists of

1. O - antigen
2. Core
3. Lipid - A

O - Antigen ⟶ Core
↓
Lipid A

The core can be further subdivided into two portions, known as the inner and outer cores.

The O - antigen is also known as somatic antigen. It is composed of a series of repeating units of linear trisaccharides or branched tetra or pentasaccharides. Each Gram negative bacterium has a characteristic O-antigen that can be used as epidemiological tool and helpful to identify the enteric bacteria such as Salmonella or E.coli.

Lipid-A is a glycophospholipid composed of a chain of D - glucosamine units. The glucosamine units are connected with long chain fatty acid(β - hydroxymyristic acid).

The subunits are linked through pyrophosphates with the lipid region.

The lipopolysaccharides are the major antigenic determinants and also the receptors for adsorption of many bacteriophages.

The inner layer contains very little amount of peptidoglycan.

Gram negative bacteria are less sensitive to antibiotics, dyes and bile salts than Gram positive bacteria because the outer layer acts as barrier. But treatment with chelating agents such as EDTA, which causes the release of most of the lipopolysaccharide makes the cells more sensitive to drugs and chemicals.

Periplasmic space

It is the space present in between the inner layer and outer layer of cell wall. It contains most proteins, oligosaccharide which play an important role in osmoregulation of the cells.

The periplasmic space protects the Gram negative bacteria from the action of certain antibiotics. This is due to the presence of enzymes. For e.g. Gram negative bacteria protect themselves from the lytic action of β - lactam antibiotics such as penicillins and cephalosporins by accumulating β - lactamase enzyme in their periplasm.

Functions of cell wall

1. To prevent the rupture of bacteria caused by osmotic pressure differences in between intracellular and extra cellular environments.
2. To provide a solid support for flagella.
3. To maintain the characteristic shape of the microorganisms.
4. To regulate a certain degree of passage of molecules into and out of the cell.
5. To serve as the sites of attachment for most bacterial viruses.

PLASMA MEMB
LL MEMBRANE
TOPLASMIC ME

Plasma membr
nbrane of cytoplasr
kness.

The trilaminar
posed of proteins (60
lipids (20-30%), an
ccharides. Sterols ar
major lipids are

- Phosphatid
- Phosphatidyl
- Phosphatidyl

The phospholipi
r head region is loca
ophobic fatty acid c
re, and proteins are
spholipid bilayer.

Intercalated

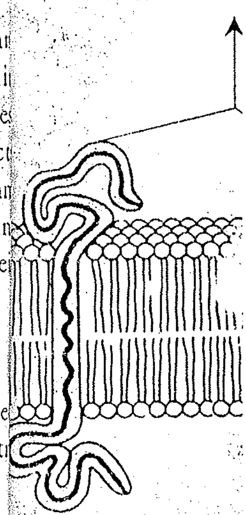


Fig 2.3 Cr
plasma

ctions

The principle f
nbrane are

Forms a protecti
lity and shape.

PLASMA MEMBRANE OR CELL MEMBRANE OR CYTOPLASMIC MEMBRANE

a. Plasma membrane is the limiting membrane of cytoplasm having 5 to 10 nm thickness.

b. The trilaminar cytoplasmic membrane composed of proteins (60-70%), lipids and phospholipids (20-30%), and small amount of oligosaccharides. Sterols are totally absent.

The major lipids are

- Phosphatidyl ethanol amine.
- Phosphatidyl serine.
- Phosphatidyl choline.

c. The phospholipids form a bilayer with the polar head region is located on the surface while hydrophobic fatty acid chains are located at the centre, and proteins are intercalated within the phospholipid bilayer.

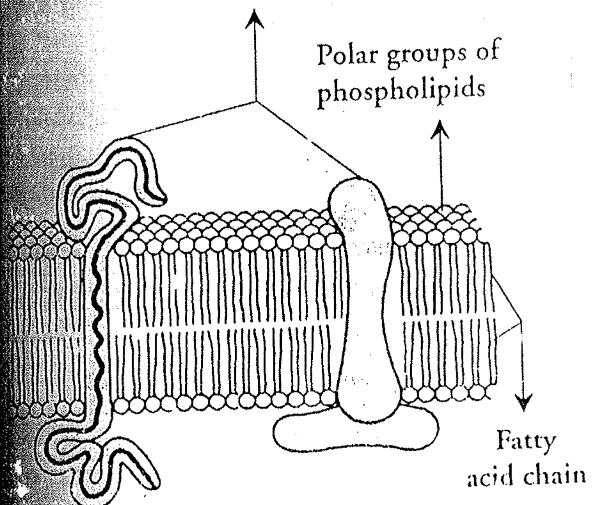


Fig 2.3 Cross section of plasma membrane

Functions

The principle functions of the plasma membrane are

1. Forms a protective covering, provides rigidity and shape.
2. It behaves as selective permeable barrier responsible for the transport of chemicals and nutrients inside and outside the cell.
3. It acts as an osmotic barrier.
4. It contains the components of the energy generation system.

It also contains cytochrome oxidase, enzymes of tricarboxylic acid cycle and polymerizing enzyme necessary for cell wall synthesis.

5. Secretion

It is involved in the release of extra cellular proteins that include various toxins, bacteriocins and enzymes produced in the cytoplasm of the cell. These products are useful and important for the survival of bacteria.

CYTOPLASM

The cytoplasm is colloidal in nature. It consists of proteins, carbohydrates, fats, minerals, enzymes, inclusions and vacuoles. It is the site of metabolic activity in bacteria. It lacks mitochondria.

RIBOSOMES

Ribosomes are the centres of protein synthesis. They are slightly smaller than eukaryotic ribosomes. The sedimentation constant is 70s. This 70s ribosomes are made up of two subunits namely a large subunit 50s and a small subunit 30s.

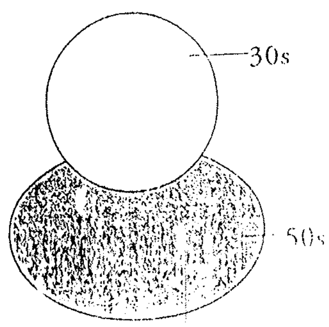


Fig 2.4 Structure of ribosome

The letter "S" refers to Svedberg unit which indicates the relative rate of sedimentation during ultra centrifugation. The sedimentation rate is greatly influenced by size, shape and weight of the particles.

During active protein synthesis the ribosomes are associated with mRNA and such associations are called **polysomes**.

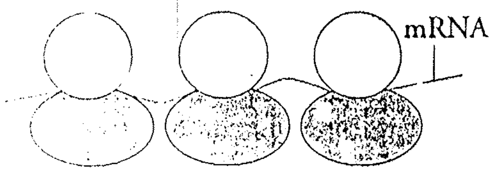


Fig 2.5 Structure of polysome

❸ MESOSOMES

Mesosomes are the respiratory sites of bacteria. These are intra cytoplasmic structure. They are vesicular, convoluted, multi laminated structure formed as invaginations of the plasma membrane into the cytoplasm. They are predominant in Gram positive bacteria.

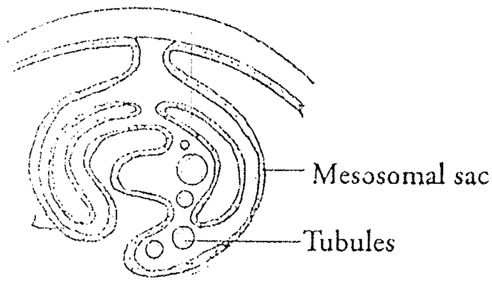


Fig 2.6 Structure of mesosome

❹ NUCLEOID

The bacterial chromosome is not surrounded by nuclear membrane so it is called nucleoid. The bacterial chromosome is made up of a single double strand circular DNA.

❺ FLAGELLA

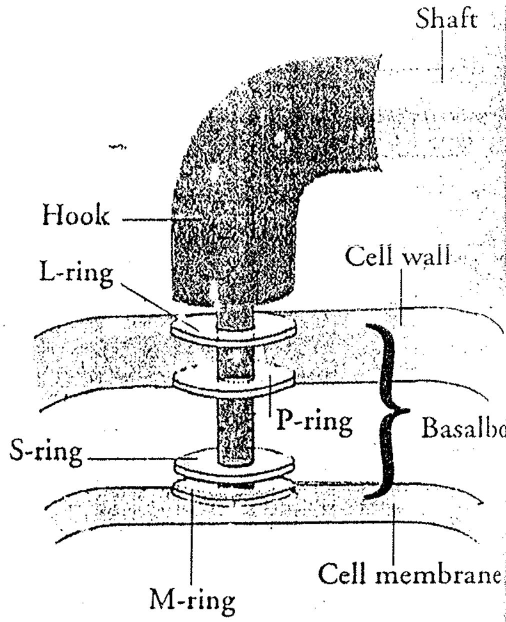
Bacteria possess one or more unbranched appendages called flagella. flagella are made up of protein called flag... They are used for locomotion.

Each flagellum consists of three compon... namely

- Basal body
- Hook
- Shaft

Basal body consists of small central ... which is inserted into a system of rings. T... are four rings namely

1. M ring (Membrane ring)
2. S ring (Super membrane ring)
3. P ring (Peptidoglycan ring)
4. L ring (Lipopolysaccharide ring)



Fit 2.7 Flagellum and its parts

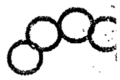
In Gram negative bacteria the basal b... bears two pairs of ring. The outer pair L an... rings are situated at the level of outer layer ... cell wall. The inner pair S and M rings are situ... near the level of the cell membrane.

In Gram posi... pair S and M rings ar... and L rings are not ... as it passes through... homogenous cell wal...

...rrangements of F...

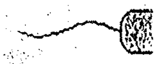
❶ *Atrichate*

Absence of f...
...g. Streptococcus.



Monotrichate

Single termi... ...ole of bacteria.
...g. V.cholerae, Pseud...



Amphitrichate

Possessing a tu...
...g. Aquaspirillum se...

Lophotrichate

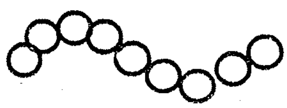
Possessing a tu...
...g. Pseudomonas flu...

In Gram positive bacteria only the lower pair S and M rings are visible. The upper pair P and L rings are not required to support the rod as it passes through the relatively thick and homogenous cell wall.

Arrangements of Flagella

1 *Atrichate*

Absence of flagella.
e.g. Streptococcus.

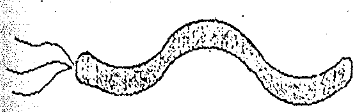


2 *Monotrichate*

Single terminal flagellum present at one pole of bacteria.
e.g. V.cholerae, Pseudomonas aeruginosa.

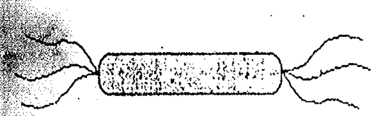
3 *Amphitrichate*

Possessing a tuft of flagella at either pole.
e.g. Aquaspirillum serpens.



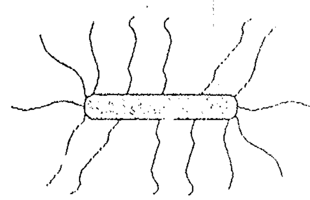
4 *Lophotrichate*

Possessing a tuft of flagella at both poles.
e.g. Pseudomonas fluorescens.



5 *Peritrichate*

Flagella arranged all round the bacterial cell.
e.g Salmonella typhi, E-coli, Cl.tetani



9 FIMBRIAE (PILI)

Some Gram negative bacilli carry very fine hair like surface appendages called fimbriae or **pili**. They are shorter and thinner than flagella. They are about 0.5nm long and less than 10 nm thickness. They project from the cell wall as straight chain.

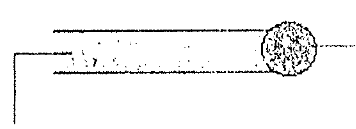
They are made up of protein sub units called pilin or fimbrin.

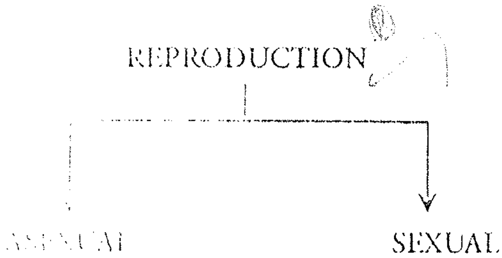
There are two types of pili namely

- Common pili
- Sex pili

Common pili, which are also called somatic pili, are thinner than sex pili. The important function of common pili is to serve as adhesins, and responsible for adhering to the surfaces of other cells.

Sex pili are hair like structure present on the surface of bacteria. They are thicker, longer than common pili. They have an axial hole and a knob at terminal end.





ASEXUAL REPRODUCTION

Binary fission

Binary fission refers to the division of one parent cell into two daughter cells. It is equal in size as parent cell.

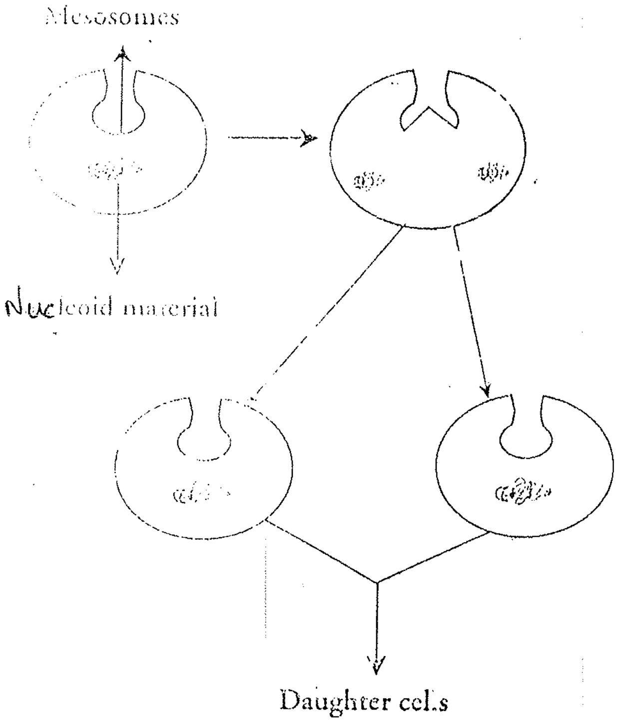
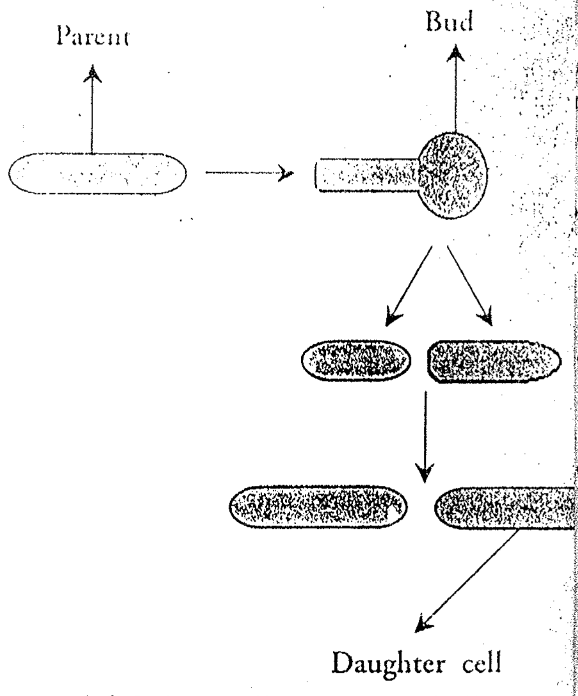


Fig 2.8 Schematic representation of binary fission

Mesosomes of bacteria initiate the binary fission. The mesosomes increase in its size and a septum is formed. First the nuclear material divides into two, followed by the cytoplasm also divides into two. Now two daughter cells are formed as parent cell.

✷ Budding

Bacteria develops a small protuberance called bud at one end of the cell. It increases in size and separates into a daughter cell.
e.g. Rhodopseudomonas.



2.9 Schematic representation of budding

✷ Fragmentation

In filamentous bacteria, the filament break into fragments and each fragment grows into daughter filaments.
e.g. Nocardia.

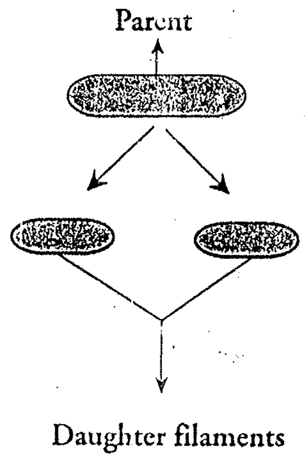


Fig 2.10 Schematic representation of fragmentation

SEXUAL RE
Most bacteria
ne bacteria reprodu
Sexual reprodu
directional passage
nor cell to recipient c
ombination.

nor
Those bacteria
terial are called don

cipient
Those bacteria
terial are called reci
ere are three metho
✷ Transforma
✷ Conjugatic
✷ Transductic

Transformatic
Transformation
genetic material fro
eous medium.

ere are two fundam
The purified dono
nsported across the
ipient.
The DNA then ur
h recipient DNA a

Frederick G
tion in 1928, who
eumonia strains. T
eumococcus bacteri
1.Virulent str
2.Avirulent str

The virulent
oduces smooth col
ain produces rough
capsule. The two str
perty.

SEXUAL REPRODUCTION

Most bacteria reproduce asexually but some bacteria reproduce sexually.

Sexual reproduction can be defined as unidirectional passage of genetic material from donor cell to recipient cell. This is otherwise called recombination.

Donor

Those bacteria which donate the genetic material are called donor.

Recipient

Those bacteria, which receive the genetic material are called recipient.

There are three methods

* Transformation
* Conjugation
* Transduction

* Transformation

Transformation process means the transfer of genetic material from donor to recipient in an aqueous medium.

There are two fundamental processes namely

* The purified donor DNA is first transported across the cell membrane into the recipient.
* The DNA then under goes recombination with recipient DNA and then it is expressed.

Frederick Griffith explained the transformation in 1928, who worked in pseudomonas pneumonia strains. There are two strains of pneumococcus bacteria namely

1. Virulent strain
2. Avirulent strain

The virulent strain has capsule, which produces smooth colonies. But the avirulent strain produces rough colonies due to the absence of capsule. The two strains differ in their antigenic property.

Griffith chose numerous mice for his experiment. He carried out the experiment in such a way by separating the mice into four groups. They are

Group A – All mice were injected with virulent strain.

Group B – All mice were injected with avirulent strain.

Group C – All mice were injected with heat killed virulent strain.

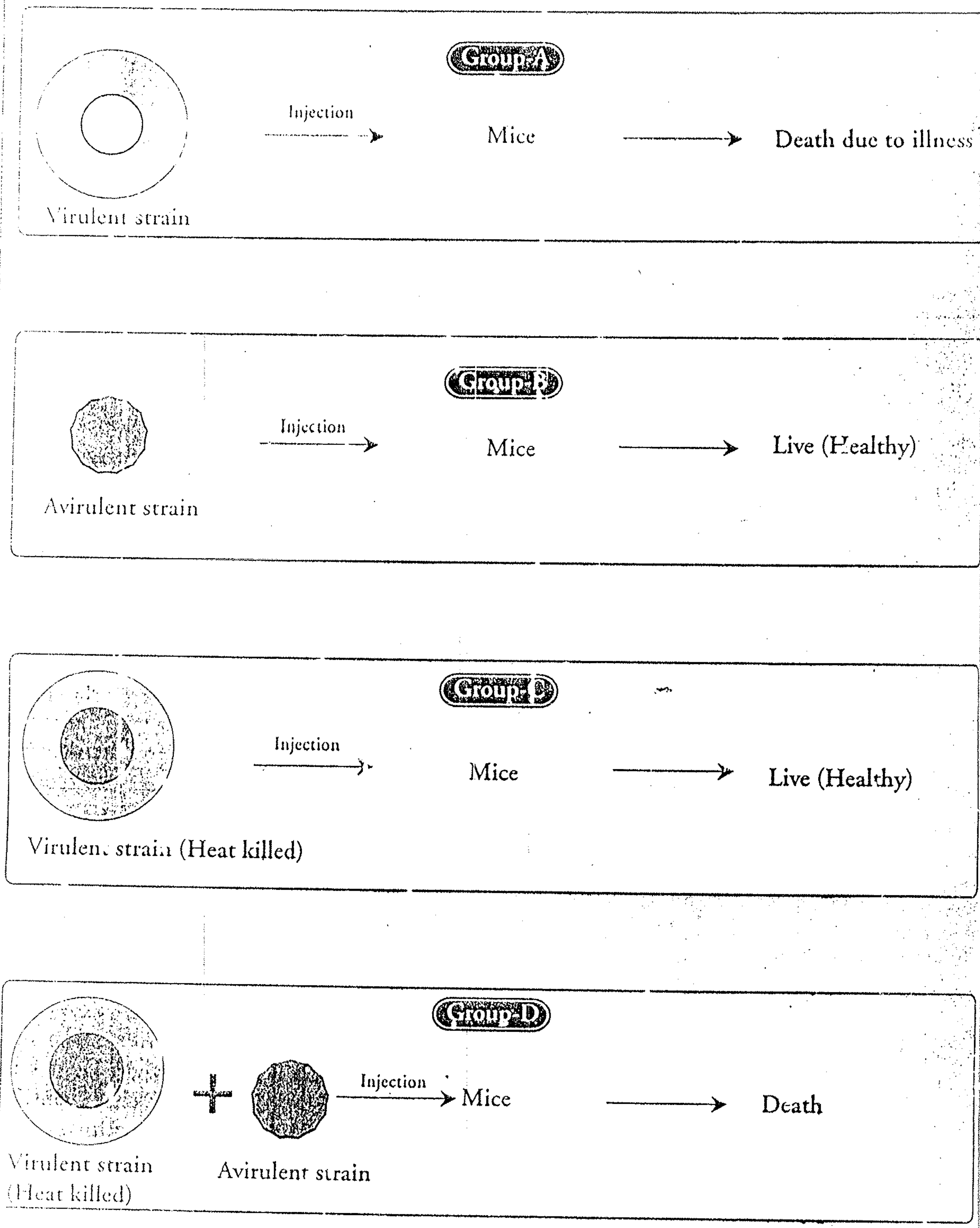
Group D – All mice were injected with the combination of heat killed virulent strain and avirulent strain.

After injection the mice were observed as follows

Groups	Strains	Animal	Observation
A	Virulent	Mice	Death due to illness
B	Avirulent	Mice	Live (healthy)
C	Heat killed virulen	Mice	Live (healthy)
D	Combination of heat killed virulent and avirulen	Mice	Death due to illness

Table 2.2 Represents Griffith's experiment

From the above observation some substances from heat killed virulent strain apparently transferred to the avirulent strain. This phenomenon is known as Griffith effect or **Bacterial transformation.** Later in 1944, O.T.Avery, C.M.MacLeod, and M. McCarty purified the pneumococcal transforming principle and proved that the DNA as genetic material.



Schematic representation of Griffith experiment

• CONJUGAT

Conjugatic
of genetic material
(recipient) througl

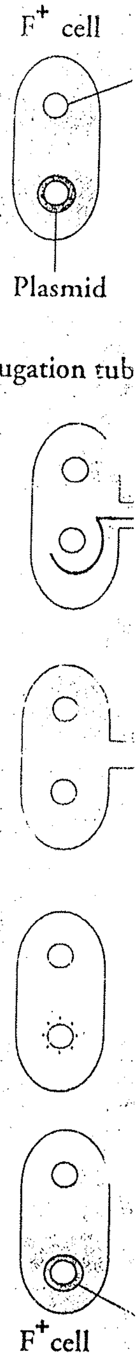


*Fig 2.11 Schem
of co*

✱ CONJUGATION

Conjugation may be defined as transfer of genetic material from male (donor) to female (recipient) through a conjugation tube.

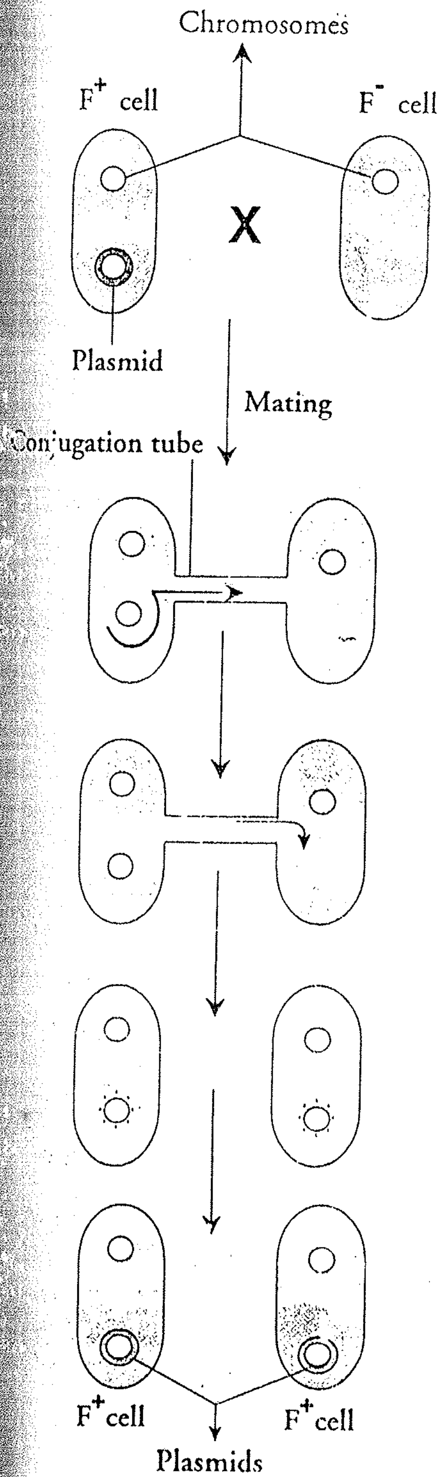


Fig 2.11 Schematic representation of conjugation

In 1946 Lederberg and E.L.Tatum found out a genetic exchange occurring between certain strains of E.coli. Some strains are designated as F^+ (Fertility plus)cells always acted as donors(males) and others designated as F (Fertility minus)cells always acted as recipients. It was found that F^+ cells contain plasmid whereas F^- cells do not have.

If F^+ cells are added to a culture of growing F^- cells, all of them rapidly become F^+ cells. The exact mechanism can be explained as

The male cells and female cells are attached by a conjugation tube. Now a nick is made on plasmid of F^+ cells.

So that one strand of plasmid is transferred to F^- cells through conjugation tube.

After transformation the single strand of DNA is present in both F^+ cells and F^- cells. The single strand DNA synthesizes a complementary strand and produces double strand circular plasmid. Thus F^- cells become F^+ cells.

✱ Transduction

The transfer of genetic material from one bacterium to another bacterium through bacteriophages is called transduction.

ENDOSPORE

The endospores are formed during unfavourable environmental conditions such as heat, cold, lack of water (or) depletion of essential nutrient in the environment. The endospores are formed in bacillus, clostridium and actinomyces species.

Endospores play a major role in the epidemiology of some diseases. Certain infections occur only when endospores are introduced into the wound site where they can germinate.
For e.g.

Cl.tetani spores are found every where in the soil, especially in feces contaminated soil. When tetanus spores are introduced into a deep wound that contains devitalized, anaerobic tissue, they germinate and the anaerobic cl.tetani multiply and release tetanus toxin, which leads to tetanus.

The shape of endospore may be spherical, ellipsoidal or cylindrical. The endospore contain a thick covering layer and central core. The covering layer is called **spore coat**. The central core is made up of nuclear body and spore cytoplasm. The cytoplasm contains DNA, RNA and proteins. The spore coat is made up of keratin like protein and impervious to antibiotics and anti bacterials. The spore coat consists of two layers, the outer layer is called exine or core wall and the inner layer is called **intine**. The central core is separated from the intine by a regular space called cortex. The spore cortex contains peptidoglycan, which is sensitive to lysozyme. It also contains Ca^{++}, dipicolinic acid. Some spores contains loose outer coating called exosporium. The exosporium contains lipoproteins and some carbohydrate residues.

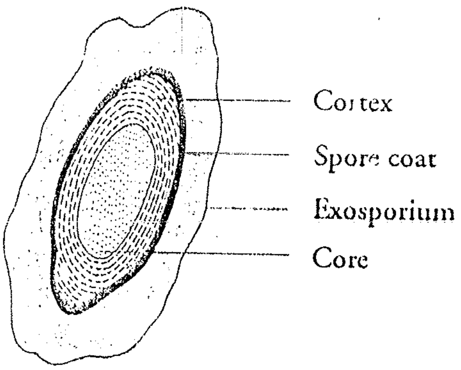
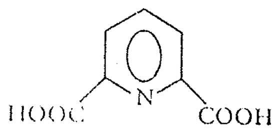


Fig 2.12 Ultra structure of endospore

The thermal resistance of endospores is caused by a variety of factors. Some of these are the presence of specific heat-resistance components, such as thermostable enzymes, high content of various minerals, particularly calcium and the presence of dipicolinic acid (DPA).

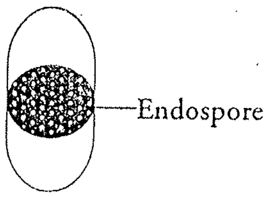


Types

- Equatorial or central
- Subterminal
- Terminal - Oval and Round

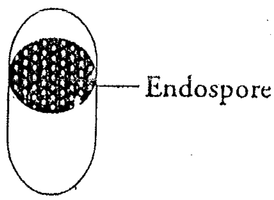
Equatorial or central

The endospore is situated at the centre of the bacterial body.
e.g. B.anthracis.



Subterminal

The endospore is situated in between centre and pole.
e.g. Cl.sporogenes, Cl.septicum

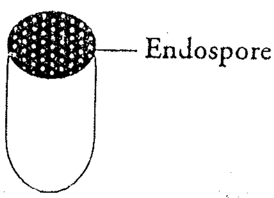


Terminal

The endospore is situated at the pole. According to the shape these are further grouped as

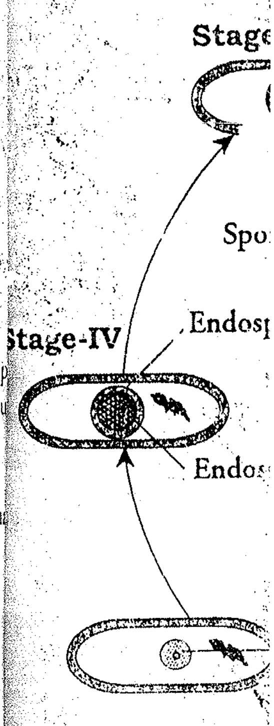
- **Round or drum stick endospore**

The round shaped endospore is situated at the pole.
e.g. Cl.tetani



- Oval endospores
The oval shape ... the pole.
e.g. Clostridium specie...

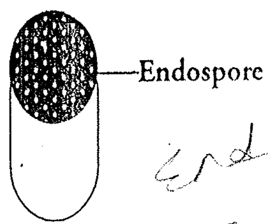
SPORULATION
The process o... called **sporulation or s...**



* Oval endospores or tennis rocket endospore

The oval shaped endospore is situated at the pole.

e.g. Clostridium species



SPORULATION

The process of endospore formation is called **sporulation or sporogenesis.**

It occurs normally when growth of b... due to lack of nutrition. Generally, s... process requires 10 hours. However, the time required for complete sporulation varies with the bacterial species and strains. During sporulation there are various stages recognized. They are

Stage I - *Formation of axial filament*

During this stage, the nuclear material undergoes structural changes to get axial filament.

Stage II - *Formation of septal double layer*

The plasma membrane is invaginated to form a septal wall. The septal wall initiates karyokinesis (division of nucleoid) followed by cytokinesis (division of cytoplasm).