ENTRI

STRUCTURE OF BACTERIAL CELL

Under microscope, it reveals several structural components outside and inside the cell wall

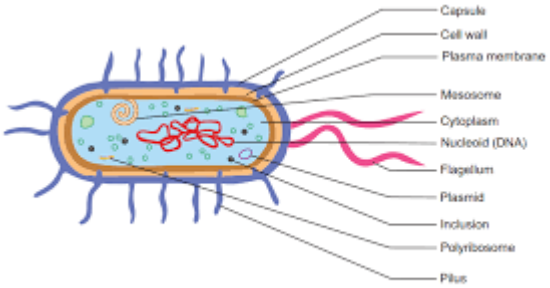


Figure 1.9: Ultrastructure of a bacterial cell

Capsule

It is the extra cellular polymeric substances (EPS) which are commonly called capsule or glycocalyx. It forms an envelope around the cell wall. Capsule is gelatinous polymer made up of either polysaccharides (it may be homopolysaccharides or heteropolysaccharides) or polypeptide or both.Capsule is species specific and so, can be used for immunological differentiation of related species.

Funtions of capsule

1. the capsule may prevent attachment of bacteriophages.

2. it protects the bacterial cells against desiccation, prevent movement of nutrients from bacterial cell.

3. they may inhibit the engulfment by WBC and contribute virulence

4. some species use its capsule as a source of energy

Flagella

The flagellum is hair like, helical and surface appendages emerging from the cell wall. It is of 20-30 nm in diameter and 4 -5 µm long and made up of protein called flagellin. The flagella of prokaryotes are several time thinner than eukaryotes. Also number and position of flagellum may vary. It provide locomotion.

- Bacteria lacks flagella is called atrichous.
- Bacteria with single flagellum at one end of the cell is called monotrichous. Eg ,*cholera vibrio*
- Bacteria with 2 or more flagella at one end or both end is called lophotrichous. Eg , *sprillum undula*
- Bacteria with many flagella at end of the cell called amphitrichous. Eg ,*spirilla*

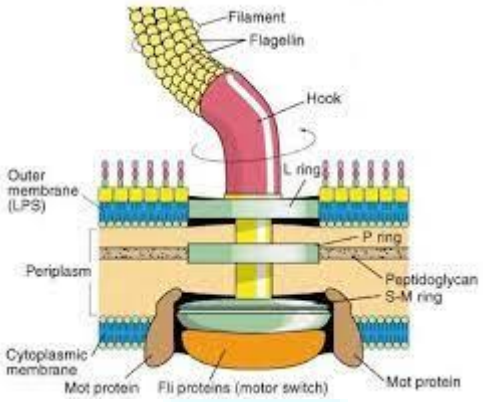
- Bacteria with many flagella attached all around body is called petrichous. Eg *salmonella sp*

Structure of flagella: consist of three parts- basic body,hook and filament

Basal body : attaches the flagellum to the cell wall and plasma membrane. It composed of small central rod inserted into a series of rings.

Hook : hook is present outside the cell wall and connects filament to basalbody. It consist of proteins.

Filament : outermost long long region of flagellum made up of globular proteins



Pili and fimbriae

Pili and fimbriae are hair like appendages found on surface of cell wall. Pili are governed by plasmid, the number of which varies from 3 to 5 and fimbriae are around 1000. They originate from cytoplasm that protrudes outside after penetrating the peptidoglycan layer of cell wall. Fimbriae made up of protein called fimbrilin and pili composed of 163 aminoacids.

Somatic pili – mainly for attachment

Sex pili – also known as F pili and controlled by sex factors

Functions of pili

1. fimbriae have the adhesive properties which attach the organism to the natural substrate or organism

2. fimbriae have antigenic properties as they acts as thermolabile nonspecific agglutinogen.

3. fimbriae affect the metabolic activity

4. sex pili act as conjugation tube

The cell wall

It is the semirigid structure present beneath the capsule and external to the plasma membrane. It is responsible for shape of cell wall and protect plasma membrane and cytoplasmic inclusions.Cell wall is made up of network of peptidoglycan.

Funtions

1. Peptidoglycan provide structural integrity to cell wall

2 . Cell envelope acts as a barrier for diffusion of certain molecules across the envelope

3. The matrix protein act as receptor site for bacteriophages

Plasma membrane

The term introduced by C. Nugeli and C. Cramer. It is situated beneath the cell wall and it consist of proteins, lipids, oligosaccharides and water. Plasma membrane consist of a continueous bilayer of phospholipid molecules in which globular proteins are embedded.

Funtions

1. Nutrients transport

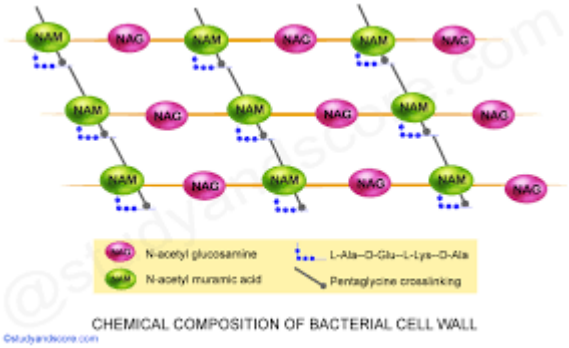
2. It possess the attachment sites for bacterial chromosome and plasmid DNA

3. Act as site for respiratory activity

4. Prevent escape of cellular materials

BACTERIAL CELL WALL

The bacterial cell wall is made up of a network of peptidoglycan. It is present on all bacterial cell wall except halobacterium and halococcus. Because these bacteria lives in marine water which contain high salt concentration. Peptidoglycan determine the shape of the cell . it account for 40-80% total dry weight of cell. Its thickness is about 30-80nm. It is insoluble and porous polymer that provide rigidity. It is a mucopolysaccharide. Its chemical composition differ from species to species. It consist of repeating disaccharides attached to chains of 4 or 5 aminoacids.The monosaccharides N – acetyl glucosamine and N-acetyl muramic acid are linked by β -1,4- glycosidic bond. These are related to glucose attached with aminoacid groups. A tetrapeptide side chain containing 4- aminoacids is attached to each NAM. The third aminoacid may vary in different bacteria and may be lysine, diamino pamitic acid. The D and L forms of aminiacids alternate to each other. The parralell tetra peptide side chains are linked by pentaglycine peptide cross bond that contain 5 aminoacid. The PPCB links L- lysine of the tetrapeptide with D-alnine at the terminal end.



CHEMICAL COMPOSITION OF BACTERIAL CELL WALL

CELL WALL OF GRAM POSITIVE BACTERIA

The cell wall contain several layer of peptidoglycan which is interconnect by side chain and cross bridges. Peptidoglycan accounts for the 40-90% total dry weight of cell. The layer of peptidoglycan are thicker in gram positive are thicker than gram negative bacteria. In most of the gram positive bacteria peptidoglycan is associated with acidic polymers containing phosphorous called teichoic acid or acid polysaccharides such as teichuronic acids. Teichoic acid are hydrophilic flexible and linear molecules. The teichoic acid possibly play arole in growth of bacterial cell by regulating the activity of enzyme autolysin. It also prevent the extensive breakdown and lysis of the cell wall. They also store phosphorous .

Cell wall of Archaeobacteria

All the archaeobacteria lack murein. Most of them posses cell wall lack peptidoglycan. The cell wall composed of protein / polysaccharide. Due to unusual chemical composition show high degree of resistance against antibiotics and lytic agents. The cell wall of gram positive archaeobacteria consists of pseudomurein and heteropolysaccharide. All gram negative archaeobacteria have all envelope which are composed of a single layerd or more complex crystalline protein

CELL WALL OF GRAM NEGATIVE BACTERIA

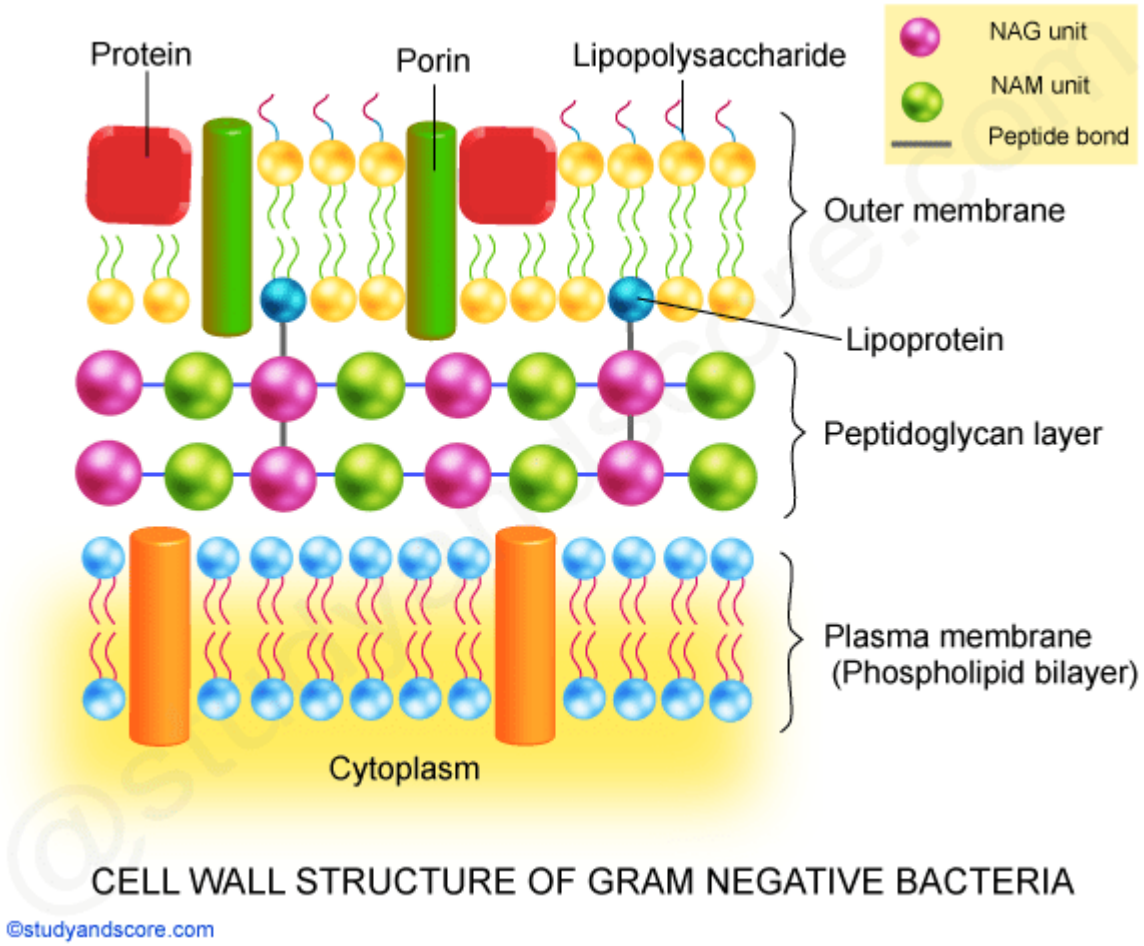
Consists of a very few layers of peptidoglycan and an outer membrane. Peptidoglycan is attached to lipoprotein. Teichoic acid is absent in gram negative bacteria.

Outer membrane of c.w consist of lipoprotein, lipopolysaccharides and phospholipids.

(1) lipoprotein- they occur freely and bound forms as well. They have molecular weight of about 7000 daltons and consist of 58 amino acids.

(2) lipopolysaccharides – which is made up of polysaccharides covalently linked to lipid A. (lipid A is composed of β – 1,6-D glucosamine disaccharide unit)

(3) polysaccharide – polysaccharide portion of salmonella cell wall is composed of 3 important components – inner core ,outerore, O- antigen side chain. The polysaccharide of LPS is also known as O- polysaccharide. O- antigen side chains have antigenic property.



CELL WALL STRUCTURE OF GRAM NEGATIVE BACTERIA

GRAM SATINING TECHNIQUES AND PRINCIPLES

Staining means colouring microbes with dye to highlight certain structures.

Stains are salt composed of a positive and negative ion, one of which is coloured and is called chromophore. Colour of basic dyes is in the positive ion, in acidic dye,it is negative ion. Bacteria are negatively charged when pH is 7. Thus in basic dye, the coloured positive ion is attracted to the negatively charged bacterial cell.

Crystal violet, methylene blue, malachite green , safranin are the commonly used dyes.

Three kinds of staining techniques

- Simple
- Differentiated
- Special

Simple stain- Simple stain is an aqueous or alcohol solution. The minimal purpose of a simple stain is to make visible the cellular shapes and structure of microorganism. Eg. methylene blue, crystal violet, safranin

Differential stain- they react differently with different kind of bacteria and thus they used to distinguish among bacterial types.

Special stain – used to isolate specific parts of microbes such as endospore and flagella.

GRAM STAINING

Christian gram in 1884

Gram positive bacteria retain crystal violet and appear deep violet

Gram negative bacteria lose crystal violet and counter stained by safranin hence appeared red

Procedure

- A heat fixed smear is covered with basic purple dye, usually crystal violet
- After short time purple dye is washed off and smear is covered with iodine (mordant)
- The slide is washed with alcohol or acetone solution.
- Alcohol is rinsed off and slide is stained with safranin

Mordant – which increase the affinity of stain to a biological specimen and can coat structure to make them thicker and visible after being stained with a dye.