Medical Microbiology

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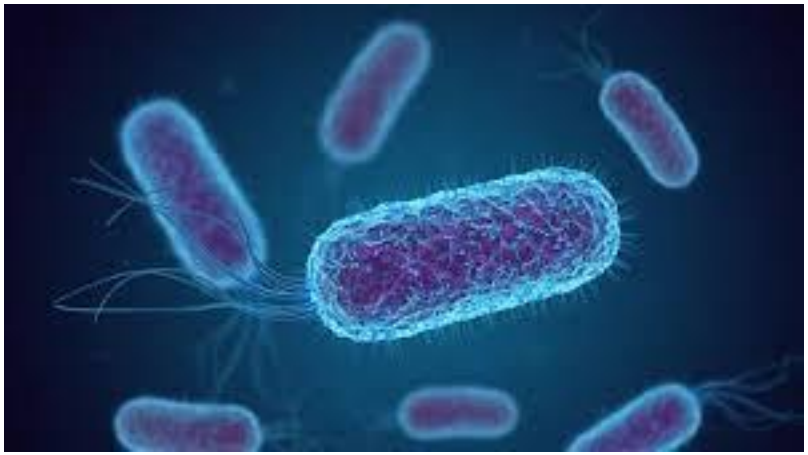
Microbiology

Is the study of Microorganisms, a large and diverse group of microscopic organisms existing as single cells or clusters. Micro-organisms include bacteria, fungi, protozoa, algae and viruses.

Table: Characteristics of Prokaryotic (bacteria) and Eukaryotic (Fungi, Protozoans) Microorganisms

Characteristic	**Prokaryotes** (bacteria)	**Eukaryotes** (fungi, protozoans)
Nuclear structure	Circular DNA molecule not covered with proteins	Complex of DNA and basic proteins
Localization of nuclear structure	Dense tangle of DNA in cytoplasm; no nuclear membrane	In nucleus surrounded by nuclear membrane
DNA	Nucleoid and plasmids	In nucleus and in mitochondria
Cytoplasm	No mitochondria and no endoplasmic reticulum, 70S ribosomes	Mitochondria and endoplasmic reticulum, 80S ribosomes
Cell wall	Usually rigid wall with murein layer; exception: mycoplasmas	Present only in fungi: glucans, mannans, chitin, chitosan, cellulose
Reproduction	Asexual, by binary transverse fission	In most cases sexual, possibly asexual

Prokaryotes are unicellular organisms that lack organelles or other internal membrane-bound structures. Therefore, they do not have a nucleus, but, instead, generally have a single chromosome: a piece of circular, double-stranded DNA located in an area of the cell called the nucleoid.



Bacteria

1 Have a cell wall.
2 May be normal flora or may be pathogenic bacteria.
3 reproduced asexually by binary trans-verse fission.

Mycoplasma

1 Mycoplasmas are bacteria without rigid cell walls**.**

2 Some, such as *Mycoplasma pneumoniae*, an agent of pneumonia, contain sterols in their membrane.

3 Mycoplasmas lack a target for cell wall-inhibiting antimicrobial agents (eg, penicillins and cephalosporins) and are therefore resistant to these drugs.

4 They are found in a wide variety of forms, the most common being the coccoid cell. Threadlike forms also occur in various lengths.

Bacterial Morphology

Shape: Along with other properties, bacterial shape is used to identify bacteria. It is determined by the mechanism of cell wall assembly.

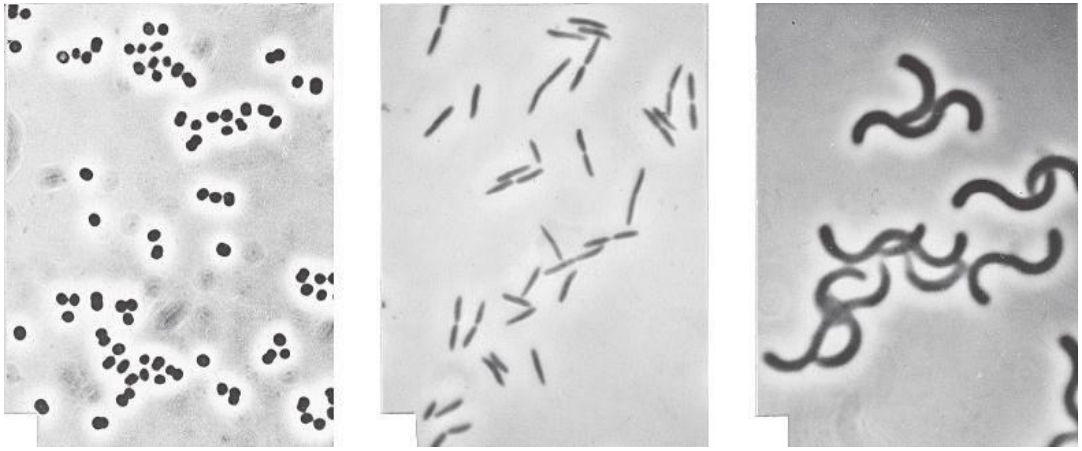
Bacteria have three basic forms:

1 **Coccus or Cocci** are bacterial cells that are spherical(e.g., *Staphylococcus aureus*)

2 **Bacilli; uniform thickness, rounded ends, pointed ends.** (e.g., *Bacillus subtilis*)

3 **Curved or spiral rods. comma shaped, spiral, screw shaped** (e.g., *Vibrio cholera*, *Treponema pallidum).*

- **Some bacterial species are pleomorphic (e.g.** *Mycoplasma pneumoniae***).**



Arrangement of bacterial cells

Bacteria are also characterized based on how cocci and bacilli aggregate themselves. The aggregation of cells is specific to each bacteria.

Arrangement of cocci cells

Singly: Bacteria that appear as single cell, is just called as cocci

Diplococci: These cells are found in pairs and they are found attached to each other

Streptococcus: These bacteria form long chains and remain attached to each other

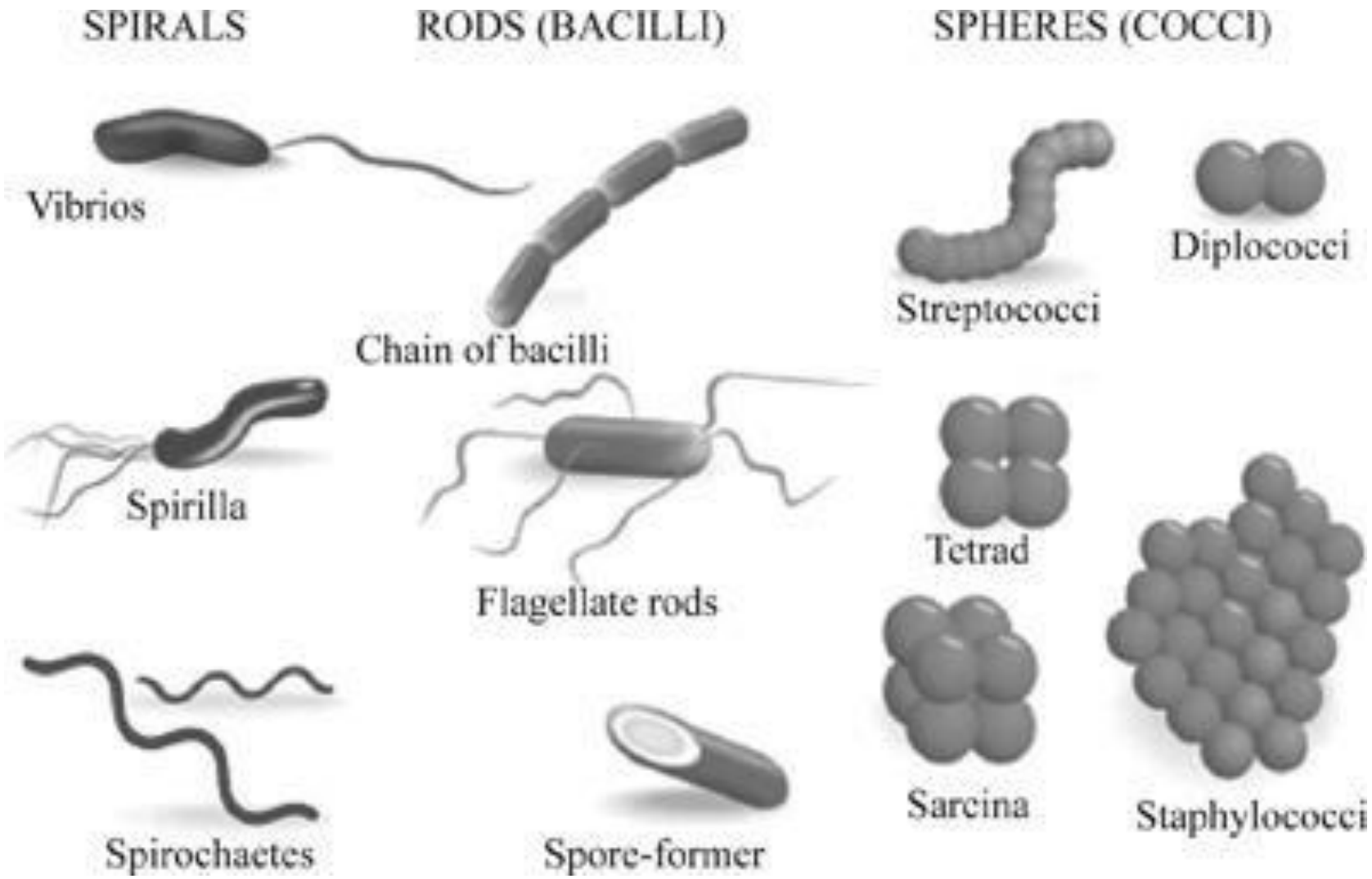
Staphylococcus: These bacteria are arranged irregularly in clusters like grapes

Arrangement of Bacilli

Singly: Bacteria that exists as single cell, called bacilli

Diplobacilli: These bacteria has two rod shaped cells which are attached to each other

Streptobacilli: Cells are arranged as long chains in these bacteria


SPIRALS
RODS (BACILLI)
SPHERES (COCCI)
Vibrios
Chain of bacilli
Streptococci
Diplococci
Spirilla
Flagellate rods
Tetrad
Sarcina
Staphylococci
Spirochaetes
Spore-former

Staining

Light microscope and chemical-staining techniques are usually used to determine bacterial shape.

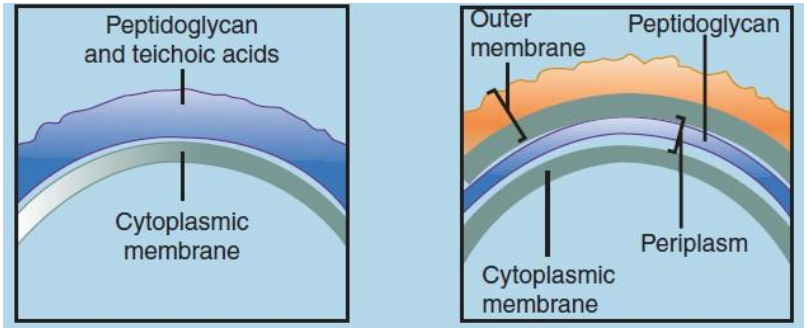
- **Simple staining:** In this technique, a single staining substance, e.g., methylene blue, is used.

- **Differential staining:** Two stains with differing affinities to different bacteria are used in differential staining techniques, the most important of which is Gram staining. Gram-positive bacteria stain blue-violet, Gram-negative bacteria stain red.

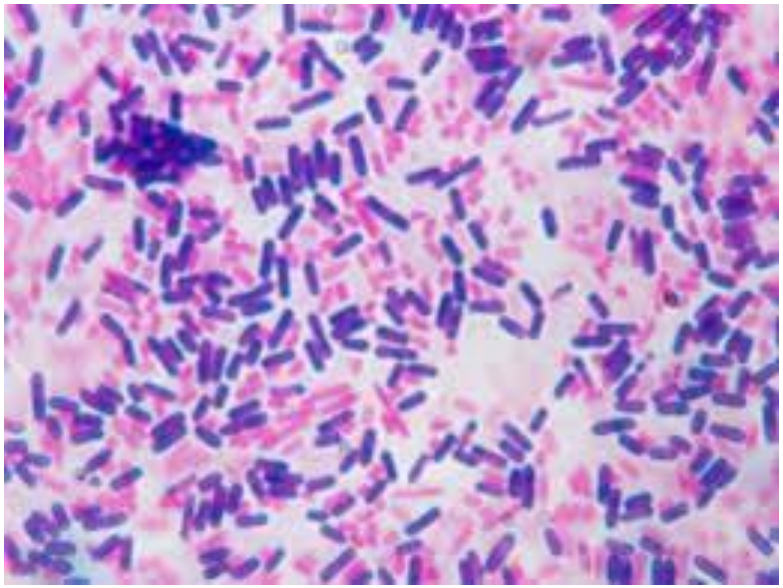
Gram Stain

Most bacteria are classified as gram positive or gram negative according to their response to the Gram-staining procedure. This procedure was named for the histologist Hans Christian Gram, who developed this differential staining procedure in an attempt to stain bacteria in infected tissues.

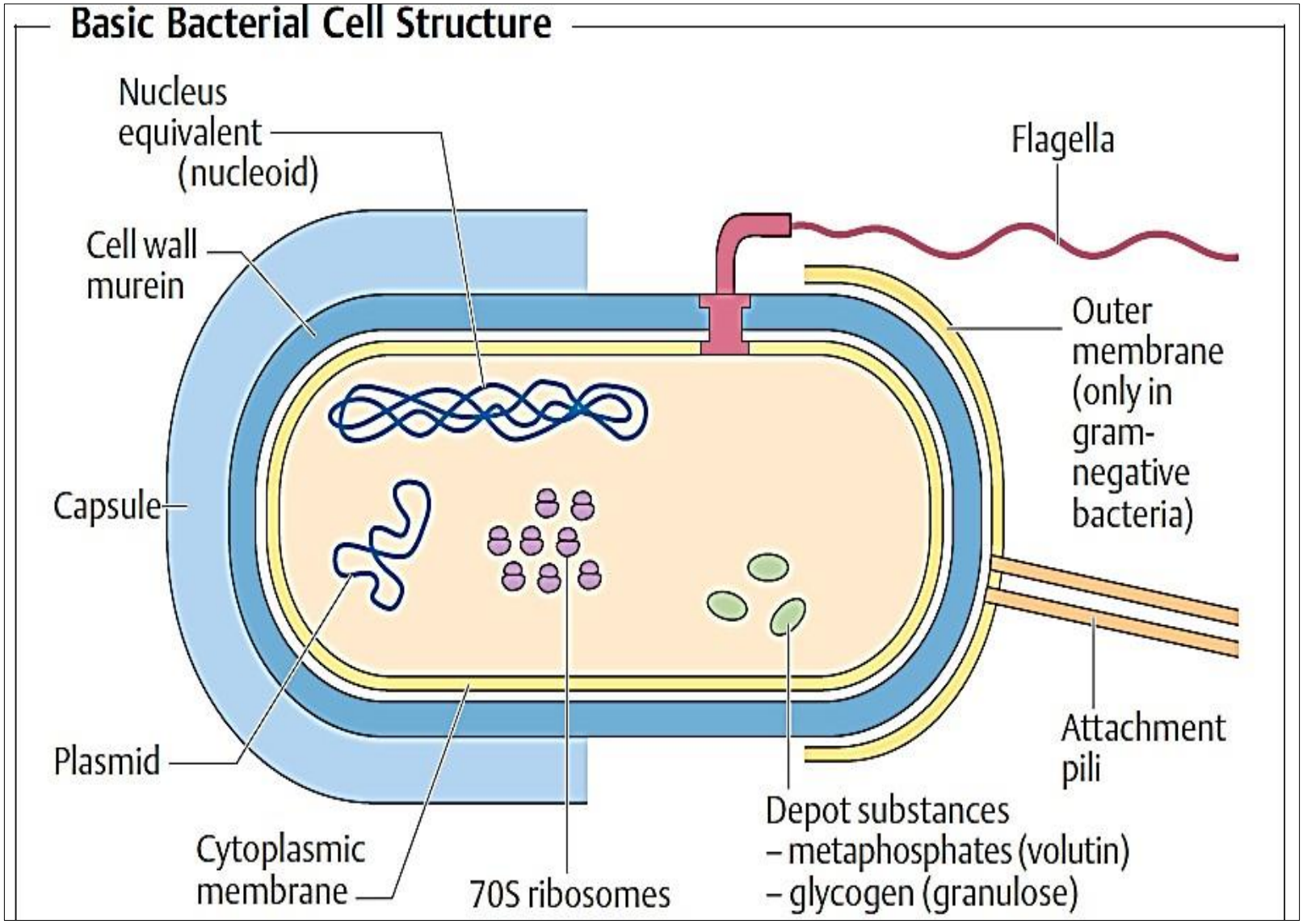
The Gram stain depends on the ability of certain bacteria (the gram-positive bacteria) to retain a complex of crystal violet (a purple dye) and iodine after a brief wash with alcohol or acetone. Gram-negative bacteria do not retain the dye–iodine complex and become translucent, but they can then be counterstained with safranin (a red dye). Thus, gram-positive bacteria look purple under the microscope, and gram-negative bacteria look red. The distinction between these two groups turns out to reflect fundamental differences in their cell envelopes



Gram-Positive	Gram-Negative
Purple	Reddish-pink



Color of Gram Stained Cell	Purple	Reddish-pink
Representative Genera	*Bacillus, Staphylococcus, Streptococcus*	*Escherichia, Neisseria, Pseudomonas*
Distinguishing Structures/Components		
Peptidoglycan	Thick layer	Thin layer
Teichoic acids	Present	Absent
Outer membrane	Absent	Present
Lipopolysaccharide (endotoxin)	Absent	Present
Porin proteins	Absent (unnecessary because there is no outer membrane)	Present; allow passage of molecules through outer membrane
Periplasm	Absent	Present


Basic Bacterial Cell Structure
Nucleus equivalent (nucleoid)
Flagella
Cell wall murein
Outer membrane (only in gram-negative bacteria)
Capsule
Plasmid
Attachment pili
Cytoplasmic membrane
70S ribosomes
Depot substances
– metaphosphates (volutin)
– glycogen (granulose)

Nucleoid

- ✓ The nucleoid consists of a tangle of double-stranded DNA, not surrounded by a membrane and localized in the cytoplasm.
- ✓ Bacterial DNA is Haploid.
- ✓ DNA is stabilized by small polyamines and Mg ions and associated with histone-like proteins.

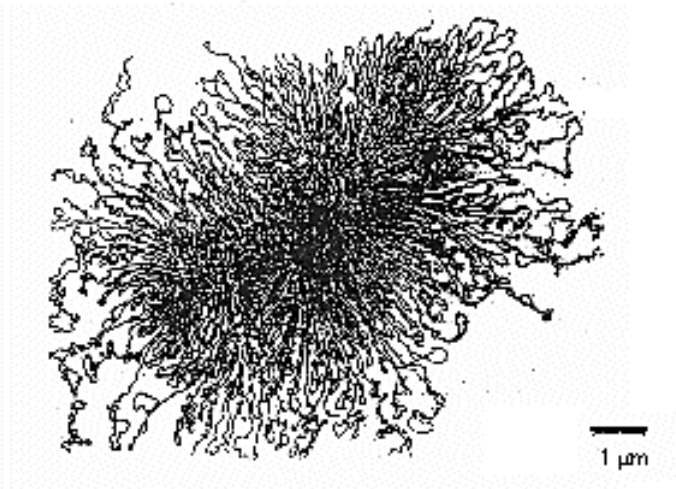
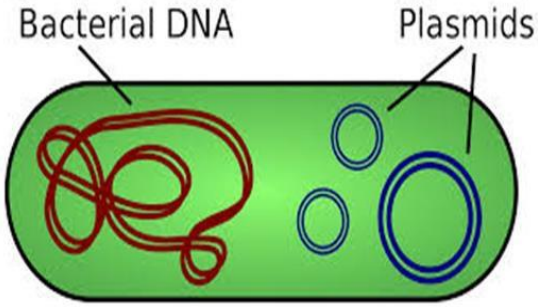


Figure : Electron Micrograph of a Bacterial Chromosome

Cytoplasm

- ✓ The cytoplasm contains a large number of solute low- and high-molecular weight substances, RNA and ribosomes.

- ✓ The cytoplasm is also frequently used to store reserve substances (glycogen depots, polymerized metaphosphates, lipids).



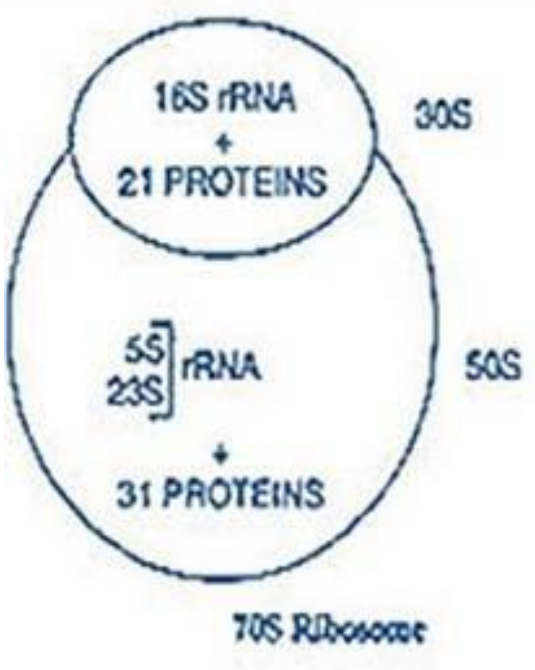
Plasmids are small, circular, non-chromosomal, double-strand DNA molecules that are capable of self-replication.

Ribosomes

Ribosomes are minute particles composed of ribosomal RNA (rRNA) and protein. Bacterial ribosomes are composed of two subunits ; 50S and 30S.

The two subunits combine during protein synthesis to form a complete 70S ribosome .

Ribosomes function to synthesize proteins



The Cell Envelope

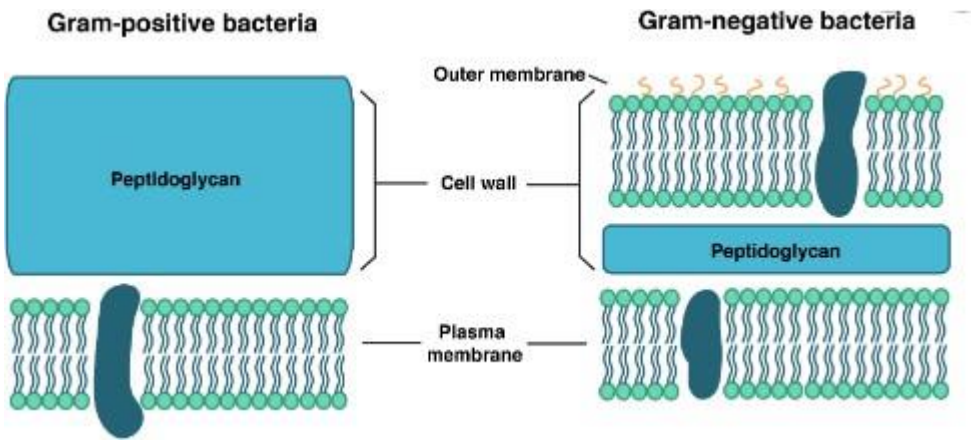
Prokaryotic cells are surrounded by complex envelope layers that differ in composition among the major groups.

- It comprises the **inner cell membrane** and the **cell wall**.

In Gram- negative bacteria an **outer membrane** is also included.

Functions

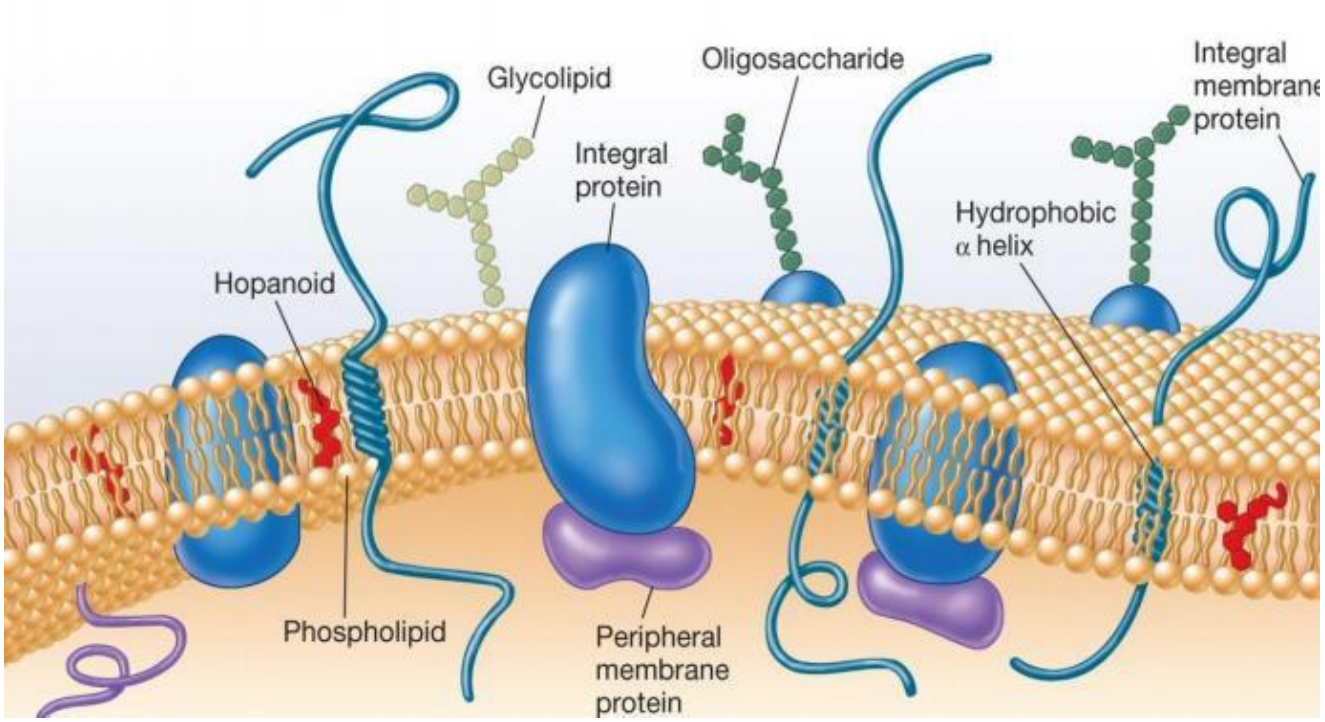
- Protect the organisms from hostile environments, such as extreme osmolarity, harsh chemicals, and even antibiotics.



The Cytoplasmic Membrane

Also known as the **plasma membrane**. It is basically a double layer of phospholipids with numerous proteins integrated into its structure.

The most important of these membrane proteins are permeases, enzymes for the biosynthesis of the cell wall, transfer proteins for secretion of extracellular proteins, sensor or signal proteins, and respiratory chain enzymes.



Cell Wall

The cell wall refers to that portion of the cell envelope that is external to the cytoplasmic membrane and internal to the capsule or glycocalex.

Function

- ✓ Protect the protoplasts from external environment.
- ✓ To withstand and maintain the osmotic pressure gradient between the cell interior and the extracellular environment,
- ✓ To give the cell its outer form.
- ✓ To facilitate communication with its surroundings.

The bacterial cell wall owes its strength to a layer composed of a substance known as **murein**, **mucopeptide**, or **peptidoglycan** (all are synonyms).

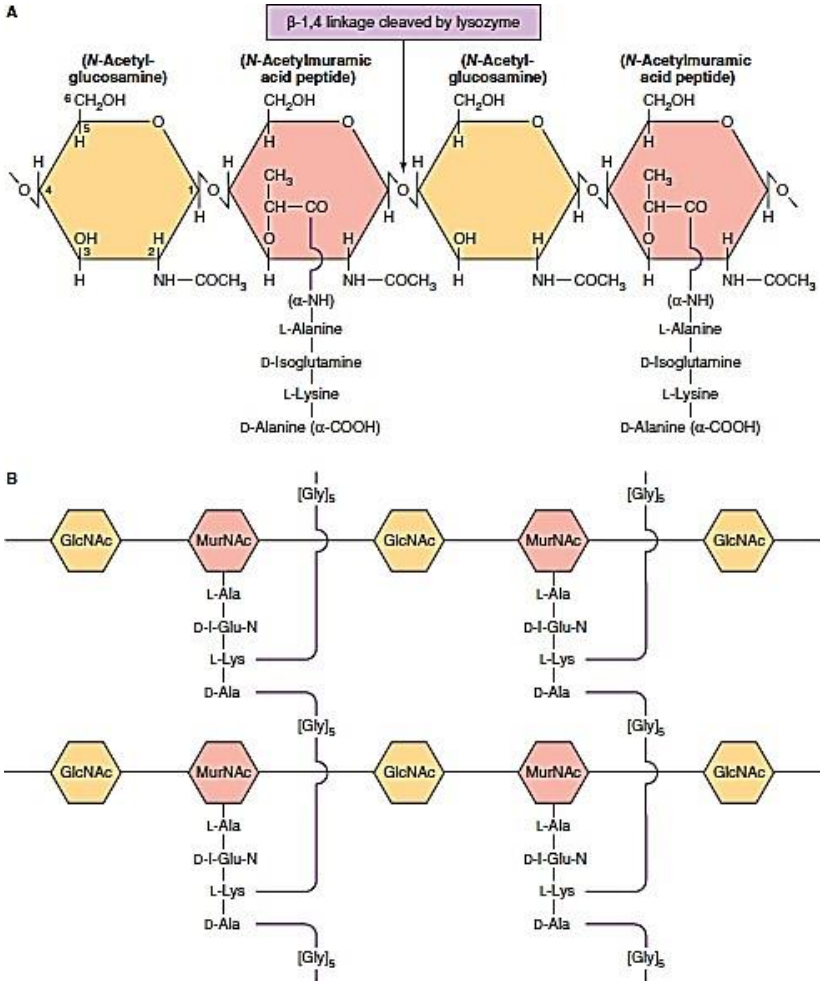
The Peptidoglycan Layer

Peptidoglycan is a complex polymer consisting of three parts:
A **backbone**, composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid connected by β1→4 linkages;

a set of identical tetrapeptide side chains attached to *N*-acetylmuramic acid; and

a set of identical peptide cross-bridges.

It may contain **Diaminopimelic acid**, an amino acid unique of bacterial cell walls.

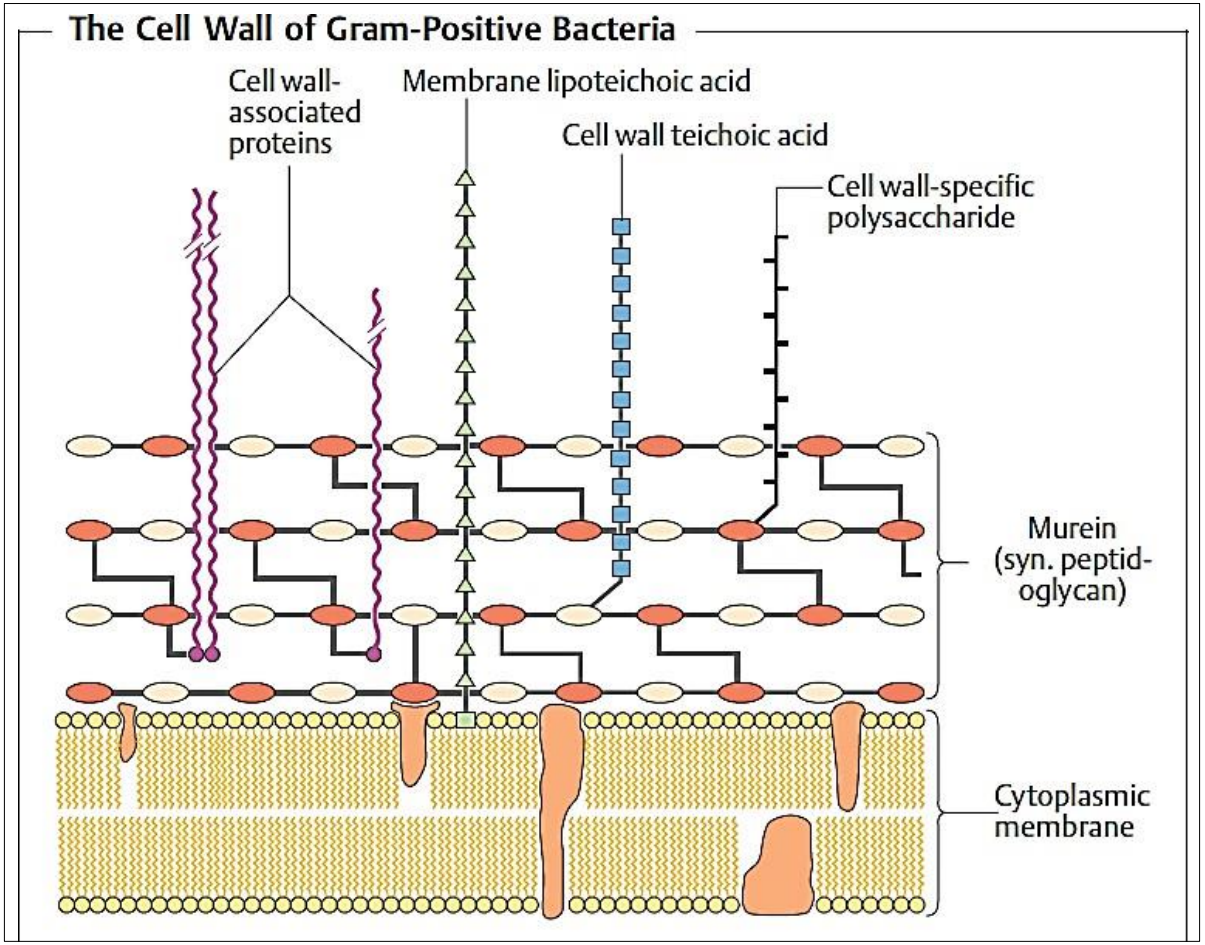


The cell wall of Gram-positive bacteria

It is composed of

- **Peptidoglycan(50% of cell wall)**
- **Teichoic acids and teichuronic acids(water -soluble polymers).**
- **Polysaccharides.**

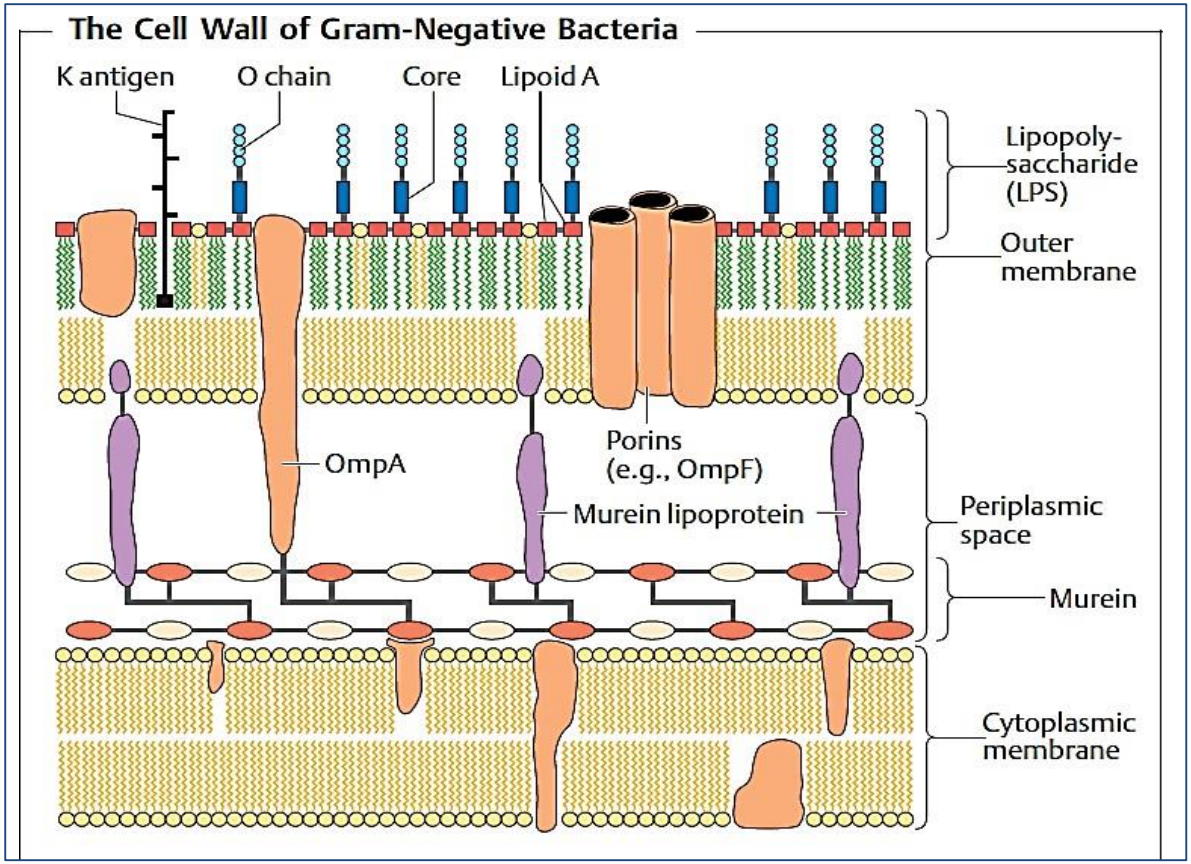
There are two types of teichoic acids:
wall teichoic acid), covalently linked to peptidoglycan, and **membrane teichoic acid**, covalently linked to membrane glycolipid. Because the latter are intimately associated with lipids, they have been called **lipoteichoic acids.**



The cell wall of Gram-negative bacteria

It is composed of:

- **Peptidoglycan(2% - 10% of cell wall)**
- **Lipoprotein**(cross links the peptidoglycan and outer membrane**).**
- **An outer membrane**



Outer membrane:

Is a phospholipid bilayer, its inner leaflet resembles in composition that of the cell membrane and its outer leaflet contains a distinctive component, a **lipopolysaccharide (LPS)**.

Function:

Protect cells from harmful enzymes, some antibiotics and to prevent leakage of periplasmic proteins.

Outer membrane proteins:

- **OmpA** (outer membrane protein A) and the **murein lipoprotein** form a bond between outer membrane and murein.
- **Porins**, proteins that form pores in the outer membrane, allow passage of hydrophilic, low-molecular-weight substances into the periplasmic space.
- **Outer membrane-associated proteins:** constitute specific structures that enable bacteria to attach to host cell receptors.
- **Transport proteins:** Example include the **LamB proteins** for maltose transport

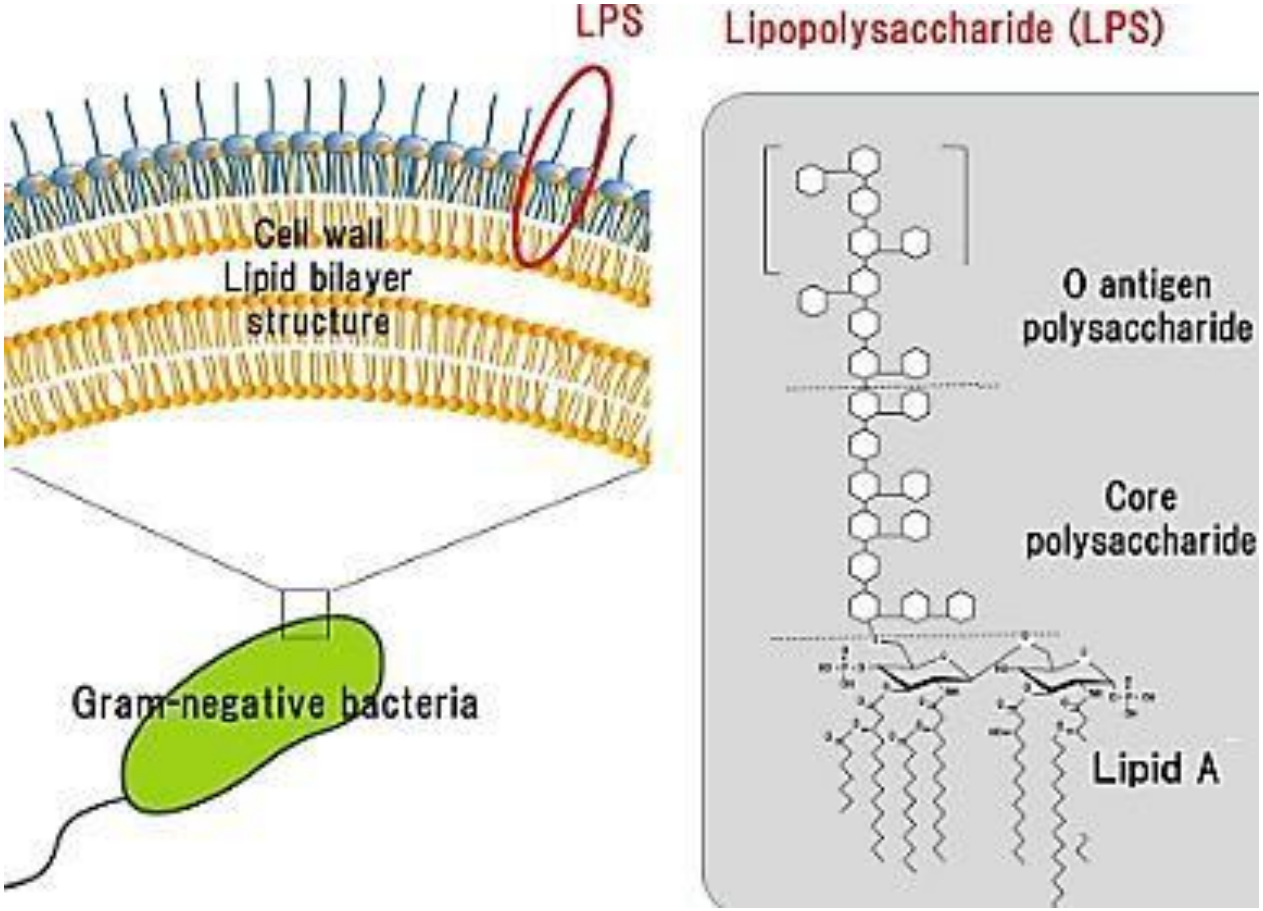
Lipopolysaccharide (LPS)

This molecular complex is comprised of the lipid A, the core polysaccharide, and the O-specific polysaccharide chain.

Function:

1 Also known as endotoxin, the toxicity is associated with the lipid A.

2 Contains major surface antigenic determinants, including O antigen found in the polysaccharide components.



The periplasmic space

- **The periplasm** is the **space** between **the inner** and **outer membrane in Gram-negative bacteria**.

In **Gram-positive bacteria** a smaller **periplasmic space** is found between the inner membrane and the peptidoglycan layer.

Glycocalyx

Is a network of polysaccharides that project from cellular surfaces of bacteria, found just outside the bacterial cell wall.

This layer can come in one of two forms:

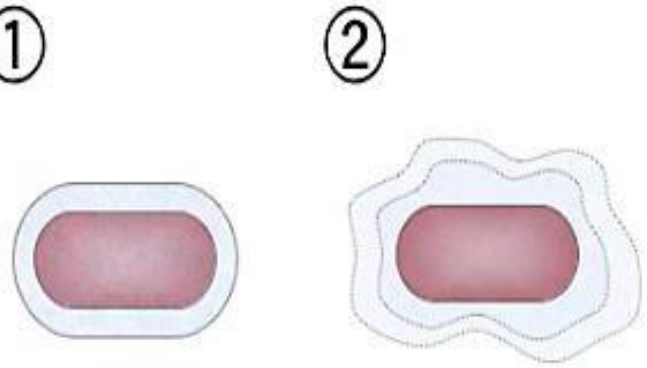
1 Capsule

The capsule is a well-defined structure of **polysaccharide** surrounding a bacterial cell and is external to the cell wall.

The one exception to the polysaccharide structure is the poly-D glutamic acid capsule of *Bacillus anthracis.*

Function:

- Protects the bacteria from phagocytosis.
- Plays a role in bacterial adherence.



2-Slime Layer

Refers to a loose network of polysaccharide fibrils that surrounds some bacterial cell walls.

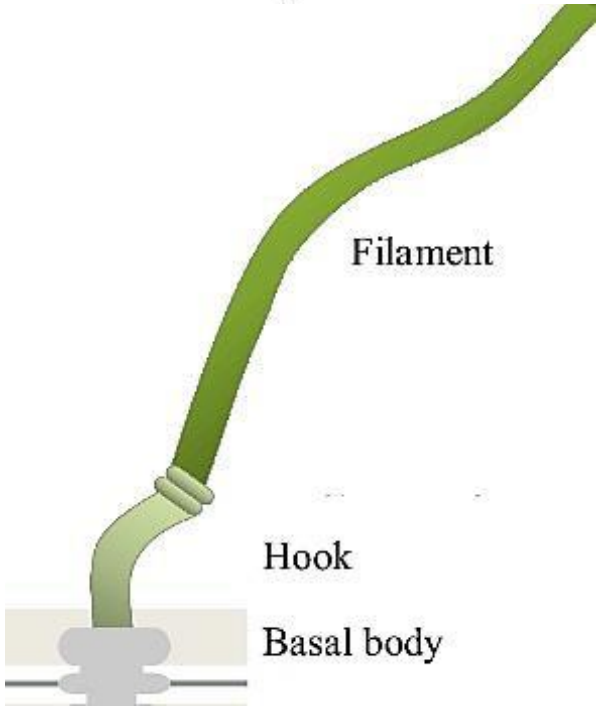
Function: associated with adhesive prosperities of the bacterial cell.

Flagella

Structure

Bacterial flagella are thread-like appendages consist of a **basal body, hook**, and a **long filament** composed of a polymerized protein called **flagellin**.

They are the organs of locomotion for the forms that possess them.



Three types of arrangement are known:

- **monotrichous** (single polar flagellum).
- **lophotrichous** (multiple polar flagella).
- **peritrichous** (flagella distributed over the entire cell).

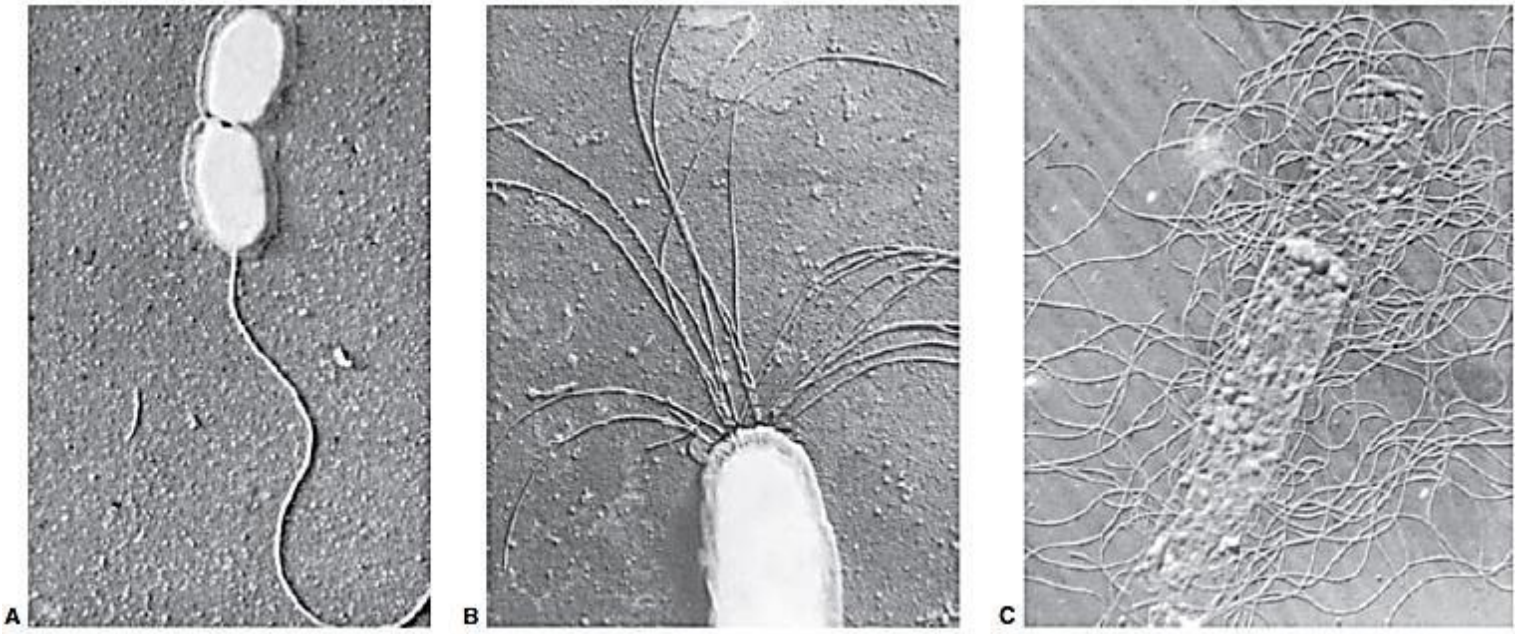
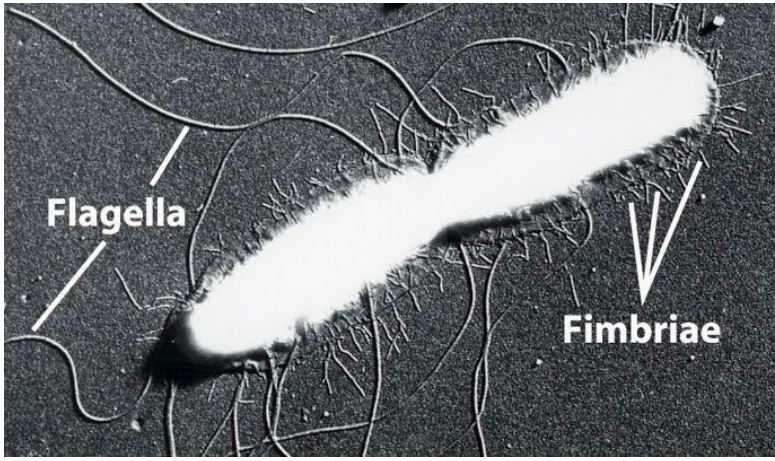


FIGURE : Bacterial flagellation. **A:** a monotrichous bacterium **B:** lophotrichous flagellation **C:** peritrichous flagellation

Pili (Fimbriae)



They are rigid surface appendages composed of structural protein subunits termed **pilins**. Minor proteins termed **adhesins** are located at the tips of pili and are responsible for the attachment properties.

Two classes can be distinguished:

- **Ordinary pili**, which play a role in the adherence of bacteria to host cells.
- **Sex pili,** which are responsible for the attachment of donor and recipient cells in bacterial conjugation.

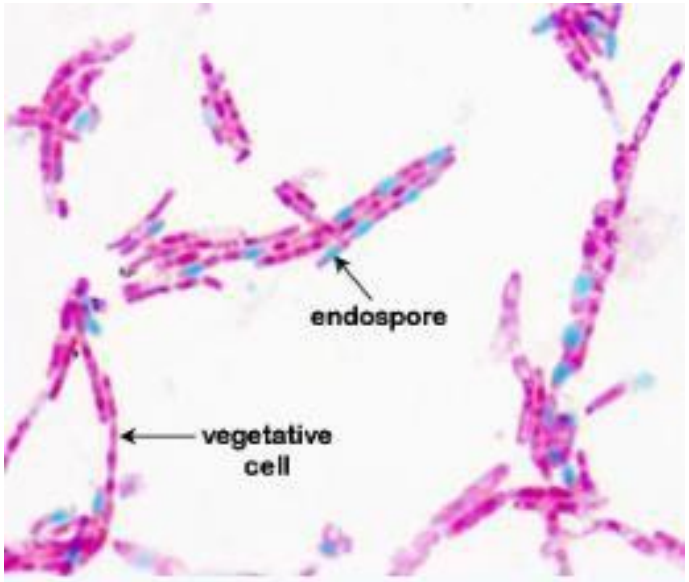
Functions

-Ordinary pili are the colonization antigens or virulence factors.

- Antiphagocytic properities.

Endospores

The spore is a resting cell, highly resistant to desiccation, heat, and chemical agents; when returned to favorable nutritional conditions and activated, the spore **germinates** to produce a single vegetative cell.



Structure:

They possess **a core** that contains many cell components(a nucleoid, some ribosomes, RNA molecules, and enzymes) **a spore wall**, a **cortex**, **a coat**, and an **exosporium**.

Biofilm

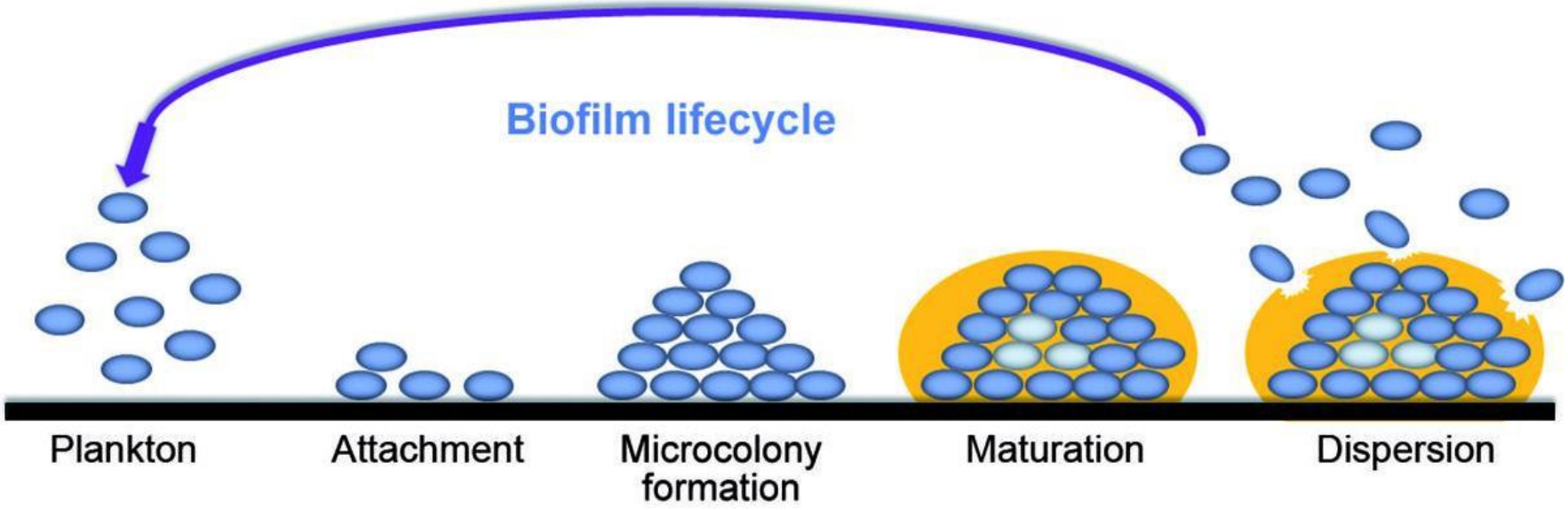
- A biofilm is an aggregate of interactive bacteria attached to a solid surface or to each other and encased in an exopolysaccharide matrix.
- The matrix is typically **composed of** polysaccharides, proteins, lipids, and DNA.

By living as a community of bacteria as a biofilm, these bacteria are better able to:

- resist attack by antibiotics
- trap nutrients for bacterial growth and remain in a favorable niche
- adhere to environmental surfaces and resist flushing
- live in close association and communicate with other bacteria in the biofilm
- protected from the host's immune mechanisms.

Stages of biofilm development

- To initiate biofilm formation, planktonic bacteria (free individual bacteria not in a biofilm) contact an environmental surface .These planktonic bacteria then attach to that surface using pili or cell wall adhesins.
- Biofilm formation is commonly considered to occur in four main stages: (1) bacterial attachment to a surface, (2) microcolony formation, (3) biofilm maturation and (4) dispersion of bacteria which may then colonize new areas.



Thanks