MSB 100: BACTERIOLOGY

Bacteria - Microscopic single-celled organisms that live in enormous numbers in almost every environment on earth.

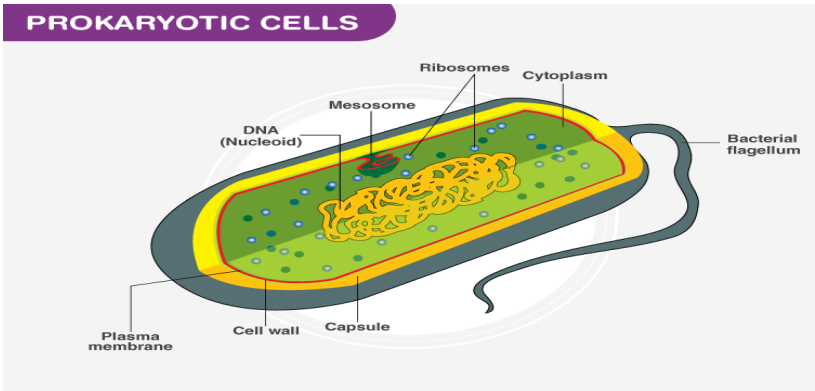
Bacteriology is the study of bacteria, including their structure, genetics, and how they interact with their environment.

Bacterial cell

A prokaryotic cell that is characterized by the absence of *a membrane-bound nucleus, lack of internal membrane-bound organelles,* ***a single circular DNA molecule located in a region called the nucleoid, a cell wall, a plasma membrane, cytoplasm, cytoplasmic inclusions and ribosomes***. Divides by binary fission.

Bacterial cell structure

- ✓ Cell envelope proper: Cell wall and cell membrane.
- ✓ Cellular elements enclosed within the cell envelope/in the cytoplasm: Mesosomes, ribosomes, nuclear apparatus/necleoid, and cytoplasmic granules.
- ✓ Cellular elements external to the cell envelope: Flagellum, Pilus and Glycocalyx.

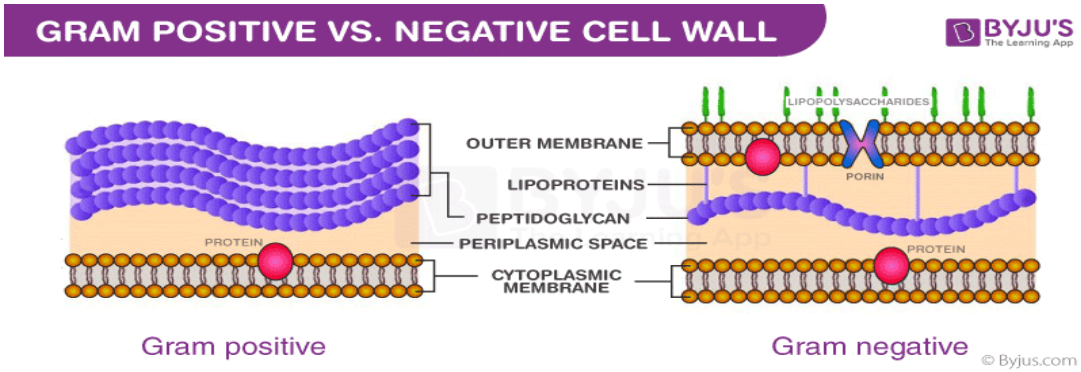


Cell envelope proper

i. Cell wall

Multi layered structure and constitutes about 20% of the bacterial dry weight.

It is composed of ***N-acetyl Muramic acid (NAM)and N-acetyl Glucosamine (NAG)*** (sugar molecules) back bones cross linked with peptide chain and pentaglycine bridge/tetrapeptide bond.



Functions of the cell wall

- ✓ Provides shape to the bacterium
- ✓ Gives rigidity to the organism
- ✓ Protects from the external environment
- ✓ Provides staining characteristics to the bacterium
- ✓ Contains receptor sites/attachment sites for phages/complements
- ✓ Contains toxic components to host

ii. Cell membrane

Also named as cell membrane or cytoplasmic membrane

It accounts for 30% of the dry weight of bacterial cell.

Functions

- ✓ Regulates the transport of nutrients and waste products into and out of the cell.
- ✓ Synthesis of cell wall components
- ✓ Carries on electron transport system for energy generation

Cellular elements enclosed within the cell envelope

i. Mesosomes

Convoluted invagination of cytoplasmic membrane often at sites of septum formation.

It is involved in DNA segregation during cell division and respiratory enzyme activity.

ii. Ribosomes

Cytoplasmic particles which are the sites of protein synthesis.

The ribosome monomer is 70s with two subunits, 30s and 50s.

iii. Cytoplasmic granules

Represent accumulated food reserves. Glycogen, Poly-beta hydroxy butyrate, volutin

iv. Nuclear apparatus

Well defined nucleus and nuclear membrane, discrete chromosome and mitotic apparatus **ARE NOT** present in bacteria, therefore, nuclear region of bacteria is named as nuclear body, nuclear apparatus and nucleoid.

Consists of single molecule of double stranded DNA arranged in a circular form.

Besides nuclear apparatus, bacteria may have extra chromosomal genetic material named as plasmids.

Plasmids do not play any role in the normal function of the bacterial cell but may confer certain additional properties (Eg. virulence, drug resistance).

Cellular element external to the cell envelope

i. Glycocalyx (capsule and slime layer)

Capsule is gel firmly adherent to cell envelope.

Slime is gel easily washed off from cell envelope – all bacteria have a slime layer.

Features of the capsule

- ✓ Usually weakly antigenic – low ability to induce antibody development.
- ✓ Not necessary for viability.
- ✓ Endows virulence - protects from phagocytosis by inhibiting receptor-mediated endocytosis.
- ✓ Capsulated strains are invariably non-motile.

ii. Flagellum

It is the organ of locomotion in bacterial cells – whiplash-like form of locomotion.

Flagellar arrangements

- ✓ **Atrichous:** Bacteria with no flagellum.
- ✓ **Monotrichous:** Bacteria with single polar flagellum.
- ✓ **Lophotrichous:** Bacteria with bunch of flagella at one pole.
- ✓ **Amphitrichous:** Bacteria with flagella at both poles.
- ✓ **Peritrichous:** Bacteria with flagella all over their surface.

iii. Pili (fimbriae)

It is hair like structure composed of protein (pilin)

Two types (Based on function)

Common pili: The structure for adherence to cell surface.

Sex pili: The structure for transfer of genetic material from the donor to the recipient during conjugation.

iv. Spores

Resting cells which are capable of surviving under adverse environmental conditions like heat, drying, freezing, actions of toxic chemicals and radiation.

Classification of bacteria

Bacterial classification depends on:

- ✓ Morphology and arrangement
- ✓ Staining
- ✓ Cultural characteristics – features that can be denoted during the growth of the bacteria.
- ✓ Biochemical reactions

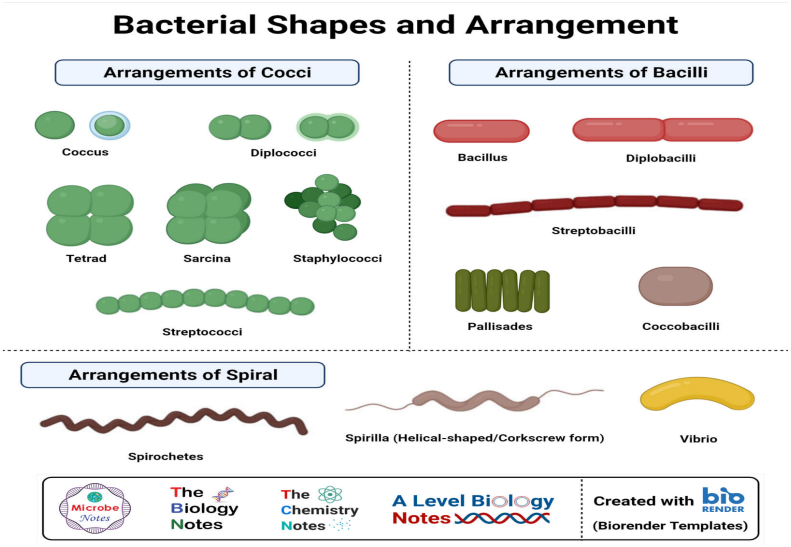
Morphology and staining of bacteria are the commonly used characteristics to classify bacteria.

i. Morphology of bacteria/Shape of the bacterial cell

Under a light microscope, bacterial morphologies include:

- ✓ **Cocci** (singular coccus): Round or oval bacteria. They are found single, pairs, chains or clusters. E.g *Streptococcus pneumoniae, Staphylococcus aureus*
- ✓ **Bacilli** (singular bacillus): Stick/rod-like bacteria with rounded, tepered, square or swollen ends. E.g *Bacillus subtilis, Escherichia coli.*
- ✓ **Coccobacilli** (singular coccobacillus): Short rods E.g *Haemophilus influenzae*.
- ✓ **Spiral:** Spiral shaped bacteria with regular or irregular distance between twisting E.g *Treponema pallidum*.
- ✓ **Vibrio:** Comma shaped bacteria E.g *Vibrio Cholerae*

The shapes are all dependent on the presence of a cell wall.



ii. Staining of bacteria

- ✓ The process of coloring of colorless bacterial structural components using stains (dyes).

- ✓ Individual variations in cell wall constituents among different groups of bacteria will consequently produce variations in colors during microscopic examination.
- ✓ Gram stain to differentiate between the gram positive and the gram negative bacterial cell walls.
- ✓ Nucleus is acidic in character and hence, it has greater affinity for basic dyes. Whereas, cytoplasm is basic in character and has greater affinity for acidic dyes.

General Mechanisms of Bacterial Pathogenesis

- ✓ Depends on the virulence of the pathogen and relative degree of resistance or susceptibility of the host.

Disease Transmission

T**ransmission** must occur for an infection to spread.

From reservoir to the individual to other susceptible individuals.

1. **Contact Transmission**

- ✓ Includes direct contact or indirect contact. **Person-to-person transmission** is a form of **direct contact transmission**.
- ✓ Direct contact can be categorized as vertical, horizontal, or droplet transmission.
- ✓ **Indirect contact transmission** involves inanimate objects called **fomites – door knobs, pens, chairs, syringes**.

2. **Vehicle Transmission**

- ✓ Carriers such as water, food, and air.

3. **Vector Transmission**

- ✓ Mechanical vector transmission – pathogen doesn't undergo a part or any of its life cycle in the vector.
- ✓ Biological vector transmission – pathogen undergoes a part of its lifecycle in the vector.

Pathogenic Mechanisms

a. The ability to adhere to host cells and resist removal

i. Fimbrial and pili adhesins

Specific adhesins on bacterial cell surfaces allows them to interact **with receptors** on host cells. This close interaction might also be detrimental for the bacteria – immune detection.

ii. Nonpilus (Non-Fimbrial) Adhesins

Most are proteins, but other structures such as lipopolysaccharides and lipoteichoic acids also have adhesive functions.

iii. Bacterial Biofilms

Biofilms enable bacteria to:

- ✓ Resist attack by antibiotics, antibodies, phagocytes and the body's complement pathways;
- ✓ 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.

b. Cellular Invasion by Bacterial Pathogens

i. Bacterial Entry Into Host Cells

Activated signaling pathways at the entry site regulate the fate of the invading microorganism – receptor-mediated endocytosis. Bacteria may **then replicate in either cytoplasmic or vacuolar** niches.

c. Nutrient Competition

Host cells have the ability to induce nutritional immunity during infection. They compete for nutrients by synthesizing **specific transport systems or cell wall components capable of binding limiting substrates (have the ability to scavenge for the available nutrients) and transporting them into the cell**.

d. Immune Evasion

i. Bacterial capsules

- ✓ Resist unenhanced attachment by preventing pathogen-associated molecular patterns from binding endocytic pattern-recognition receptors on the surfaces of phagocytes – inhibit phagocytosis.

ii. Bacterial Resistance to Antimicrobial Peptides

Host defence peptides (HDP) are small molecules produced by all organisms as a first line of defence against microbial invasion.

- ✓ Capsules prevent antibacterial peptides from reaching the cytoplasmic membrane of some bacteria.
- ✓ The *lipopolysaccharide* of the gram-negative cell wall binds cationic antibacterial peptides and prevents them from reaching the cytoplasmic membrane.
- ✓ Some bacteria secrete peptidases that break down antibacterial peptides.

iii. Phagosomes

Bacteria prevent phagocytosis by:

- ✓ Preventing fusion of the lysosome with the phagosome.
- ✓ Escaping from the phagosome before the lysosome fuses.
- ✓ Preventing acidification of the phagosome.
- ✓ Resisting killing by lysosomal chemicals.
- ✓ Killing phagocytes.

iv. Induction of Autoimmune Responses

- ✓ Stimulating the production of cross-reacting antibodies.

d. **Host Damage**

i. Toxins

Bacterial cytolytic pore-forming protein toxins (PTFs) are produced by both Gram-positive and -negative bacteria.

ii. Chronic inflammation

CULTIVATION OF BACTERIA IN CULTURE MEDIA

Culture media/medium for the growth of bacteria

It is the media containing the required nutrients for bacterial growth.

Used for: Isolation and identification of micro-organisms
Performing anti-microbial sensitivity tests

Common ingredients of culture media

- ✓ **Peptone:** Hydrolyzed product of animal and plant proteins: Free amino acids, peptides and proteoses (large sized peptides). It provides nitrogen; as well carbohydrates, nucleic acid fractions, minerals and vitamins.
- ✓ **Meat extract:** supply amino acids, vitamins and mineral salts.
- ✓ **Yeast extract:** It is bacterial growth stimulants.
- ✓ **Mineral salts:** Sulfates as a source of sulfur, iron sources, pottassium.
- ✓ **Phosphates** as a source of phosphorus. Other elements
- ✓ **Carbohydrates:** Simple and complex sugars are a source of carbon and energy.

  Agar: It is an inert polysaccharide of seaweed. Gells to form a solid for the growth of bacteria. It is not metabolized by micro-organism.

Sterilization and sterility testing

Always sterilize a medium at the correct temperature and for the correct length of time as instructed in the method of preparation.

Methods used to sterilize culture media:

- ✓ **Autoclaving -** Used to sterilize most agar and fluid culture media – steam under pressure.
- ✓ **Steaming at 100 ^{0}C -** Used to sterilize media containing ingredients that would be inactivated at temperature over 100 ^{0} **C**
- ✓ **Filtration - U**sed to sterilize additives that are heat-sensitive and cannot be autoclaved.

Agar and Broth

Bacterial Genetics

Genetics is the study of inheritance. Bacterial inherited characteristics are encoded in DNA.

Bacteria have two types of DNA that contain their genes:

- ✓ Chromosome
- ✓ Extra chromosome: Plasmid

The bacterial chromosome is circular, double stranded DNA attached to bacterial cell membrane.

Plasmids are self-replicating extra chromosomal DNA molecules (code for proteins –toxins, dyes, antibiotics - that are necessary for the survival of the bacteria/have antagonistic effects against other bacteria).

It multiplies independent of the host cell.

Plasmid types

- ✓ **R factors:** Plasmids which contain genes that code for antibiotic resistance.
- ✓ **Col factors:** Plasmids which contain genes that code for extracellular toxins (colicines).
- ✓ **F (fertility) factors:** Plasmids that can recombine itself with the bacterial chromosome. It promotes transfer of the chromosome at a high frequency of recombination into the chromosome of a second (recipient) bacterial cell during mating.

Genetic variations in Bacteria

Mechanisms: Mutations and gene transfer

- ✓ **Mutations:** It is due to alterations in DNA. It could be spontaneous or induced by chemical and physical means.

Types of mutations

1. Substitution: Change of a single base.
2. Deletion: Loss of a base.
3. Insertion: Addition of a base.

- ✓ **Gene transfers**

There are three types of gene transfer that alter the DNA gene content of bacteria.

- ✓ **Transformation** occurs when fragments of exogenous bacterial DNA in the environment are taken up and absorbed into recipient bacterial cells. The recipient bacterium must be competent to absorb the exogenous fragments of bacterial DNA.

- ✓ **Transduction** occurs when fragments of chromosomal DNA is transferred or transduced into a second bacterium by phage – virus that infects bacteria.
  During phage replication, the bacterial DNA may be accidentally enclosed instead of the normal phage DNA, and when this particle which enclosed the bacterial DNA infects a second bacterial cell, the DNA from the first bacterium is released and incorporated into the chromosome of the second bacterium.
- ✓ **Conjugation** occurs when plasmid DNA is transferred from donor to recipient bacterium by direct contact via a sex pilus.

STERILIZATION AND DISINFECTION

Sterilization: Destruction of all forms of microbial life, including spores.

Disinfection: Destruction of microbes that cause disease; may not be effective in killing spores.

Sterilizing and disinfecting agents are divided into two groups.

- ✓ Chemical methods of sterilization and disinfection
- ✓ Physical methods of sterilization and disinfection

i. Chemical methods of sterilization and disinfection

These chemical agents destroy any type of microbes without showing any form of selectivity, unlike antibiotics. Ethanol, chlorine compounds

iii. Physical methods of sterilization and disinfection

1. Heat: The most reliable and universally applicable method of sterilization.
   - ✓ Dry heat – denatures protein. Incineration, Flaming, ovens
   - ✓ Moist heat – denatures and coagulates protein. Boiling, autoclaving, pasteurization.
2. Radiation – Gamma radiation, X-rays (rays have energy that can be able to break the hydrogen bonds in the DNA double helix structure).
3. Filtration- filters that are less that 0.2 micrometers in diameter.

Host-Microbe Relations

Host-microbe interactions are the relationships between a host and microorganisms, such as bacteria, viruses, fungi, and protozoans. These interactions can be beneficial, neutral, or harmful to the host.

- ✓ **Mutualism**: Both the host and the microbe benefit from the relationship. For example, some gut bacteria help humans and animals digest food.

- ✓ **Commensalism**: A type of symbiosis where the microbe benefits but the host is not affected.
- ✓ **Parasitism:** One organism benefits while the other suffers – pathogenic microorganisms.