NARUMALAR ACADEMY – ONLINE COACHING CENTRE

DHARMAPURI – 8122190917, 9988404061

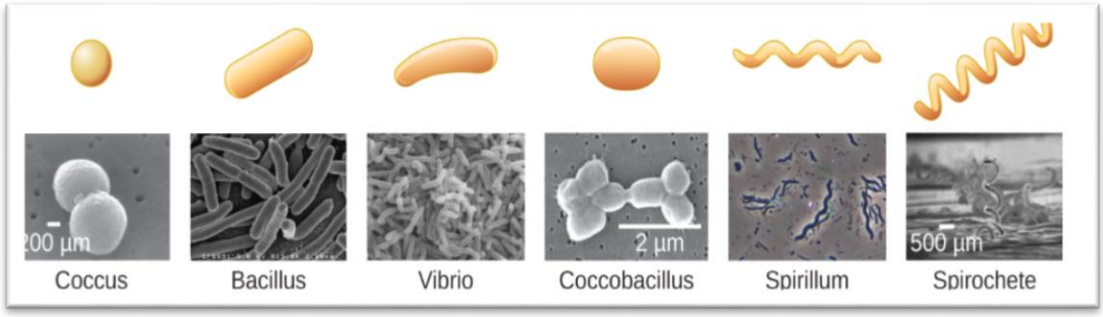
COLLEGE TRB – MICROBIOLOGY

STUDY MATERIAL- demo file

Prokaryotic Microorganisms

Bacteria are found in nearly every habitat on earth, including within and on humans. Most

bacteria are harmless or helpful, but some are pathogens, causing disease in humans and other animals. Bacteria are prokaryotic because their genetic material (DNA) is not housed within a true nucleus. Most bacteria have cell walls that contain peptidoglycan.



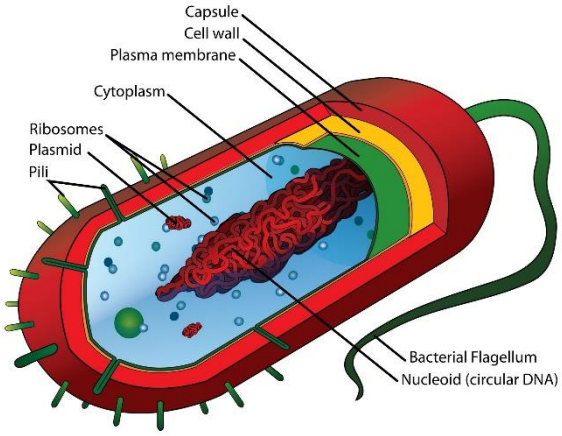
Bacteria are often described in terms of their general shape. Common shapes include spherical (coccus), rod-shaped (bacillus), or curved (spirillThey have a wide range of metabolic capabilities and can grow in a variety of environments,

using different combinations of nutrients. Some bacteria are photosynthetic, such as oxygenic

Cyanobacteria and anoxygenic green sulfur and green non-sulfur bacteria; these bacteria use energy derived from sunlight, and fix carbon dioxide for growth. Other types of bacteria are non-photosynthetic, obtaining their energy from organic or inorganic compounds in their environment.

BACTERIA

Bacteria, any of a group of microscopic single-celled organisms that live in enormous numbers in almost every environment on Earth, from deep-sea vents too deep below Earth's surface to the digestive tracts of humans.



Bacteria lack a membrane-bound nucleus and other internal structures and are therefore ranked among the unicellular life-forms called prokaryotes. Prokaryotes are the dominant living creatures on Earth, having been present for perhaps three-quarters of Earth history and having adapted to almost all available ecological habitats.

As a group, they display exceedingly diverse metabolic capabilities and can use almost any organic compound, and some inorganic compounds, as a food source. Some bacteria can cause diseases in humans, animals, or plants, but most are harmless and are beneficial ecological agents whose metabolic activities sustain higher life-forms.

Other bacteria are symbionts of plants and invertebrates, where they carry out important functions for the host, such as nitrogen fixation and cellulose degradation. Without prokaryotes, soil would not be fertile, and dead organic material would decay much more slowly.

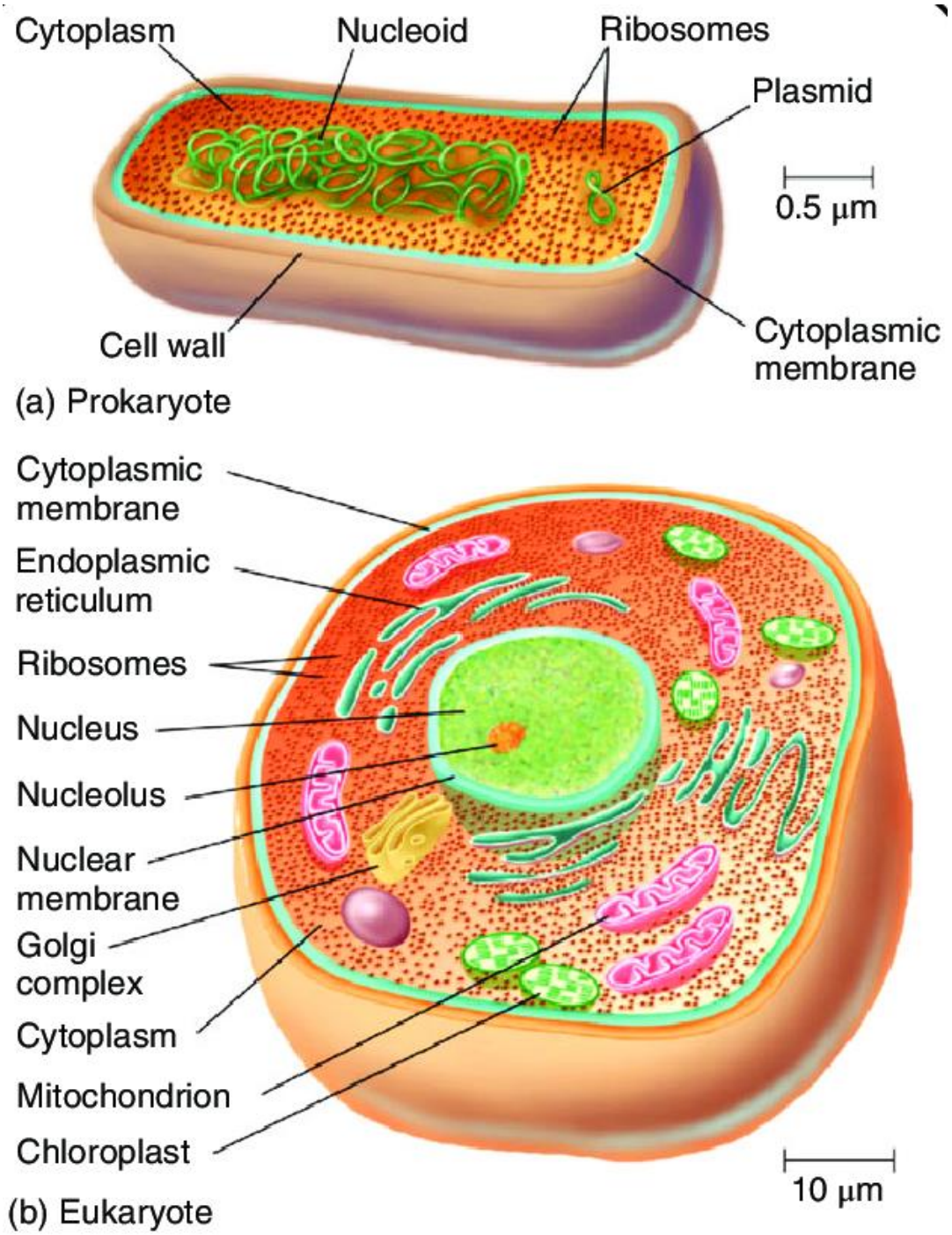
Some bacteria are widely used in the preparation of foods, chemicals, and antibiotics. Studies of the relationships between different groups of bacteria continue to yield new insights into the origin of life on Earth and mechanisms of evolution.

The bacterial cell

Bacteria as prokaryotes

Bacterial cells differ from animal cells and plant cells in several ways.

One fundamental difference is that bacterial cells lack intracellular organelles, such as mitochondria, chloroplasts, and a nucleus, which are present in both animal cells and plant cells.



All living organisms on Earth are made up of one of two basic types of cells: eukaryotic cells, in which the genetic material is enclosed within a nuclear membrane, or prokaryotic cells, in which the genetic material is not separated from

the rest of the cell. Traditionally, all prokaryotic cells were called bacteria and were classified in the prokaryotic kingdom Monera.

However, their classification as Monera, equivalent in taxonomy to the other kingdoms—Plantae, Animalia, Fungi, and Protista—understated the remarkable genetic and metabolic diversity exhibited by prokaryotic cells relative to eukaryotic cells. In the late 1970s American microbiologist Carl Woese pioneered a major change in classification by placing all organisms into three domains—Eukarya, Bacteria (originally called Eubacteria), and Archaea (originally called Archaebacteria)—to reflect the three ancient lines of evolution.

The prokaryotic organisms that were formerly known as bacteria were then divided into two of these domains, Bacteria and Archaea. Bacteria and Archaea are superficially similar; for example, they do not have intracellular organelles, and they have circular DNA. However, they are fundamentally distinct, and their separation is based on the genetic evidence for their ancient and separate evolutionary lineages, as well as fundamental differences in their chemistry and physiology. Members of these two prokaryotic domains are as different from one another as they are from eukaryotic cells.

Prokaryotic cells (i.e., Bacteria and Archaea) are fundamentally different from the eukaryotic cells that constitute other forms of life. Prokaryotic cells are

defined by a much simpler design than is found in eukaryotic cells. The most-apparent simplification is the lack of intracellular organelles, which are features characteristic of eukaryotic cells.

Organelles are discrete membrane-enclosed structures that are contained in the cytoplasm and include the nucleus, where genetic information is retained, copied, and expressed; the mitochondria and chloroplasts, where chemical or light energy is converted into metabolic energy; the lysosome, where ingested proteins are digested and other nutrients are made available; and the endoplasmic reticulum and the Golgi apparatus, where the proteins that are synthesized by and released from the cell are assembled, modified, and exported. All of the activities performed by organelles also take place in bacteria, but they are not carried out by specialized structures.

In addition, prokaryotic cells are usually much smaller than eukaryotic cells. The small size, simple design, and broad metabolic capabilities of bacteria allow them to grow and divide very rapidly and to inhabit and flourish in almost any environment.

Prokaryotic and eukaryotic cells differ in many other ways, including lipid composition, structure of key metabolic enzymes, responses to antibiotics and toxins, and the mechanism of expression of genetic information.

Eukaryotic organisms contain multiple linear chromosomes with genes that are much larger than they need to be to encode the synthesis of proteins. Substantial portions of the ribonucleic acid (RNA) copy of the genetic information (deoxyribonucleic acid, or DNA) are discarded, and the remaining messenger RNA (mRNA) is substantially modified before it is translated into protein.

In contrast, bacteria have one circular chromosome that contains all of their genetic information, and their mRNAs are exact copies of their genes and are not modified.

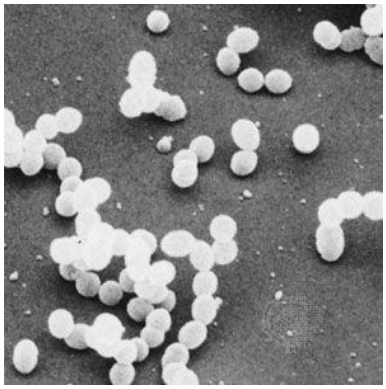
Diversity of structure of bacteria

Although bacterial cells are much smaller and simpler in structure than eukaryotic cells, the bacteria are an exceedingly diverse group of organisms that differ in size, shape, habitat, and metabolism.

Much of the knowledge about bacteria has come from studies of disease-causing bacteria, which are more readily isolated in pure culture and more easily investigated than are many of the free-living species of bacteria. It must be noted that many free-living bacteria are quite different from the bacteria that are adapted to live as animal parasites or symbionts.

Thus, there are no absolute rules about bacterial composition or structure, and there are many exceptions to any general statement.

Streptococcus mutans



The bacterium *Streptococcus mutans* is an example of a spherical (coccus) bacterium. This species of bacteria commonly aggregates into pairs and short chains.

Individual bacteria can assume one of three basic shapes: spherical (coccus), rodlike (bacillus), or curved (vibrio, spirillum, or spirochete). Considerable variation is seen in the actual shapes of bacteria, and cells can be stretched or compressed in one dimension.

Bacteria that do not separate from one another after cell division form characteristic clusters that are helpful in their identification. For example, some cocci are found mainly in pairs, including *Streptococcus pneumoniae*, a pneumococcus that causes bacterial lobar pneumonia, and *Neisseria gonorrhoeae*, a gonococcus that causes the sexually transmitted disease gonorrhea. Most streptococci resemble a long strand of beads, whereas the staphylococci form random clumps (the name "staphylococci" is derived from the Greek word *staphyle*, meaning "cluster of grapes").

In addition, some coccal bacteria occur as square or cubical packets. The rod-shaped bacilli usually occur singly, but some strains form long chains, such as rods of the corynebacteria, normal inhabitants of the mouth that are frequently attached to one another at random angles.

Some bacilli have pointed ends, whereas others have squared ends, and some rods are bent into a comma shape. These bent rods are often called vibrios and include *Vibrio cholerae*, which causes cholera. Other shapes of bacteria include the spirilla, which are bent and rebent, and the spirochetes, which form a helix similar to a corkscrew, in which the cell body is wrapped around a central fiber called the axial filament.

Bacteria are the smallest living entities. An average-size bacterium—such as the rod-shaped *Escherichia coli*, a normal inhabitant of the intestinal tract of humans and animals—is about 2 micrometers (μm; millionths of a meter) long and 0.5 μm in diameter, and the spherical cells of *Staphylococcus aureus* are up to 1 μm in diameter.

A few bacterial types are even smaller, such as *Mycoplasma pneumoniae*, which is one of the smallest bacteria, ranging from about 0.1 to 0.25 μm in width and roughly 1 to 1.5 μm in length; the rod-shaped *Bordetella pertussis*, which is the causative agent of whooping cough, ranging from 0.2 to 0.5 μm

in diameter and 0.5 to 1 μm in length; and the corkscrew-shaped *Treponema pallidum*, which is the causative agent of syphilis, averaging only 0.1 to 0.2 μm in diameter but 6 to 15 μm in length. The cyanobacterium *Synechococcus* averages about 0.5 to 1.6 μm in diameter.

Some bacteria are relatively large, such as *Azotobacter*, which has diameters of 2 to 5 μm or more; and *Achromatium*, which has a minimum width of 5 μm and a maximum length of 100 μm, depending on the species. Giant bacteria can be visible with the unaided eye, such as *Thiomargarita namibiensis*, which averages 750 μm in diameter; *T. magnifica*, which averages 700 μm in diameter and 1 cm in length; and the rod-shaped *Epulopiscium fishelsoni*, which ranges from 30 to more than 600 μm in length.

Bacteria are unicellular microorganisms and thus are generally not organized into tissues. Each bacterium grows and divides independently of any other bacterium, although aggregates of bacteria, sometimes containing members of different species, are frequently found. Many bacteria can form aggregated structures called biofilms.

Organisms in biofilms often display substantially different properties from the same organism in the individual state or the planktonic state. Bacteria that have aggregated into biofilms can communicate information about population size and

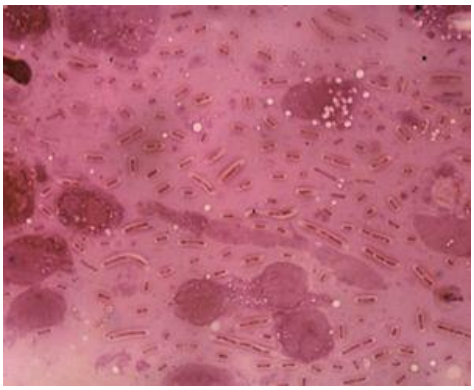
metabolic state. This type of communication is called quorum sensing and operates by the production of small molecules called autoinducers or pheromones.

The concentration of quorum-sensing molecules—most commonly peptides or acylated homoserine lactones (AHLs; special signaling chemicals)—is related to the number of bacteria of the same or different species that are in the biofilm and helps coordinate the behavior of the biofilm.

Morphological features of bacteria

The Gram stain

***Klebsiella pneumoniae* in pneumonia**



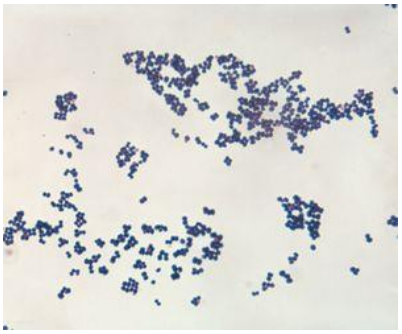
Gram-negative bacilli, *Klebsiella pneumoniae*, isolated from a lung abscess in a patient with pneumonia.

Bacteria are so small that their presence was only first recognized in 1677, when the Dutch naturalist Antonie van Leeuwenhoek saw microscopic organisms in a variety of substances with the aid of primitive microscopes (more similar in design to modern magnifying glasses than modern microscopes), some of which were capable of more than 200-fold magnification. Now bacteria are usually examined under light microscopes capable of more than 1,000-fold

magnification; however, details of their internal structure can be observed only with the aid of much more powerful transmission electron microscopes.

Unless special phase-contrast microscopes are used, bacteria have to be stained with a colored dye so that they will stand out from their background.

Staphylococcus aureus



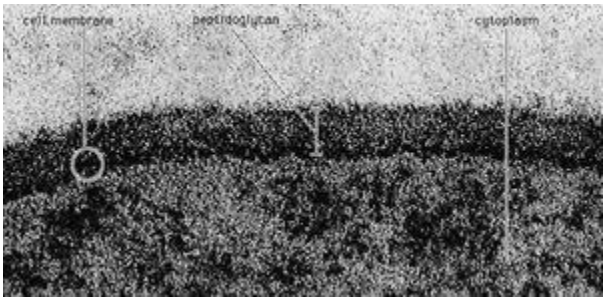
Gram-positive cocci, *Staphylococcus aureus*, in a laboratory culture.

One of the most useful staining reactions for bacteria is called the Gram stain, developed in 1884 by the Danish physician Hans Christian Gram. Bacteria in suspension are fixed to a glass slide by brief heating and then exposed to two dyes that combine to form a large blue dye complex within each cell.

When the slide is flushed with an alcohol solution, gram-positive bacteria retain the blue color and gram-negative bacteria lose the blue color. The slide is then stained with a weaker pink dye that causes the gram-negative bacteria to become pink, whereas the gram-positive bacteria remain blue.

The Gram stain reacts to differences in the structure of the bacterial cell surface, differences that are apparent when the cells are viewed under an electron microscope.

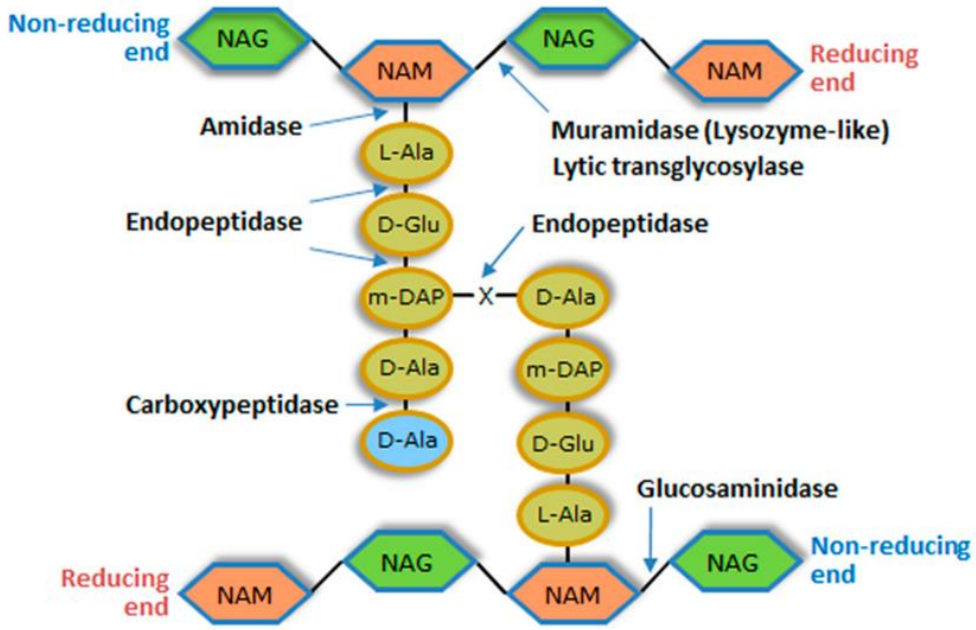
The cell envelope



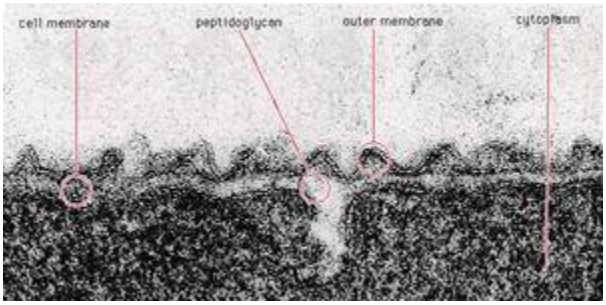
Peptidoglycan layer of *Bacillus coagulans*

A portion of the gram-positive bacterium *Bacillus coagulans* showing the cell wall's thick peptidoglycan layer that surrounds the cell membrane.

The bacterial cell surface (or envelope) can vary considerably in its structure, and it plays a central role in the properties and capabilities of the cell. The one feature present in all cells is the cytoplasmic membrane, which separates the inside of the cell from its external environment, regulates the flow of nutrients, maintains the proper intracellular milieu, and prevents the loss of the cell's contents.



The cytoplasmic membrane carries out many necessary cellular functions, including energy generation, protein secretion, chromosome segregation, and efficient active transport of nutrients. It is a typical unit membrane composed of proteins and lipids, basically similar to the membrane that surrounds all eukaryotic cells. It appears in electron micrographs as a triple-layered structure of lipids and proteins that completely surround the cytoplasm.



Peptidoglycan layer of *Aquaspirillum serpens*

The gram-negative bacterium *Aquaspirillum serpens* has a thin peptidoglycan layer that lies between the cell membrane and the outer membrane.

Lying outside of this membrane is a rigid wall that determines the shape of the bacterial cell. The wall is made of a huge molecule called peptidoglycan (or murein). In gram-positive bacteria the peptidoglycan forms a thick meshlike layer that retains the blue dye of the Gram stain by trapping it in the cell. In contrast, in gram-negative bacteria the peptidoglycan layer is very thin (only one or two molecules deep), and the blue dye is easily washed out of the cell.

Peptidoglycan occurs only in the Bacteria (except for those without a cell wall, such as *Mycoplasma*). Peptidoglycan is a long-chain polymer of two repeating sugars (n-acetylglucosamine and n-acetyl muramic acid), in which adjacent sugar chains are linked to one another by peptide bridges that confer rigid stability.

The nature of the peptide bridges differs considerably between species of bacteria but in general consists of four amino acids: l-alanine linked to d-glutamic acid, linked to either diaminopimelic acid in gram-negative bacteria or l-lysine, l-ornithine, or diaminopimelic acid in gram-positive bacteria, which is finally linked to d-alanine.

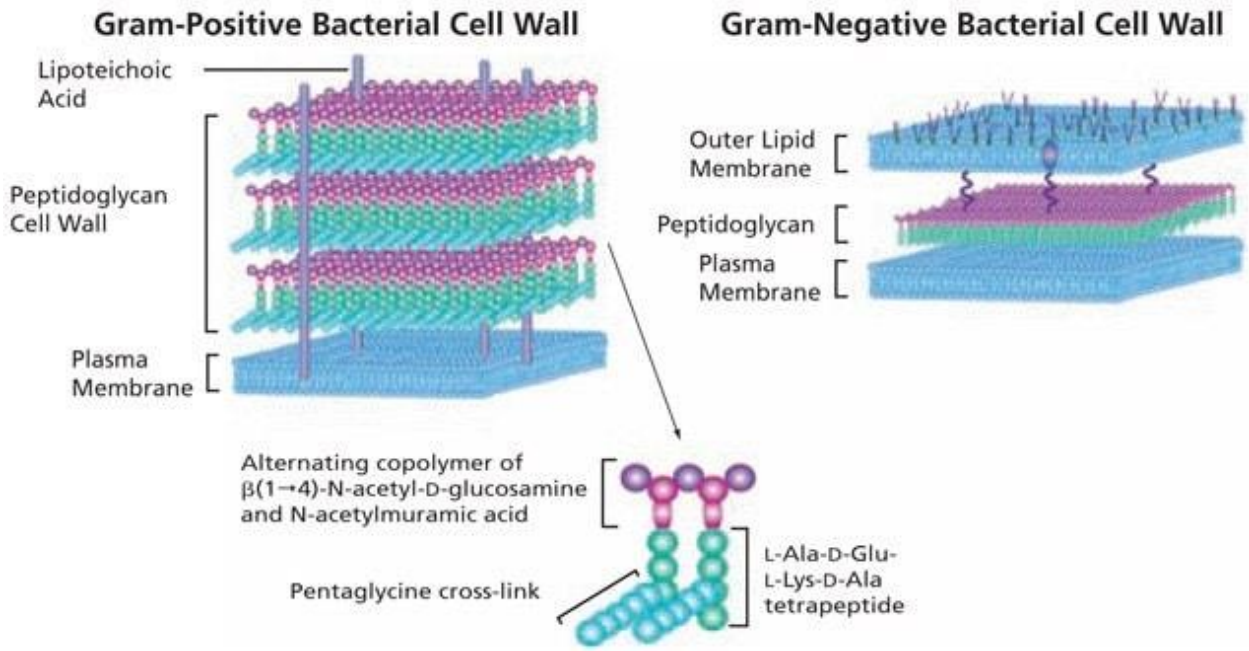
In gram-negative bacteria the peptide bridges connect the d-alanine on one chain to the diaminopimelic acid on another chain. In gram-positive bacteria there can be an additional peptide chain that extends the reach of the cross-link; for example, there is an additional bridge of five glycines in *Staphylococcus aureus*.

Peptidoglycan synthesis is the target of many useful antimicrobial agents, including the β-lactam antibiotics (e.g., penicillin) that block the cross-linking of the peptide bridges. Some of the proteins that animals synthesize as natural antibacterial defense factors attack the cell walls of bacteria. For example, an enzyme called lysozyme splits the sugar chains that are the backbone of peptidoglycan molecules. The action of any of these agents weakens the cell wall and disrupts the bacterium.

In gram-positive bacteria the cell wall is composed mainly of a thick peptidoglycan meshwork interwoven with other polymers called teichoic acids (from the Greek word *teichos*, meaning "wall") and some proteins or lipids. In contrast, gram-negative bacteria have a complex cell wall that is composed of multiple layers in which an outer membrane layer lies on top of a thin peptidoglycan layer.

This outer membrane is composed of phospholipids, which are complex lipids that contain molecules of phosphate, and lipopolysaccharides, which are complex

lipids that are anchored in the outer membrane of cells by their lipid end and have a long chain of sugars extending away from the cell into the medium.



Lipopolysaccharides, often called endotoxins, are toxic to animals and humans; their presence in the bloodstream can cause fever, shock, and even death. For most gram-negative bacteria, the outer membrane forms a barrier to the passage of many chemicals that would be harmful to the bacterium, such as dyes and detergents that normally dissolve cellular membranes.

Impermeability to oil-soluble compounds is not seen in other biological membranes and results from the presence of lipopolysaccharides in the membrane and from the unusual character of the outer membrane proteins. As evidence of the

ability of the outer membrane to confer resistance to harsh environmental conditions, some gram-negative bacteria grow well in oil slicks, jet fuel tanks, acid mine drainage, and even bottles of disinfectants.

Bacterial Staining

Staining techniques use dyes to enhance the visibility of microorganisms and their components under a microscope. Since most microbes are colorless, staining allows microbiologists to observe cell morphology, size, and arrangement. There are several types of staining methods:

Staining Methods

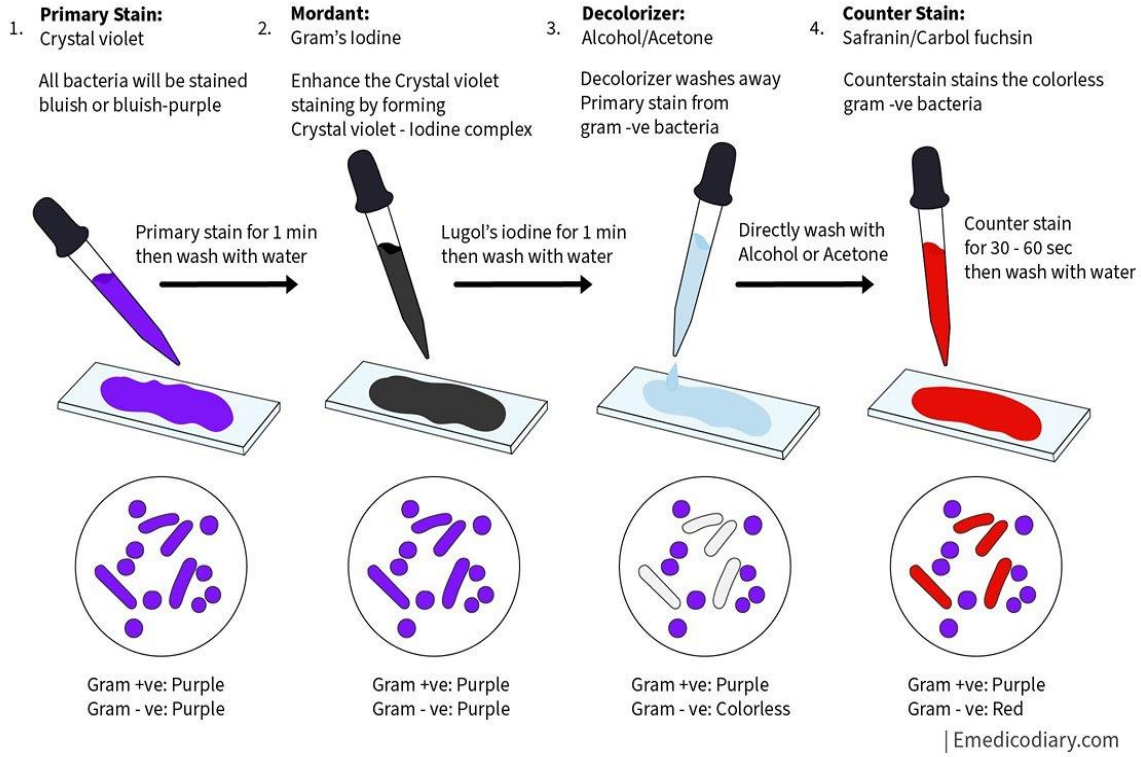
1. Simple staining

- **Method**: Uses a single basic dye, such as methylene blue, crystal violet, or carbolfuchsin, to stain all cells uniformly.
- **Purpose**: To quickly determine the basic shape (cocci, bacilli, spirilla) and arrangement (clusters, chains) of bacteria.

2. Differential staining

- **Method**: Employs more than one chemical dye to distinguish between different types of cells or structures. The most common examples are Gram staining and acid-fast staining.

- **Gram staining**: This technique differentiates bacteria into two major groups based on their cell wall composition.



Procedure:

Primary stain: Crystal violet is applied, coloring all cells purple.

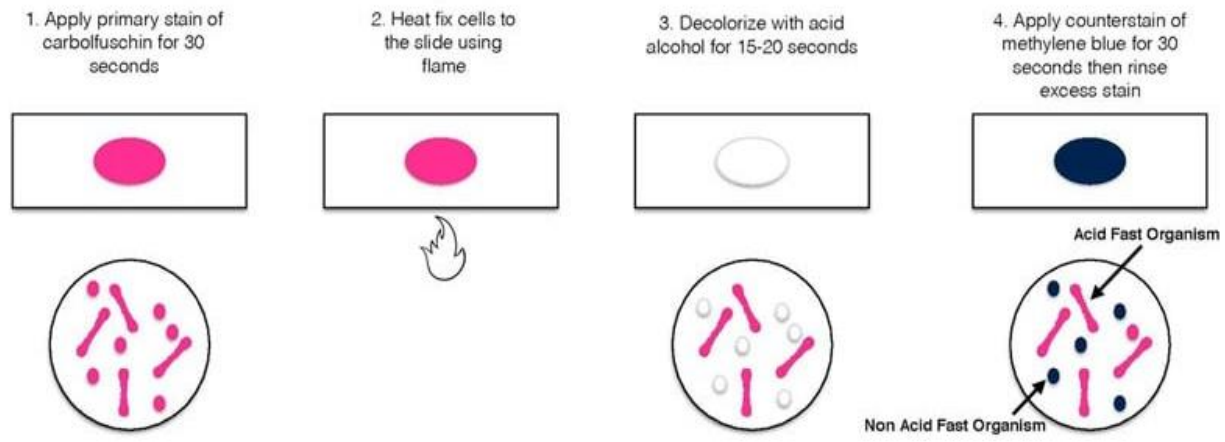
Mordant: Gram's iodine is added to form a complex with the crystal violet, anchoring the dye inside the cell.

Decolorizer: Alcohol or acetone is used to wash out the stain. Gram-positive bacteria, with thick peptidoglycan cell walls, retain the crystal violet and remain

purple. Gram-negative bacteria, with thinner cell walls and an outer membrane, lose the purple stain.

Counterstain: Safranin is applied, staining the decolorized Gram-negative cells pink or red.

Acid-fast staining (Ziehl-Neelsen method): This method is used to identify bacteria with waxy, lipid-rich cell walls, such as *Mycobacterium tuberculosis*.



Procedure: The primary stain, carbol fuchsin, is applied with heat to penetrate the waxy cell wall. Acid-alcohol is then used as a decolorizer, but acid-fast bacteria resist decolorization. Methylene blue is used as a counterstain.

Result: Acid-fast bacteria appear red, while non-acid-fast cells appear blue.

3. Special staining

- **Method**: These techniques are used to highlight specific bacterial structures that are not visible with simple or differential stains.
    - **Negative staining**: Uses an acidic dye (e.g., India ink or nigrosin) that stains the background but is repelled by the negatively charged bacterial cell. This leaves the cells clear against a dark background and is particularly useful for observing capsules.
    - **Endospore staining**: The Schaeffer-Fulton method uses malachite green with heat to stain the spore, while a counterstain like safranin stains the vegetative cell. This differentiates between endospores (green) and vegetative cells (red or pink).
    - **Flagella staining**: This technique coats the flagella with a mordant and stain to increase their thickness and make them visible under a light microscope.