The Microbial World

The microbial world includes the kinds of cells that van Leeuwenhoek observed looking through his simple microscope. Although he could not realize it at the time, the microbial world, infact all living organisms can be classified into one of three major groups called domains. Organisms in each domain share properties of their cells that distinguish them from members of the other domains. The three domains are the **Bacteria** (formerly called Eubacteria), the **Archaea** (meaning ancient), and the **Eukarya**.

Microscopically, members of the Bacteria and Archaea look identical (Figure 1-1). Both are single-celled organisms that do not contain a membrane-bound nucleus nor any other intracellular lipid-bound organelles. Their genetic information is stored in fibrils composed of deoxyribonucleic acid (DNA) in a region called the nucleoid. These simple cell types have their cytoplasm surrounded by a rigid cell wall and are termed prokaryotes, which means "**prenuclcus**". All bacteria and archaea are prokaryotes. These two groups of prokaryotes, however, differ significantly in their chemical composition.

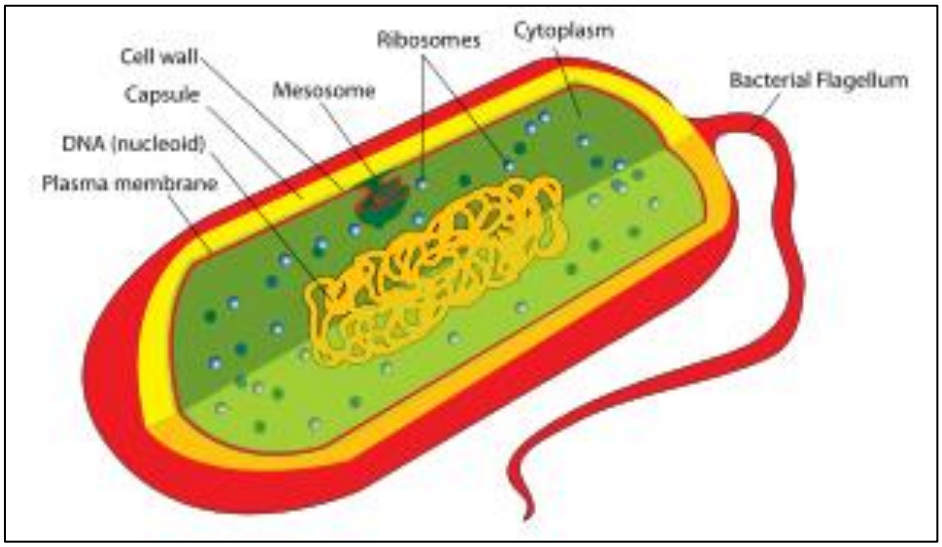


Figure 1-1: The structure of bacterial cell

Members of the Eukarya, termed eukaryotes, which means "**true nucleus**, are distinctly different from members of the Bacteria and Archaca. Eukaryotes may be single-celled or multicellular, but they always contain a true membrane-bound nucleus and other internal cell organelles, making them far more complex than the simple prokaryotes (figure 1-2). These structures include mitochondria, organelles for generating energy, and chloroplasts, which harvest light energy' in plants.

Eukaryotes also have an internal scaffolding, the cytoskeleton, which gives the cells their shape. All algae, fungi, protozoa, and multicellular parasites considered in this book are eukaryotes. The prokaryotes and eukaryotes are compared in table 1-1.

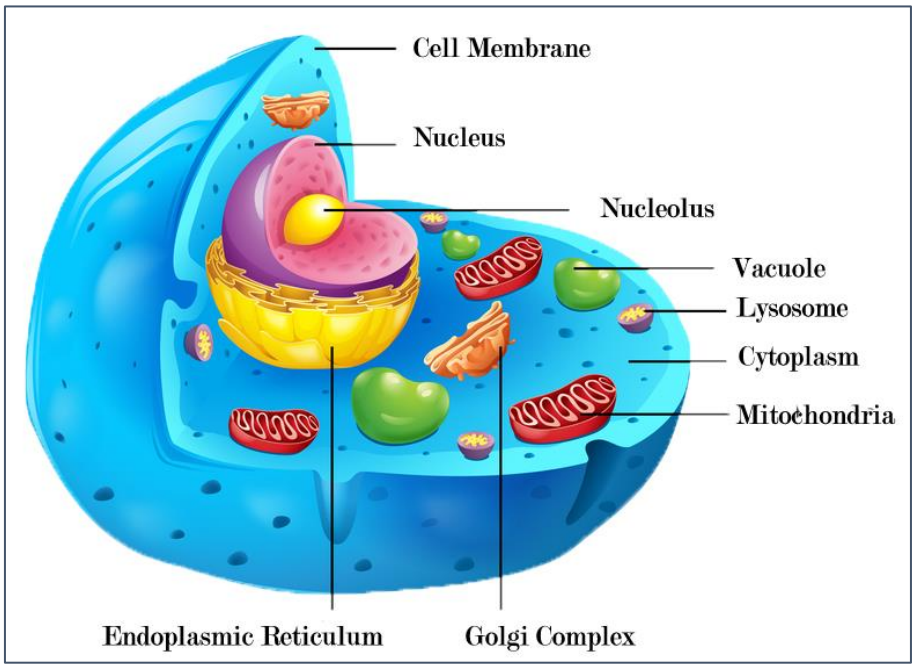


Figure 1.2: Eukaryotic Cell .

The Bacteria

Most of the prokaryotes covered in this text are members of the domain bacteria. even within this group, much diversity is seen in the shape and properties of the organisms. At this time we will only describe the properties of the typical bacteria that most frequently cause disease and carry out the biochemical reactions necessary to support life on earth. Their most prominent features are:

- They are all single-celled prokaryotes.
- They have specific shapes, most commonly, cylindrical (rod-shaped), spherical (round), or spiral
- They have rigid cell walls, which are responsible for the shape of the organism. The walls contain an unusual chemical compound called peptidoglycan, which is not found in organisms in the other domains.
- They multiply by binary fission in which one cell divides into two cells, each identical to the original cell.
- Many can move using appendages extending from the cell, called flagella (sing: flagellum).

The Archaea

The Archaea have the same shape, size, and appearance as the Bacteria like the Bacteria, the Archaea multiply by binary fission and move primarily by means of flagella. They also have rigid cell walls. The chemical composition of their cell wall, however, differs from that in the Bacteria. The Archaea do not have peptidoglycan as part of their cell walls. Other chemical differences also exist between these two groups. Perhaps the most interesting and distinguishing feature of the Archaea as a group is their ability to grow in environments in which most organisms including the

Bacteria, cannot survive. For example, some archaea can grow in salt concentrations 10 times as high as that found in seawater. These organisms grow in such habitats as the Great Salt Lake and the Dead Sea. Other archaea grow best at extremely high temperatures. One member grows best at temperatures above 105°C (100°C is the temperature at which water boils at sea level). Some archaea can be found in the boiling hot springs at Yellowstone National Park. Members of the Archaea, however, are spread far beyond extreme environments. They are widely distributed in the oceans, and they are found in the cold surface waters of Antarctica and Alaska.

Table 1-1 Comparisons among Bacteria, Archaea, and Eukarya

character	**Bacteria**	**Archaea**	**Eukarya**
Size	0.3-2µm	0.3-2µm	2-20µm
Nuclear Membrane	No	No	Yes
Cell Wall	Peptidoglycan present	No peptidoglycan	No peptidoglycan
Cytoplasmic structures	No	No	Yes
Mitochondria	No	No	In plant and that cells
Chloroplasts	No	No	Yes
Cytoskeleton	In all environments that	Extreme environments	In all environments that
Where found	are not extreme		are not extreme

II. Historical Microbiology

A. Reasons why Microbiology was slow to develop as a science

1. tendency to explain natural phenomena with superstition , magic and chance
2. lack of professional organization essential to sharing information
3. lack of microscopes adequate enough to view microscopic life
4. lack of scientific method
5. Existence of the concept of spontaneous generation (Abiogenesis); which is a belief that life could arise from non-living things that first stated by Aristotle.

B. Redi and Spontaneous Generation

1. Aristotle state this belief of abiogenesis
2. Redi (1668) worked with decaying meat and cheese cloth to disprove spontaneous generation as it related to organism visible with the naked eye
3. Redi's experiment when the flask remained unsealed, maggots covered the meat within a few days. When the flask was sealed, flies were kept away and no maggots appeared on the meat. When the flask opening was covered with gauze, flies were kept away and no maggots appeared on the meat, although a few maggots appeared on top of the gauze.
4. The cheesecloth was used to appease those who said that the "vital force" in air was needed for spontaneous generation.

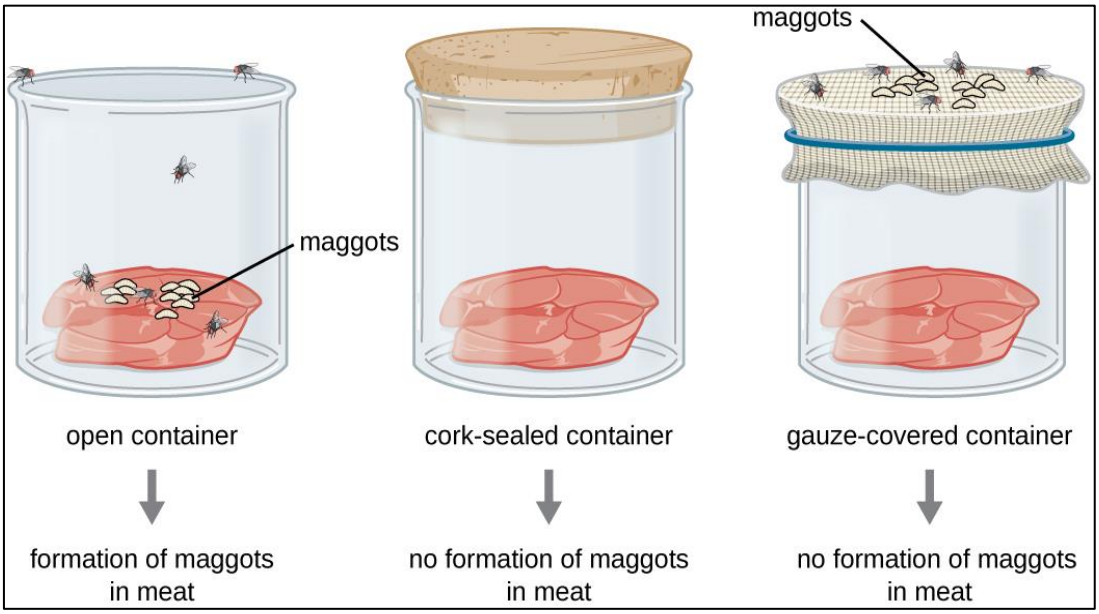


Figure 1-3: Redi's experiment; unsealed flask, maggots covered the meat within a few days. Sealed flask, flies were kept away and no maggots appeared on the meat. Covered flask with gauze, flies was kept away and no maggots appeared on the meat, although a few maggots appeared on top of the gauze.

Anton Van Leeuwenhoek (1674)

a. call by many as the "father of microbiology" but is given the title of father of bacteriology and protozoology

b. improved the microscope and was the first to see microscopic organism and called then animalcules
c. he saw protozoans, fungi, and bacteria
d. described them in a letter to the Royal Society of London
e. His microscope open up the debate related to microscopic life forms.

Louis Pasteur (1861)

a. boiled broth in swan neck flask
b. these flask allowed air to enter
c. the purpose of the bent neck was to prevent uncreated air with dust and microbes could not enter.
d. the contents of the flask remained sterile
e. note that Pasteur was fortunate that the foods he boiled into broths did not contain bacterial spores
f. His experiment disproved spontaneous generation.

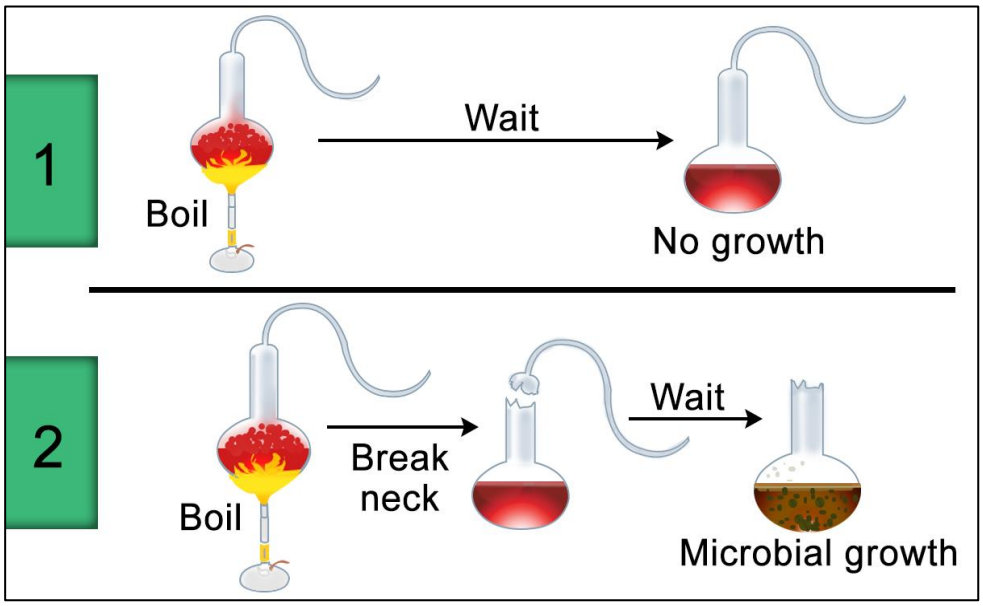


Figure 1-4: Pasture's swan flask experiment; 1) boiling broth media within swan flask left no contamination. 2) Breaking the neck of swan flask led to contaminate the broth with bacteria

Buchner s Experiment

In 1897 the German scientist Eduard Buchner (1860 – 1917) resurrected the chemical by showing that **fermentation does not require living cells**. Buchner's experiments demonstrated the presence of *enzymes* (Figure 1-5), which are cell-produced proteins that promote chemical reactions. Buchner's work began the field of **biochemistry** and the study of metabolism, a term that refers to the sum of all chemical reactions within an organism.

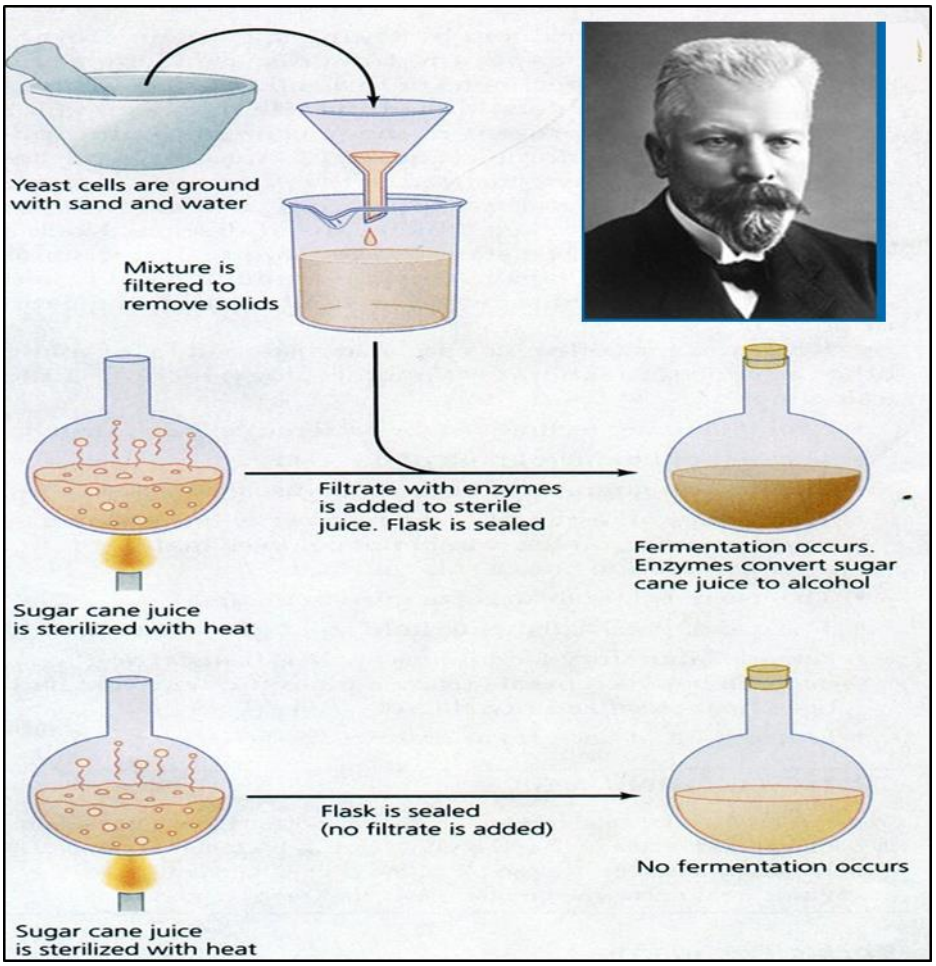


Figure1-5: Buchner's experiment; **fermentation occurred in cell-free yeast extract showing the role of enzyme.**

What Causes disease?

Pasteur's discovery that bacteria are responsible for spoiling wine led naturally to his hypothesis in 1857 that microorganisms are also responsible for diseases. This idea came to be known as the **germ theory of disease**. Since a particular disease is

typically accompanied by the same symptoms in all affected individuals. Early investigators suspected that diseases such as cholera, tuberculosis, and anthrax are each caused by a specific germ, called a pathogen. Today we know that some diseases are genetic and that allergic reactions and environmental toxins cause others, so the germ theory applies only to infectious diseases.

Just as Pasteur was the chief investigator in disproving spontaneous generation and determining the cause of fermentation, investigations in etiology (the study of causation of disease) were dominated by **Robert Koch** (1843-1910).

Koch and his colleagues are also responsible for many other advances in laboratory microbiology, including the following:

- Simple staining techniques for bacterial cells and flagella
- The first photomicrograph of bacteria
- The first photograph of bacteria in diseased tissue
- Techniques for estimating the number of bacteria in a solution based on the number of colonies that form after inoculation onto a solid surface
- The use of steam to sterilize growth media
- The use of Petri dishes to hold solid growth media
- Aseptic laboratory techniques such as transferring bacteria between media using a platinum wire that had been heat-sterilized in a flame.
- Elucidation of bacteria as distinct species

Koch's Postulates

After discovering the anthrax bacterium, Koch continued to search for disease agents. In two pivotal scientific publications in 1882 and 1884, he announced that the cause of tuberculosis was a rod-shaped bacterium, *Mycobacterium tuberculosis*. In 1905 he received the Nobel Prize in Physiology or Medicine for this work.

In his publications on tuberculosis, Koch elucidated a series of steps that must be taken to prove the cause of any infectious disease. These steps, now known as

Koch's postulates, are one of his more important contributions to microbiology. The postulates, are the following:

1. The suspected causative agent must be found in every case of the disease and be absent from healthy hosts.
2. The agent must be isolated and grown outside the host.
3. When the agent is introduced to a healthy, susceptible host, the host must get the disease.
4. The same agent must be reisolated from the diseased experimental host.

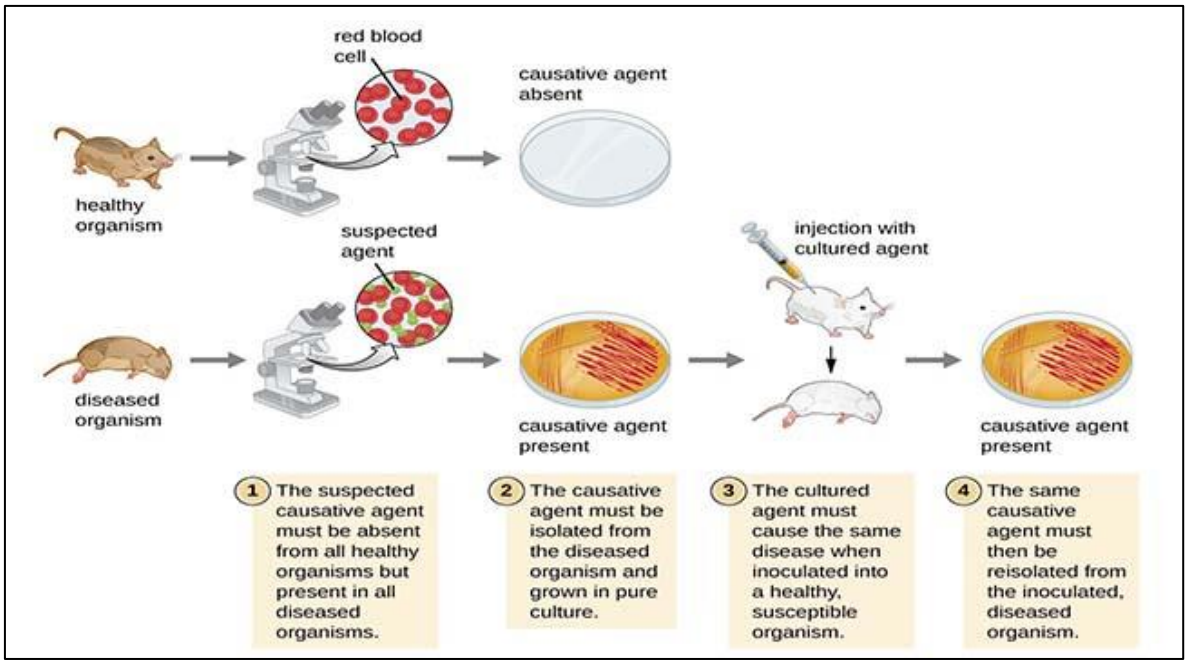


Figure 1-6: Four steps of Koch's postulates

Semmelweis and Hand-washing

Ignaz Semmelweis (1818-1865) was a physician in the obstetric ward of a physician teaching hospital in Vienna. He is the first person who began requiring medical students to wash their hands with chlorinated lime water, a substance long used to eliminate the smell of cadavers.

Jenner's Vaccine

In about 1789, the English physician Edward Jenner (1749-1823) tested the hypothesis that a mild disease called cowpox provided protection against potentially fatal smallpox.

Jenner invents vaccination (the term *immunization* is often synonymously today) reestablished a safe treatment for preventing smallpox, and began the field of immunology "the study of the body's specific defenses against pathogen".

Ehrlich's "Magic Bullets"

Gram's discovery that bacteria could be differentiated into two types by staining suggested to the German microbiologist Paul Ehrlich (1854-1915) that chemicals could be used to kill microorganisms differentially. To investigate this idea Ehrlich undertook an exhaustive survey of chemicals to find "magic bullet" that would destroy pathogens while remaining nontoxic to humans. By 1908, he had discovered chemicals active against trypanosomes (the protozoan parasites that cause sleeping sicknesses) and against *Treponema pallidum* (trep-6-ne'ma pal'li-dum), the causative agent of syphilis. His discoveries began the branch of medical microbiology known as chemotherapy.

The structure of prokaryotic cell

Bacterial Cell Wall

The bacterial cell wall is a unique structure which surrounds the cell membrane. Although not present in every bacterial species, the cell wall is very important as a cellular component.

Structurally, the wall is necessary for:

- **Maintaining the cell's characteristic shape**- the rigid wall compensates for the flexibility of the phospholipid membrane and keeps the cell from assuming a spherical shape
- **Countering the effects of osmotic pressure**- the strength of the wall is responsible for keeping the cell from bursting when the intracellular osmolality is much greater than the extracellular teichoic Osmolality.
- **Providing attachment sites for bacteriophages**-teichoic acids attached to the outer surface of the wall are like landing pads for viruses that infect bacteria
- **Providing a rigid platform for surface appendages**- flagella, fimbriae, and pili all emanate from the wall and extend beyond it.

The cell walls of all bacteria are not identical. In fact, cell wall composition is one of the most important factors in bacterial species analysis and differentiation. There are two major types of walls: **Gram-positive and Gram-negative**. The cell wall of **Gram-positive bacteria** consists of many polymer layers of peptidoglycan connected by amino acid bridges. A schematic diagram provides the best explanation of the structure. The peptidoglycan polymer is composed of an alternating sequence of **N-acetylglucosamine** and **N-acetyl-muramic** acid; it's a lot easier to just remember NAG and NAMA. Each peptidoglycan layer is connected, or crosslinked, to the other by a bridge made of amino acids and amino acid derivatives. The particular amino acids vary among different species. However, the cross-linked

peptidoglycan molecules form a network which covers the cell like a grid. Also, 90% of the Gram-positive cell wall is comprised of peptidoglycan.

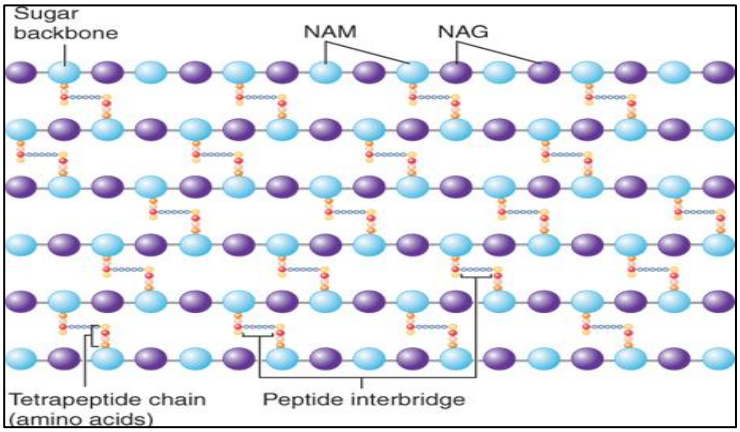


Figure 2-1: the structure of peptidoglycan in bacterial cell wall

The cell wall of Gram-negative bacteria is much thinner, being comprised of only 20% peptidoglycan. Gram-negative bacteria also have two unique regions which surround the outer plasma membrane: the periplasmic space and the **lipopolysaccharide layer (LPS)**. The **periplasmic space** separates the outer plasma membrane from the peptidoglycan layer. It contains proteins which destroy potentially dangerous foreign matter present in this space. The lipopolysaccharide layer is located adjacent to the exterior peptidoglycan layer. It is a phospholipid bilayer construction similar to that in the cell membrane and is attached to the peptidoglycan by lipoproteins. The lipid portion of the LPS contains a toxic substance, called **Lipid A**, which is responsible for most of the pathogenic affects associated with harmful Gram-negative bacteria. **Polysaccharides** which extend out from the bilayer also contribute to the toxicity of the LPS. The LPS, lipoproteins, and the associated polysaccharides together form what is known as the outer membrane.

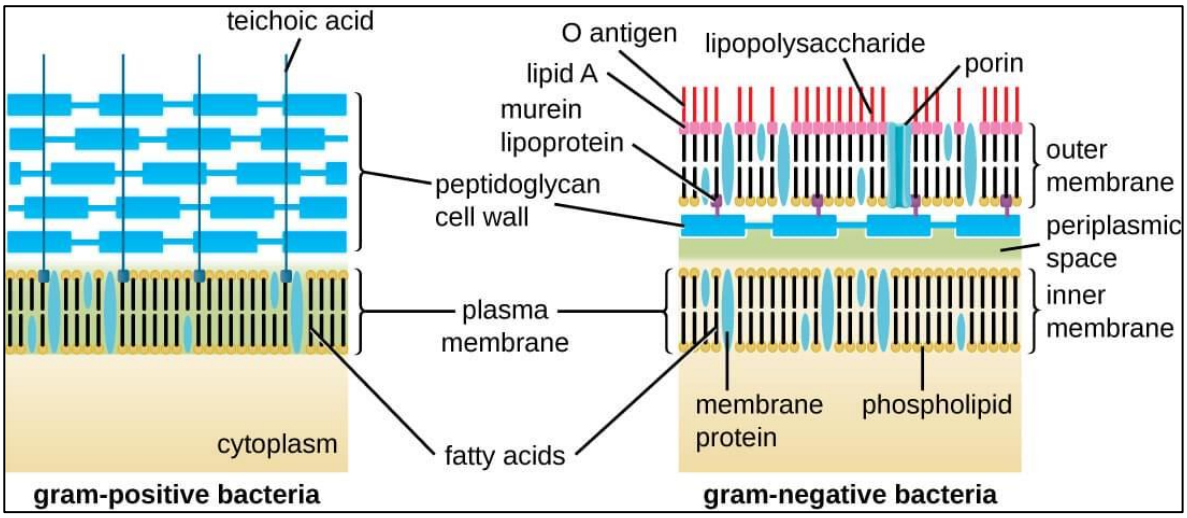


Figure 2-2 : The differences between gr+ve and gr-ve cell wall composition

Antibacterial Compounds that Target Peptidoglycan

These compounds include the antibiotic penicillin and the enzyme lysozyme, which is found in many body fluids including tears and saliva.

Penicillin

Penicillin is the most thoroughly studied of a group of antibiotics that interfere with peptidoglycan synthesis. Penicillin binds to proteins involved in cell wall synthesis and subsequently prevents the cross-linking of adjacent glycan chains. These proteins are called penicillin-binding proteins, a name that reflects their medical importance rather than their role in peptidoglycan synthesis.

Generally, but with notable exceptions, penicillin is far more effective against Gram-positive cells than Gram-negative cells. This is because the outer membrane of Gram-negative cells prevents the medication from reaching its site of action, the peptidoglycan layer. However, the structure of penicillin can be modified to create penicillin derivatives that can pass through porin channels. These drugs are effective against a range of Gram-negative bacteria.

Lysozyme

Lysozyme breaks bond that links the alternating N-acetylglucosamine and the N-acetylmuramic acid molecules and thus destroys the structural integrity of the glycan chain, the backbone of the peptidoglycan molecule.

Lysozyme is sometimes used in the laboratory to remove the peptidoglycan layer from bacteria for experimental purposes. Removing that layer from a Gram-positive bacterium creates a **protoplast** that lacks a cell wall. In contrast, removing the peptidoglycan layer from Gram- negative bacterium creates a **spheroplast**. Spheroplasts retain some portions of the outer membrane. Bacteria they lack their rigid cell wall, protoplasts and spheroplasts both become spherical regardless of the original cell shape. Due to osmosis, they will burst unless maintained in a solution that has the same relative concentration of ions and small molecules as the cytoplasm.

Differences in Cell Wall Composition and the Gram Stain

Differences in the cell wall composition of Gram-positive and Gram-negative bacteria account for their staining characteristics. It is not the cell wall, however, but the inside of the cell that is stained by the crystal violet-iodine complex. The Gram-positive cell wall somehow retains the crystal violet-iodine complex even when subjected to the trauma of acetone-alcohol treatment, whereas the Gram-negative cell wall cannot (table 2.1). The precise mechanism that accounts for the differential aspect of the Gram stain is not entirely understood. Presumably, the decolorizing agent dehydrates the thick layer of peptidoglycan and in this dehydrated state the wall acts as a permeability barrier, retaining the dye. In contrast, the solvent action of acetone-alcohol easily damages the outer membrane of Gram-negative bacteria; their relatively thin layer of peptidoglycan cannot retain the dye complex. These bacteria lose the dye complex more readily than their Gram-positive counterparts. Also, as

Gram-positive cells age, they often lose their ability to retain the dye. This probably results from damage to their peptidoglycan layer that occurs as a consequence of aging.

Table 2-1: Correlation of Grams Main with other properties of Bacteria

Property	Gram-positive	Gram-negative
Thickness of wall	thick (20-80 nm)	thin (10) nm)
Number of layers	1	2
Peptidoglycan (murein) content	50%	10-20%
Teichoic acids in wall	present	absent
Lipid and lipoprotein content	0-3 %	58%
Protein content	0	9%
Lipopolysaccharide content	0	13%

Characteristics of Bacteria that Lack a Cell Wall

Some bacteria naturally lack a cell wall. Species of *Mycoplasma,* one of which causes a mild form of pneumonia, have an extremely variable shape because they lack a rigid cell wall (figure2-3). As expected, neither penicillin nor lysozyme affects these organisms. *Mycoplasma* and related bacteria can survive without a cell wall because their cytoplasmic membrane is stronger than that of most other bacteria. They have sterols in their membrane; these rigid, planar molecules stabilize membranes making them stronger.

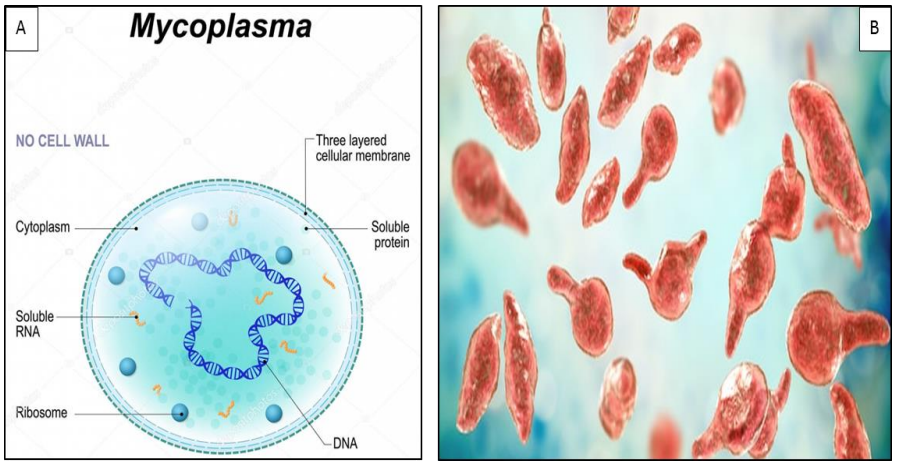


Figure 2-3: A) The shape of *Mycoplamsa* lacking cell wall.

B) The plastic shape of *Mycoplasma*, which lacks a rigid cell wall

Cell Wall of the domain Archaea

As a group, members of the Archaea inhabit a wide range of extreme environments, and so it is not surprising they contain a greater variety of cell wall types than do members of the Bacteria. However, because most of these organisms have not been studied as extensively as the Bacteria, less known about the structure of their walls. None contain peptidoglycan, but some do have a similar molecule **pseudopeptidoglycan**.

Cell membrane

The cell membrane, also called the **plasma membrane** or **plasmalemma**, is a **semipermeable lipid bilayer** common to all living cells. It contains a variety of biological molecules, primarily **proteins and lipids**, which are involved in a vast array of cellular processes. It also serves as the attachment point for both, the intracellular cytoskeleton and, if present, the cell wall. **Robert Hooke** was the first one to name the cells parts including the plasma membrane.

The cell membrane surrounds the cytoplasm of a cell and physically separates the intracellular components from the extracellular environment, thereby serving a mechanical function similar to that of skin. This barrier is able to regulate what enters

and exits the cell as it is selectively permeable - cells require a variety of substances to survive and the cell membrane serves as "**gatekeeper**" to what, and how much, enters and exits. The movement of substances across the membrane can be either **passive**, occurring without the input of cellular energy, or **active**, requiring the cell to expend energy moving it across the membrane.

Functions

Functions of the cell membrane include, but are not limited to:

- Controlling what goes in and out of the cell
- Anchoring of the cytoskeleton to provide shape to the cell
- Attaching to the extracellular matrix to help group cells together in the formation of tissues
- Transportation of particles by way of ion pumps, ion channels, and carrier proteins
- Containing receptors that allow chemical messages to pass between cells and systems
- Participation in enzyme activity important in such things as metabolism and immunity.

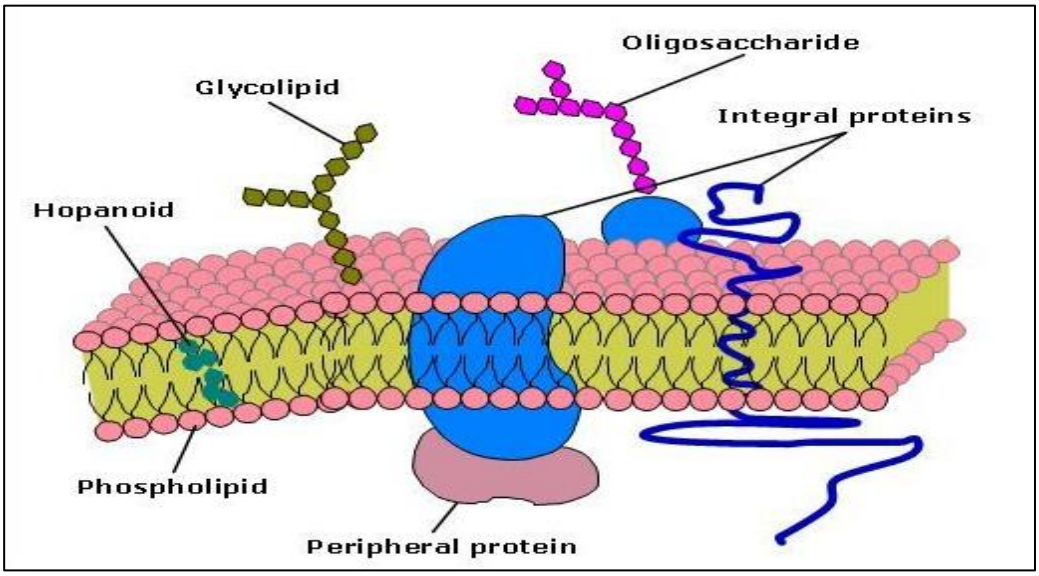


Figure 2-4: The structure of bacterial cell membrane

Surface Layers External to the Cell Wall

Bacteria may have one or more layers outside of the cell wall. The functions of some of these are well established, but that of others are unknown.

Glycocalyx

Many bacteria envelop themselves with a gel-like layer called a **glycocalyx** that generally functions as a mechanism of either protection or attachment (figure 2-5). If the layer is **distinct and gelatinous**, it is called a **capsule**; recall that capsules can be seen microscopically using a capsule stain. If, instead, the layer is **diffuse and irregular**, it is called a **slime layer**. Colonies of bacteria that form either of these extracellular layers often appear moist and glistening.

Capsules and slime layers vary in their **chemical composition** depending on the species of bacteria. Most are composed of **polysaccharides such as dextrans** and **glucans**. These take the form of tiny, short, hair-like structures or fibrils, which form a network on the outside of the cell wall. A few capsules consist of **polypeptides** made up of repeating subunits of only one or two amino acids.

Some types of capsules and slime layers enable bacteria to adhere to specific surfaces, including teeth, rocks, and other bacteria. These often enable microorganisms to grow as a **biofilm**, a mass of bacteria coating a surface. One example is dental plaque, a biofilm on teeth. *Streptococcus mutans uses* sucrose to synthesize a capsule, which enables it to adhere and grow in the crevices of the tooth. Other bacteria can then adhere to the layer created by the growth of *S. mutans*. Acid production by bacteria in the biofilm damages the tooth surface.

Some capsules enable bacteria to **thwart innate defense systems** that otherwise protect against infection. This is well illustrated in the case of the organism that causes bacterial pneumonia, *Streptococcus pneumoniae*. This organism can only cause disease if it has a capsule. Unencapsulated cells are quickly engulfed and killed by phagocytes an important cell of our innate defense system.

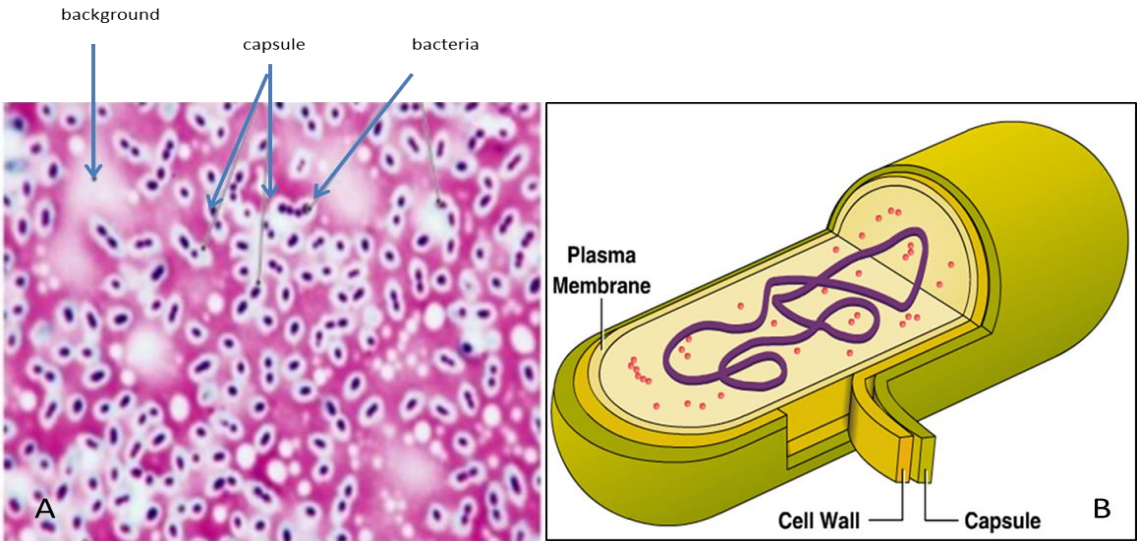


Figure 2-5: bacterial capsule: A) negative staining, bacteria in black, capsule are colorless and the pink is the slide background. B) Bacterial capsule outer cell wall.

Filamentous Protein Appendages

Many bacteria have protein appendages that are anchored in the membrane and protrude out from the surface. These structures are not essential to the life of the cell, but they do allow some bacteria to exist in certain environments in which they otherwise might not survive.

Flagella

The flagellum (plural: flagella) is a long protein structure that is responsible for most types of bacterial motility (figure 2-6).

In some case, flagella are important in the ability of an organism to cause disease. For example, *Helicobacter pylori* the bacterium that causes **gastric ulcers**, has powerful multiple flagella at one end of its spiral-shaped cell. These flagella allow *H. pylori* to penetrate the viscous mucous gel that coats the Stomach epithelium.

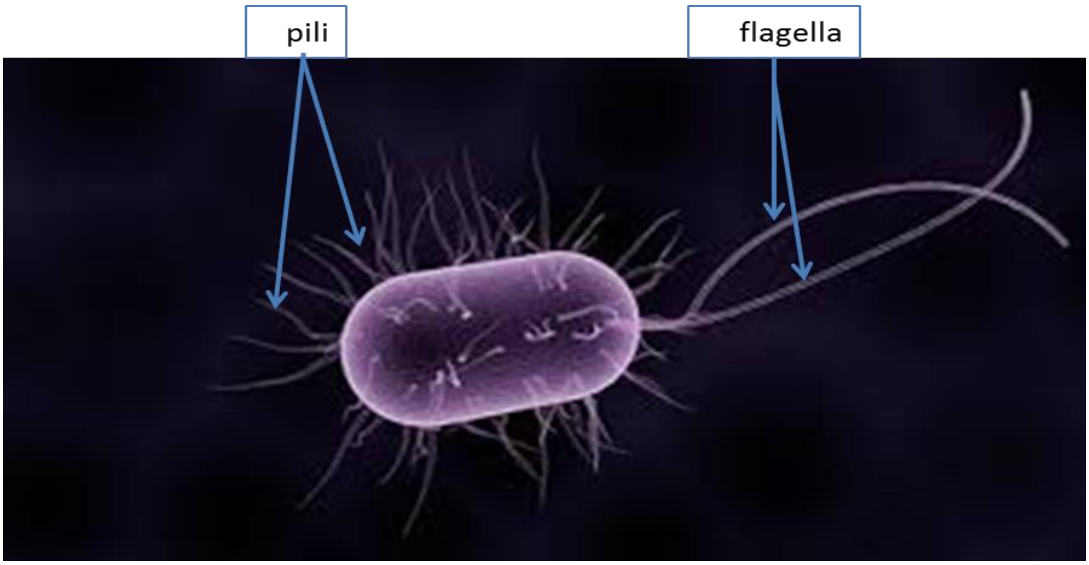


Figure 2-6: flagella in one polar of bacteria

Structure and Arrangement of Flagella

Flagella are composed of three basic parts (figure 2-7). The filament is the portion that extends into the exterior environment. It is composed of identical subunits of a protein called **flagellin**. These subunits form a chain that twists into a helical structure with a hollow core. Connecting the filament to the cell surface is a curved structure, the **hook**; the **basal body** anchors the **flagellum** to the cell wall and cytoplasmic membrane.

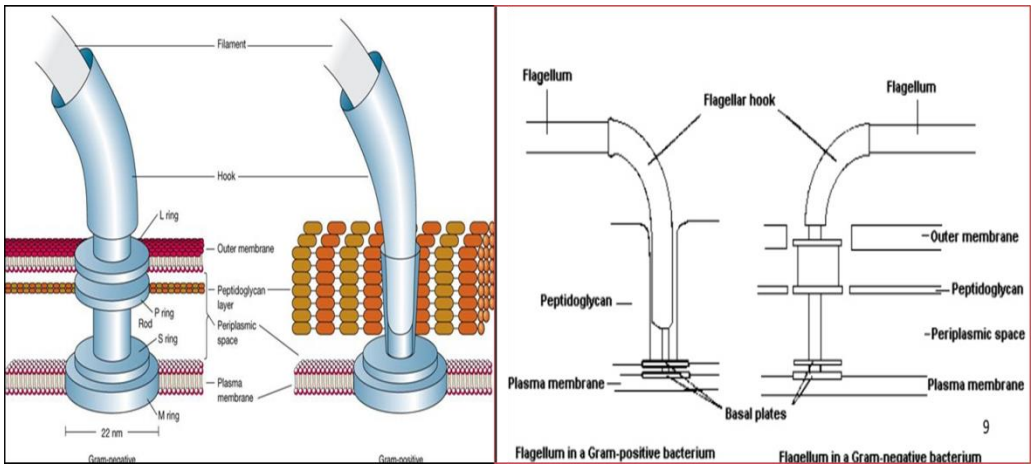


Figure 2-7: structure and insertion of flagella in gram positive and negative bacteria

The numbers and arrangement of flagella can be used to characterize flagellated bacteria. For example, *E. coli* have flagella distributed over the entire surface, an arrangement called peritrichous (Peri means "around"). Other common bacteria have a polar flagellum, a single flagellum at one end of the cell. Less commonly, bacteria may have a tuft of flagella at one or both ends of a cell (figure 2-8).

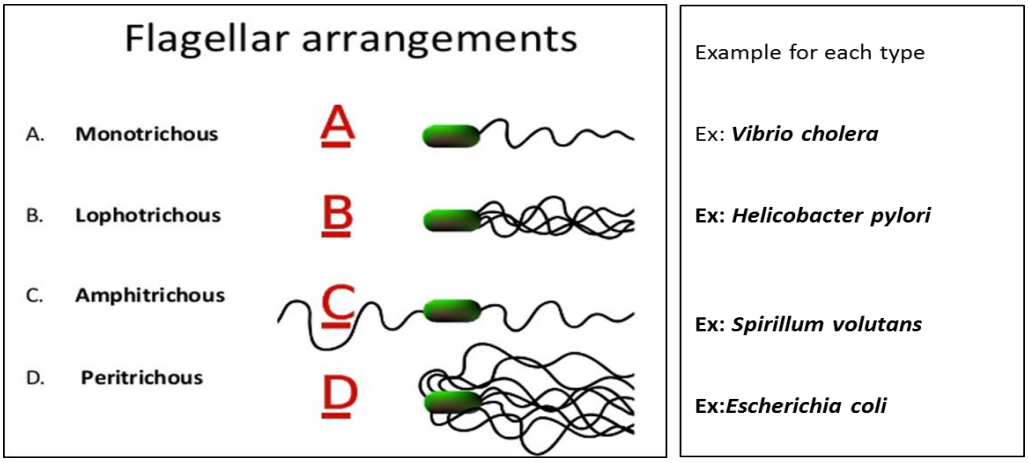


Figure 2-8: the arrangement of flagella on bacterial cell with example for each of them.

Chemotaxis

Motile bacteria sense the presence of chemicals and respond by moving in a certain direction, a phenomenon called **chemotaxis**. If a compound is a nutrient, it may serve as an attractant, enticing cells to move toward it. On the other hand, if the compound is toxic, it may act as a repellent, causing cells to move away.

In addition to reacting to chemicals, some bacteria can respond to variations in **light**, **phototaxis**. Other bacteria can respond to the concentration of **oxygen**, **aerotaxis**. Organisms that require oxygen for growth will move toward it, whereas bacteria that grow only in its absence tend to be repelled by it. Certain motile bacteria can react to the **earth's magnetic field** by the process of **magnetotaxis**. They actually contain a row of magnetic particles that cause the cells to line up in a north-to-south direction much as a compass does (figure 2-9). The magnetic forces of the earth attract the organisms so that they move downward and into sediments where the

concentration of oxygen is low, which is the environment best suited for their growth.

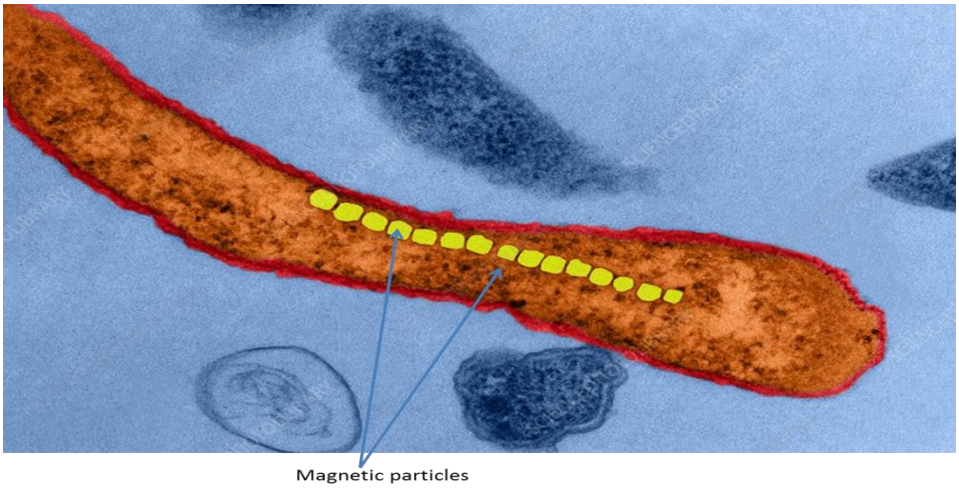


Figure 2-9: Chain of magnetic particles within a magnetotactic spirillum bacteria
Magnetospirillum (*Aquaspirillum*) *magnetotacticum* serve to align the cell along geomagnetic lines (TEM).

Pili

Pili are considerably shorter and thinner than flagella, but they have a similar structural theme to the filament of flagella-a string of protein subunits arranged helically to form a long cylindrical molecule with a hollow core (figure 2-6). The functions of pili, however, are distinctly different from those of flagella.

Many types of pili enable attachment of cells to specific surfaces; these pili are also called **fimbriae**. At the tip or that along the length of the molecule is located another protein, an adhesion, that adheres by binding to a very specific molecule. For example, certain strains of *E. coli* that cause a severe watery diarrhea can attach to the cells that line the small intestine. They do this through specific interactions between adhesions on their pili and the intestinal cell surface. Without the ability to attach, these cells would simply be propelled through the small intestine along with the other intestinal contents.

Pili also appear to play a role in the movement of populations of cells on solid media. Some types of bacteria show a short, jerking movement called twitching, whereas other bacteria move smoothly, called **gliding**. Both of these forms of motility occur on solid media and appear to require some form of cell-to-cell contact. They do not, however, involve flagella.

Another type of pilus is involved in conjugation a mechanism of DNA transfer from one bacterial cell to another. **Sex pili** are used to join those two cells. An example is the **F pili** of *E. coli*. Typically, sex pili are somewhat longer than the types of pili that mediate other characteristics.

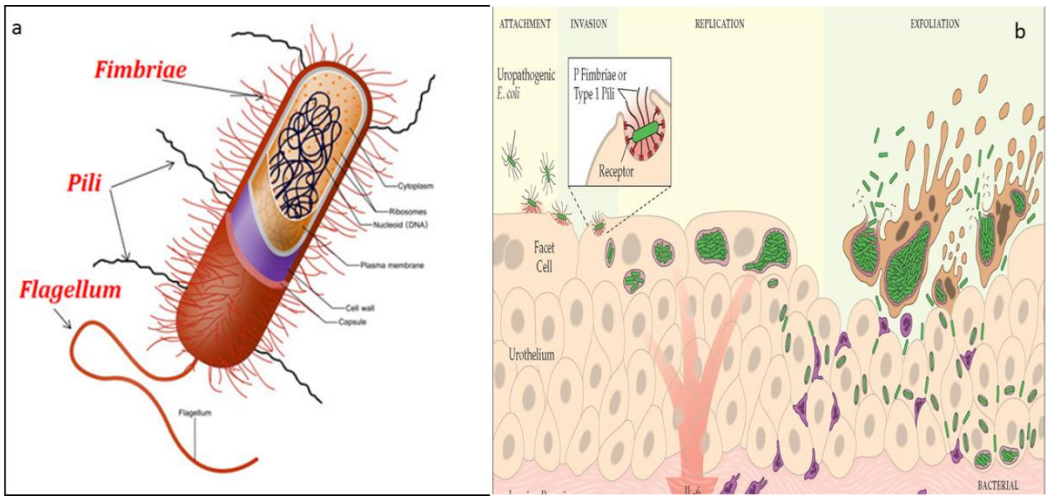


Figure 2-10: a)Pili on an *E. coli* cell. The short pili (fimbriae) mediate adherence; the F pilus is involved in DNA transfer. (b) *E.coli* attaching to epithelial cells in the small intestine of a pig.

Internal Structures

Prokaryotic cells have a variety of structures within the cell. Some, such as the **chromosome and ribosomes**, are essential for the life of all cells. Other such as **plasmids**, are optional but confer certain selective advantages. **Storage granules**, **vesicles**, and **endospores** are characteristic of only certain types of bacteria.

The Chromosome

The chromosome of prokaryotes is not contained within a membrane-bound nucleus. Instead, it irregular mass within the cytoplasm, forming a gel-like region called the **nucleoid**. Typically, it is a single, circular, double-stranded DNA molecule that contains all the genetic information required by a cell. If that circular molecule is cut to form a linear piece and extended to its full length, it is about 1 mm long, approximately 1,000 times as the cell itself. Plasmids are circular double-stranded DNA molecules that are present in many bacteria. They are generally 0.1% to 10% of the size of the chromosome and carry from a few to several hundred genes. A single cell can carry multiple types of plasmids.

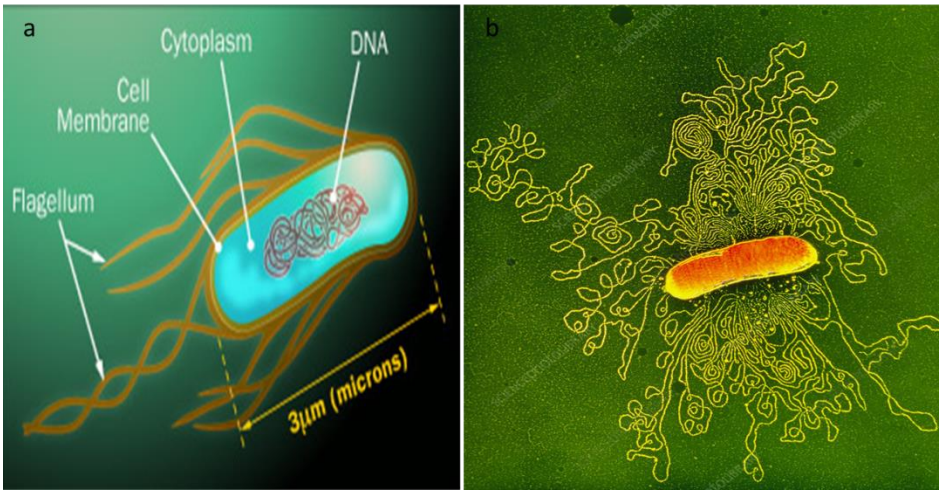


Figure 2-11: The Chromosome (a) Color diagram of DNA in *Escherichia coli*, with the DNA shown in red. (b) Chromosome released from a gently lysed cell of *E*

Note how tightly packed the DNA must be inside the bacterium.

Ribosomes

Ribosomes are intimately involved in protein synthesis, where they serve as the structures that facilitate the joining of amino acids. They are not passive workbenches; rather, they play an "active role in the complex process of protein synthesis. The number of ribosomes in *E. coli* varies from approximately 7,000 to more than 25,000, depending on how rapidly the cell is multiplying. The faster the cell is growing, the faster proteins being synthesized and the greater numbers of

ribosomes are needed. Each ribosome is composed of large and small sub-unit, which are made up of ribosomal proteins and ribosomal RNAs.

The relative size and density- of ribosomes and their subunits are expressed as a distinct unit, S (for Svedberg), that reflects how fast they move when they are spun at very high speeds in an ultracentrifuge. The faster they move toward the bottom, the higher the S value and greater density. Prokaryotic ribosomes are 70S ribosomes. Note that S units are not strictly arithmetic; the 70S ribosome is composed of a 30S and a 50S subunit (figure 2-12). Prokaryotic ribosomes differ from the eukaryotic ribosomes, which are 80S. This variance serves as a target for antibiotics, which preferentially bind to the 70S ribosome and thus inhibit protein synthesis in bacteria.

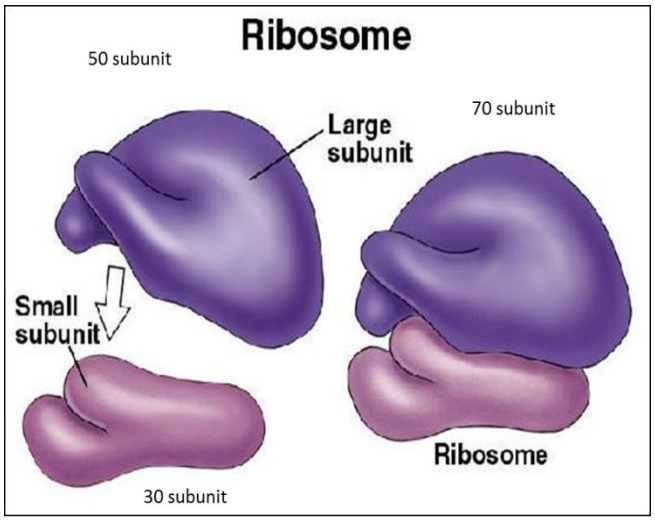


Figure 2-12: The Ribosome. The 70S ribosome is composed of 50S and 30S subunits 50S subunit.

Storage Granules

Storage granules are accumulations of high molecular height polymers, which are synthesized from a nutrient that a cell has in relative excess. For example, if nitrogen and/or phosphorus are lacking, *E. coli* cannot multiply even if a carbon and energy source such as glucose is plentiful. Rather than waste the carbon/energy source, cells use it to produce **glycogen**, a glucose polymer. A single large molecule such as glycogen has little osmotic effect on the cell. Later, when conditions appropriate,

cells degrade and use the glycogen granule. Other bacterial species store carbon and energy as **poly β-hydroxybutyrate** (figure 2-13). This microbial compound is now being employed to produce a biodegradable polymer, which can be used in place of petroleum-based plastics. As a general rule, only one type of storage granule is produced by a given organism. Some types of granules can be readily detected by light-microscopy. **Volutin** granules, a storage form of phosphate, stain red with blue dyes such as methylene blue, whereas the surrounding cellular material stains blue. Because of this, they are often called **metachromatic granules** (meta: means "change" and chromatic means "color"). Bacteria that store volutin are beneficial in wastewater treatment because they scavenge phosphate, which is an environmental pollutant.

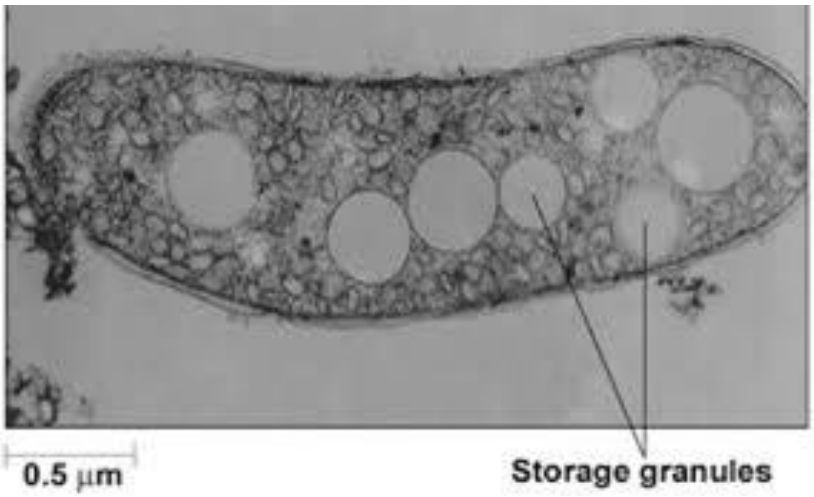


Figure 2-13: Storage Granules, the large unstained areas in the photosynthetic bacterium *Rhodospirillum nibrum* are granules of poly-B-hydroxybutyrate.

Spore

A unique resting structure called spore (either endo- or exo spore) produce by spore-forming bacteria. Spore forming bacteria are Gram-positive and usually rod-shaped, but there are exceptions. The two most important genera are; *Bacillus*, the members of which are aerobic endospore-forming bacteria in the

soils, and *Clostridium*, whose species are anaerobic spore-formers of soils, sediments and the intestinal tracts of animals.

Endospore heat resistance probably is due to several factors: calcium-dipocolonic acid, acui dsoluble protein, stabilization of DNA, the spore coat, DNArepair. The greater stability of cell proteins in bacteria adapted to growth at high temperatures.

Spore maybe central Bacillus subtilis; subterminal Clostridium perfringens or terminal such as Clostridium tetani. The appearance maybe spherical, ovoid or elongated and being narower than the cell.

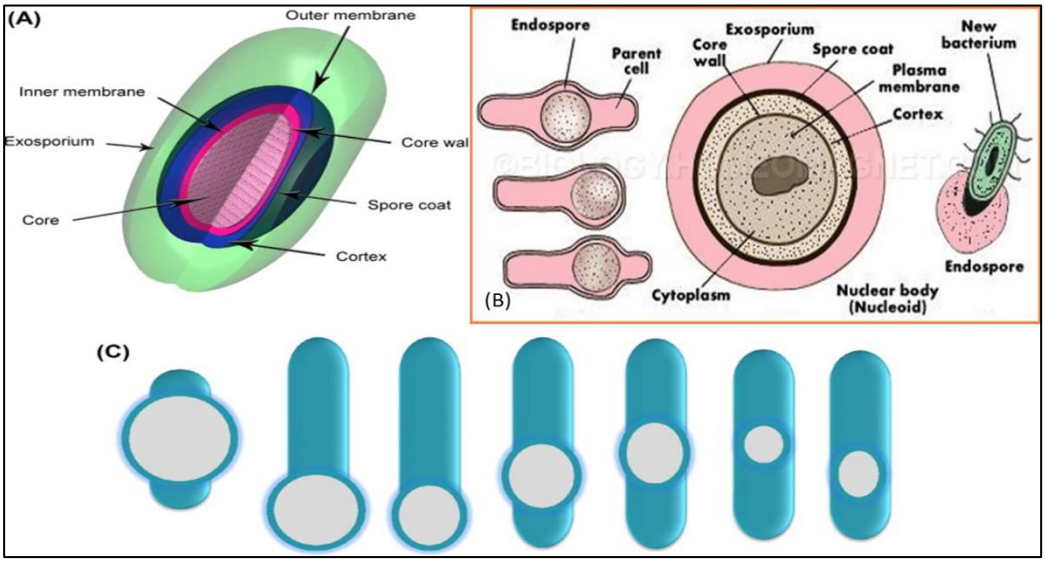


Figure2-13: bacterial spore (A) structure of spore, (b) position of spore in bacterial cell, cross section of spore and spore generation, (C) position of spore in different bacteria.

Table 2-2: Summary of prokaryotic cell structures

Structure	Chemical Composition	Function
Essential components		
Cell wall		
Peptidoglycan	Glycan (sugar) backbone with peptide side chains that are cross-linked	Gives rigid support, protects against osmotic pressure, is the site of action of penicillins and cephalosporins, and is degraded by lysozyme
Outer membrane of gram-negative bacteria	Lipid A	Toxic component of endotoxin
	Polysaccharide	Major surface antigen used frequently in laboratory diagnosis
Surface fibers of gram-positive bacteria	Teichoic acid	Major surface antigen but rarely used in laboratory diagnosis
Plasma membrane	Lipoprotein bilayer without sterols	Site of oxidative and transport enzymes
Ribosome	RNA and protein in 50S and 30S subunits	Protein synthesis; site of action of aminoglycosides, erythromycin, tetracyclines, and chloramphenicol
Nucleoid	DNA	Genetic material
Mesosome	Invagination of plasma membrane	Participates in cell division and secretion
Periplasm	Space between plasma membrane and outer membrane	Contains many hydrolytic enzymes, including β-lactamases
Nonessential components		
Capsule	Polysaccharide[1]	Protects against phagocytosis
Pilus or fimbria	Glycoprotein	Two types: (1) mediates attachment to cell surfaces; (2) sex pilus mediates attachment of two bacteria during conjugation
Flagellum	Protein	Motility
Spore	Keratinlike coat, dipicolinic acid	Provides resistance to dehydration, heat, and chemicals
Plasmid	DNA	Contains a variety of genes for antibiotic resistance and toxins
Granule	Glycogen, lipids, polyphosphates	Site of nutrients in cytoplasm
Glycocalyx	Polysaccharide	Mediates adherence to surfaces

[1]Except in *Bacillus anthracis*, in which it is a polypeptide of D-glutamic acid.

Dynamic of Prokaryotic growth

Obtaining a Pure Culture

Bacteria generally multiply by the process of binary fission. After a bacterial cell has increased in size and double the amount of each of its parts, it divides. One cell divides to become two, those two divide to become four, those four, become eight and so on. As such, **microbial growth** is defined as an increase in the number of cells in a population.

In nature, many different organisms, including bacteria, live together as a mixed population, jointly contributing to numerous activities and processes in their surroundings. In the laboratory, bacteria are isolated and grown as a pure culture in order to study the functions of a particular species. **A pure culture** is defined as a population of organisms that descended from a single cell and species.

Cultivating bacteria on a solid medium

The basic requirements for obtaining a pure culture are a solid medium (medium that bacteria can grow in or on is a mixture of nutrients dissolved in water and could be a liquid form or solidified gel-like form), a media container, that could be maintained in aseptic condition, and a method to separate individual cells. A single bacterium supplied with the right nutrients, will multiply on the solid medium in a limited area to form a colony, which is a mass of cells all descended from the original one. About 1 million calls are required for a colony to be easily visible to the naked eye.

Agar, a polysaccharide extracted from marine algae, is employed to solidify a specific nutrient solution to create the ideal medium on which to grow cells. Unlike other gelling agents such as gelatin, **very few bacteria can degrade agar**, **it is not destroyed at high temperatures** and can therefore be sterilized by heating, a process that also liquefies it. Melted agar will stay liquid until it is cooled to a temperature below 45°C. Therefore, nutrients that might be destroyed at high temperatures can be

added at lower temperatures before the agar hardens. Once solidified, an agar medium will remain so until it is heated above 95°C. Thus, unlike gelatin, which is liquid at 37°C, **agar remains solid over the entire temperature** range at which the majority of bacteria grow. Agar is also **translucent**, enabling colonies imbedded in the solid medium to be seen more easily.

The streak-plate method

The streak-plate method is the simplest and most commonly used technique for isolating bacteria (figure 2-14). A sterilized inoculating loop is dipped into a solution containing the organism of interest and is then lightly drawn several times across an agar plate, creating a set of parallel streaks covering approximately one-third of the plate. The loop is then sterilized and a new series of parallel streaks are made across and at an angle on the previous ones, covering another one-third of a plate. This action drags some of those cells streaked over the first portion of the plate un-inoculated portion, creating a region containing a more dilute inoculum. The loop is sterilized again and another set of parallel streaks are made, dragging into area some of the organisms that had been moved into the second section. The object of this is to reduce the number of cells being spread with each successive series of streak diluting the concentration of cells. By the third cells should be separated enough so that distinct well-isolated colonies will form.

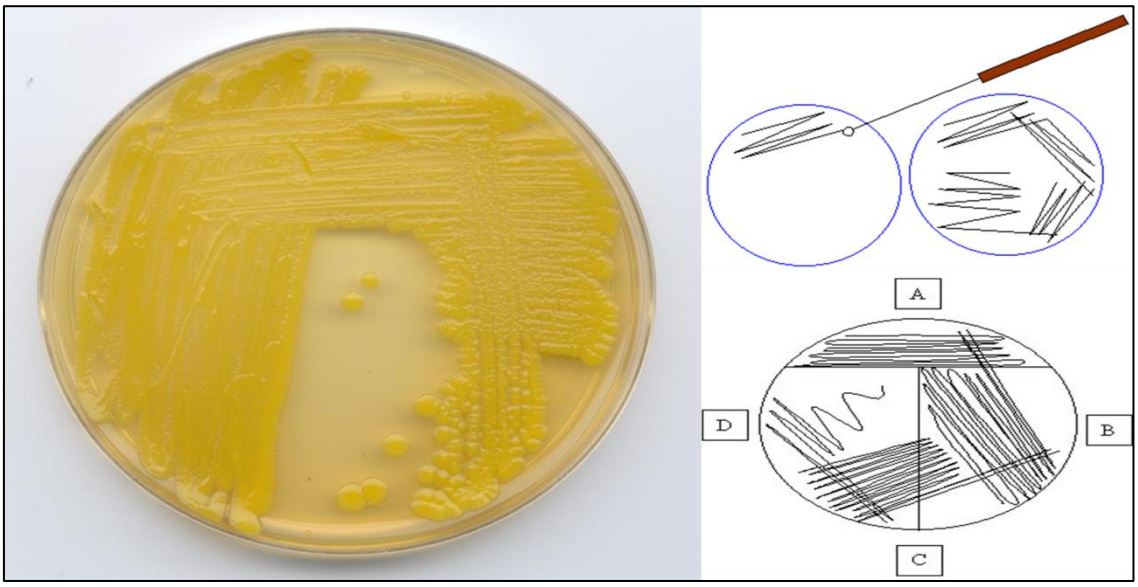


Figure 2-14: The Streak-Plate Method. A sterilized inoculating loop is dipped into a culture and then (A) lightly is drawn several times across an agar plate (the loop is sterilized again) (B) and a new series of streaks is made at an angle to the first set (the loop is sterilized again). (C) and another set of parallel streaks is made (sometimes another parallel streak can be done as in d or you can finish the streak). The successive streaks dilute the concentration of cells. By the third set of streaks, cells should be separated enough so that isolated colonies develop after incubation (as shown in the plate with growing bacteria).

Maintaining Stock Cultures

Once a pure culture has been obtained, it can be maintained as a stock culture, a culture stored for use as an inoculum in later procedures. Often, stock cultures are stored in the refrigerator as growth on an **agar slant**. This is agar medium stored in a tube that was held at a shallow angle as the medium solidified, creating a larger surface area. For **long-term storage**, stock cultures can be frozen at **-70°C** in a glycerol solution. The glycerol prevents ice crystals from damaging cells. Alternatively, cells can be lyophilized, or freeze-dried.

Environmental factors that influence microbial growth

The major environmental conditions that influence the growth of microorganism are summarized in table 2-3. Temperature Requirements

Temperature Requirements

Each species of prokaryote has a well-defined upper and lower temperature limit within which they grow and outside of which growth stops. The temperature span between these limits is usually about 25°C. Within this range lies their optimum growth temperature, the temperature at which the organism multiplies most rapidly. As a general rule, this optimum temperature is close to the upper limit of the organism's range. This is because the speed of enzymatic reactions in the cell approximately doubles for each 10°C rise in temperature. At a critical point, however, the temperature becomes too high and enzymes required for growth are denatured and can no longer function. As a result, the cells will die.

Prokaryotes are commonly divided into four groups based on their optimum growth temperatures (figure 2-15). Note, scheme. In reality, there is no sharp dividing line between each group. Furthermore, not every organism in a group can grow-in the entire temperature range typical for its group.

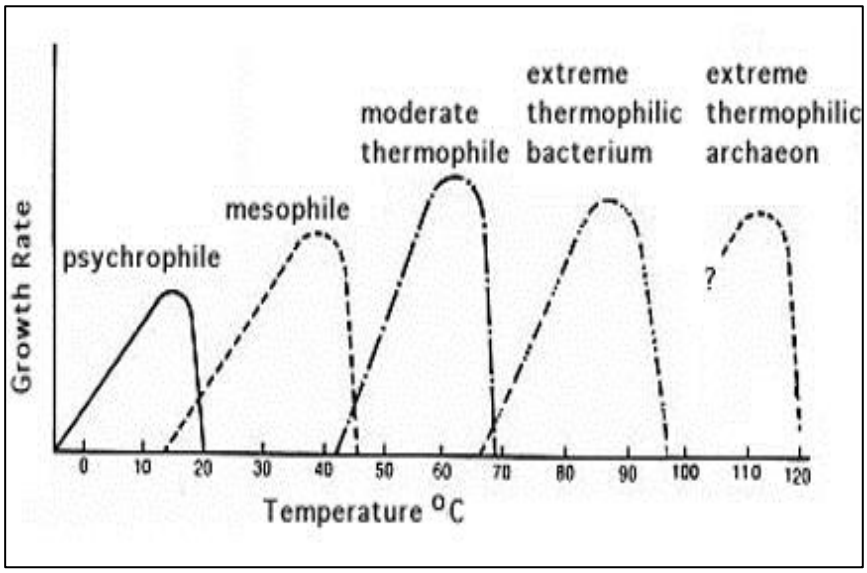


Figure 2-15: Optimal growth temperatures

- **Psychrophiles** have their optimum between -5°C and 15°C. These organisms are usually found in such environments as the Arctic and Antarctic regions and in lakes fed by glaciers. Some, but not all members of the genus *Pseudomonas* are psychrophiles. Psychrotrophs have a temperature optimum of>15°C, but grow well at lower temperatures.

- **Mesophiles**, which include *E. coli* and most other common bacteria, have their optimum temperature within the range of 25°C to about 45°C. Disease-causing bacteria, which are adapted to growth in the human body, typically have an optimum between 35°C and 40°C. Mesophiles that inhabit soil, a colder environment, generally have a lower optimum, close to 30°C.

-**Thermophiles** have an optimum temperature between 45°C and 70°C. These organisms commonly occur in hot springs and compost heaps. They also are found in artificially created thermal environments such as water heaters and nuclear power plant cooling towers. *Lactobacillus delbrueckü* is a thermophile used in yogurt production.

- **Thermeduric** organisms: are **mesophile** that can survive brief period at high temperature.

- **Hyperthermophiles** have an optimum growth temperature between 70°C and 110°C, these usually members of the **Archaea**. ***Pyrolobus fumarimii***, which was isolated from the wall of a hydrothermal vent at an ocean depth of 3650 meters, has a maximum growth temperature of 113°C, the highest yet recorded.

Why some prokaryotes can withstand in such a high temperatures but most cannot? As a general rule, proteins from thermophiles are not denatured at high temperatures. This thermostability is due the sequence of the amino acids in the protein. These control the number and position of the bonds that form within the protein, which in turn determines its three-dimensional structure. For example, the formation of many covalent bonds, as well as many hydrogen and other weak bonds,

prevents denaturation of proteins. Heat-stable proteins, which include enzymes that degrade fat and other proteins, are being used in high-temperature detergents.

Oxygen (O_2) Requirements

Based on their O_2 requirements, prokaryotes can be separated into the following groups (Figure 2-16):

- **Obligate aerobes:** have an absolute or obligate requirement for oxygen (O_2). They use it to generate energy in the process of aerobic respiration. Obligate aerobes include members of the genus *Pseudomonas*, a diverse group of Gram-negative rods that are common in the environment.

- **Obligate anaerobe**: cannot multiply if any O_2 is present; in fact, they are often killed by traces of O_2 because of its toxic derivatives, which will be discussed shortly. Obligate anaerobes may transform energy by fermentation or by anaerobic respiration. Obligate anaerobes include members of the genus *Bacteroides*, which are the major inhabitants of the large intestine. Another obligate anaerobe is *Clostridium botulinum*, the causative agent of botulism. It is estimated that one-half of all the cytoplasm on earth is in anaerobic bacteria

- **Facultative anaerobes**: grow better if O_2 is present, but can also grow without it. The term facultative means that the organism is flexible, in this case in its requirements for O_2. Facultative anaerobes use aerobic respiration if oxygen is available, but use fermentation or aerobic respiration in its absence. Growth is more rapid when oxygen is present because aerobic respiration yields the most ATP of all these processes. Examples of facultative anaerobes include *E.coli*, a common inhabitant of the large intestine, and the yeast *Saccharomyces* (eukaryote), which is used to make bread and alcoholic beverages.

- **Microaerophiles**: require small amounts of O_2 (2-10%) for aerobic respiration; higher concentrations are inhibitory. Examples include *Spirillum volutans*, which is

common in aquatic habitats, and *Helicobacter pylori*, which cause gastrointestinal ulcers.

- Aerotolerant anaerobes: are indifferent to O_2. They can grow in its presence, but they do not use it to transform energy. Because they do not use aerobic or anaerobic respiration, they are also called **obligate fermenters**. They include *Lactobacillus bulgaricus*, which is used in cheese-making and *Streptococcus pyogenes*, which causes sore throat.

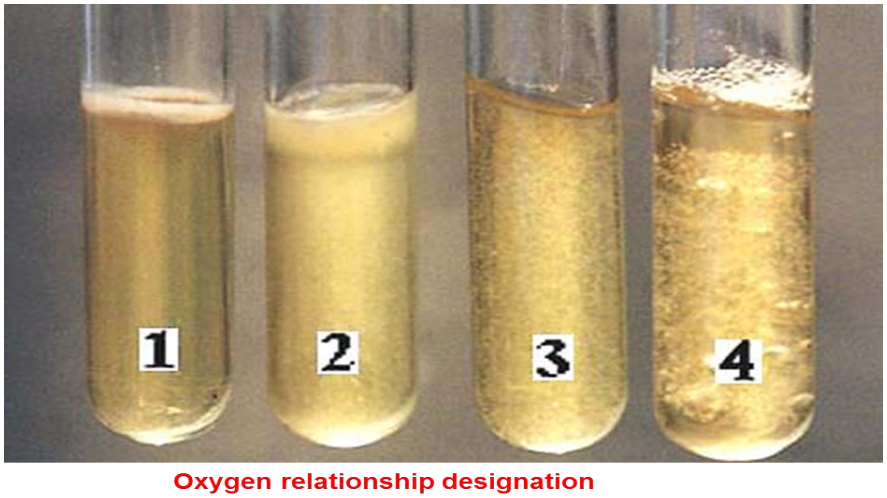
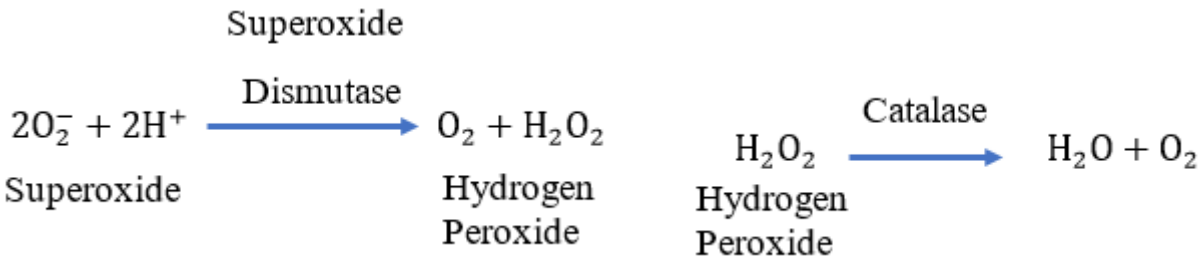


Figure 2-16: Using liquid growth medium to identify the oxygen requirements of organisms. The surface of a thioglycollate medium, which is exposed to atmospheric oxygen, is aerobic. Oxygen concentration decreases with depth; the bottom of the tube is anaerobic, (1) Obligate aerobes cannot survive below the depth to which oxygen penetrates the medium, (2) Facultative anaerobes can grow with or without oxygen, but their ability to use aerobic respiration pathways enhances their growth near the surface, (3) Aerotolerant aerobes can grow equally well with or without oxygen; their growth is relatively evenly distributed throughout the medium, (4) Obligate anaerobe? cannot tolerate any oxygen Where in such a test tube would be the growth of microaerophilic aerobe?

Toxic Derivatives of Oxygen (O2)

Although not toxic itself, O_2 can be converted into a number of compounds that are highly toxic. Some of these, such as superoxide (O_2), are produced both as a part of normal metabolic processes and as chemical reactions involving oxygen in light. Others, such as hydrogen peroxide (H_2O_2), result from metabolic processes involving oxygen. To survive in an environment containing O_2, cells must have

enzymes that can convert these toxic compounds to nontoxic forms. The enzyme superoxide dismutase degrades superoxide to produce hydrogen peroxide. Catalase breaks down hydrogen peroxide to H_2O and O_2. Together, these two enzymes detoxify these reactive products of O.



Although most strict anaerobes do not have superoxide dismutase, some do, while a few aerobes lack it. Therefore, other factors must also be playing a role in protecting organisms from the toxic forms of oxygen.

pH

Each bacterial species pH can survive within a range of pH values, within this range, it has a pH optimum. Despite the pH of the external environment, cells maintain a constant internal pH, typically near neutral.

Most bacteria can live and multiply within the range of pH 5 (acidic) to pH 8 (basic) and have a pH optimum near neutral (pH 7). These bacteria are called neutrophiles. Preservation methods that acidify foods, such as pickling are intended to inhibit these organisms. Surprisingly, some neutrophiles have adapted special mechanisms that enable them to grow at a very low pH. For example, *Helicobacter pylori* grows in the stomach where it can cause ulcers. To maintain the pH close to neutral in its immediate surroundings, *H. pylori* produces the enzyme urease, which splits urea in the stomach into carbon dioxide and ammonia. The ammonia neutralizes the stomach acid in the bacterium's immediate surroundings

Acidophiles grow optimally at a pH below 5.5. For example, *Thiobacillus ferroxidans* grows best at a pH of approximately 2.0. This bacterium obtains its energy by oxidizing sulfur compounds, producing sulfuric acid in the process. It maintains its internal pH near neutral by pumping out protons (H^+) as quickly as they enter the cell.

Alkaliphiles grow optimally pH above 8.5. For example, *Bacillus alcalophilus* grows best at at a pH 10.5. It appears alkaliphiles maintain a relatively neutral internal pH by using an antiporter that exchanges internal sodium ions for external protons. Alkaliphiles often live in alkaline lakes and soils.

Water Availability

All microorganisms require water for growth. Even if water is present, however, it may not be available in certain environments. For example, dissolved substances such as salt (NaCl) and sugars interact with the water molecules and make that water unavailable to the cell. In any environment, particularly in certain natural habitats such as salt marshes, prokaryotes are faced with this situation. If the solute concentration is higher in the medium than in the cell, water diffuses out of the cell. This can cause the cytoplasm to dehydrate and shrink from the cell wall, a phenomenon called plasmolysis

Bacteria that can tolerate relatively high salt concentrations, up to approximately 10% NaCl, are called **osmotolerant**. *Staphylococcus* species, which reside on the dry salt environment of the skin, are osmotolerant. Certain members of the Archaea can live in very high salt solutions. These are found in such organisms, called **halophiles** (halo means "salt" and phile means "loving"), environments as the salt flats in Utah and the Dead Sea.

Cell responses to solution of differing osmotic content

Isotonic:

Diffusion of water proceeds at the same rate in both directions, there is no net change in cell volume. Isotonic solutions are generally the most stable environments for cells, because they are already in an osmotic steady- state with the cell. Parasites living in host tissues are most likely to be living in isotonic habitats (fig 2-17).

Under hypotonic* conditions, the solute concentration of the external environment is lower than that of the cell's internal environment. Pure water provides the most hypotonic environment for cells because it has no solute. The net direction of osmosis is from the hypotonic solution into the cell, and cells without walls swell and can burst.

Hypertonic* conditions are also out of balance with the tonicity of the cell's cytoplasm, but in this case, the environment has a higher solute concentration than the cytoplasm. Because a hypertonic environment will force water to diffuse out of a cell, it is said to have high *osmotic pressure* or potential. The growth- limiting effect of hypertonic solutions on microbes is the principle behind using concentrated salt and sugar solutions as preservatives for food, such as in salted hams.

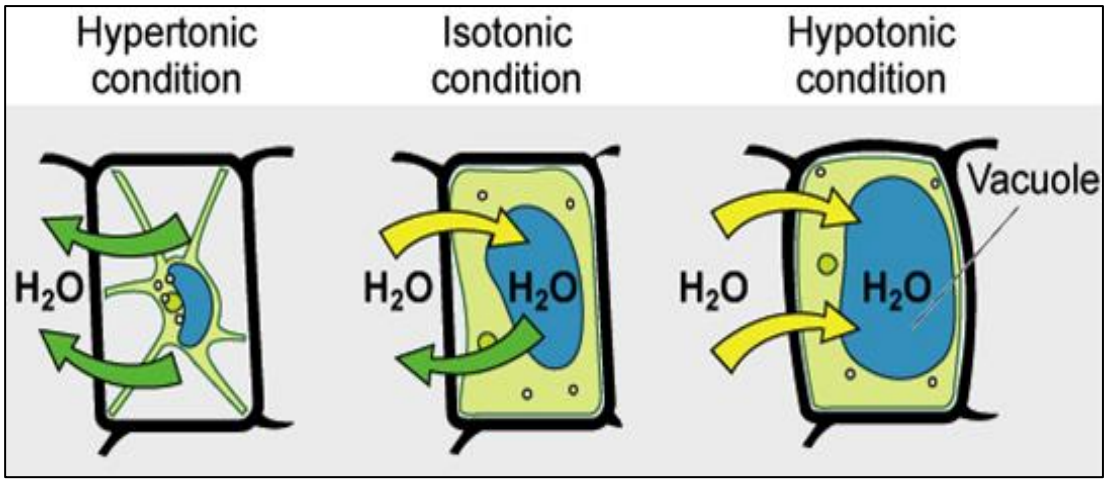


Figure 2-17: Bacterial cell response to solution. Hypertonic: the water goes from inside to outside more than that inter the bacteria**. Isotonic:** the amount of water that inter equal to the exist water**. Hypotonic:** The amount of interring water to inside more than the exist water.

Table 2-3: Environmental factors that influence microbial growth.

Environmental factor descriptive terms	Characteristics
Temperature	Thermostability appears to be due to protein structure
Psychrophile	Optimum temperature between -5°C and 15°C
Mesophile	Optimum temperature between 25°C and 45°C
Thermophile	Optimum temperature between 45°C and 70°C
Hyperthermophile	Optimum temperature between 70°C and 110°C
Oxygen(O_2)Availability	Oxygen (O_2) requirement/ tolerance reflect the organism's energy-converting mechanisms (aerobic respiration, anaerobic respiration, and fermentation) and its ability to detoxify O_2 derivatives.
Obligate aerobe	Requires O_2
Obligate anaerobe	Cannot multiply in the presence of O_2
Facultative anaerobe	Crows best if O_2, is present, but can also grow without it.
Microaerophile	Requires small amounts of 02 but higher concentrations are inhibitory
Aerotolerant anaerobe (obligate fermenter)	Indifferent to O_2
PH	Prokaryotes that live in pH extremes appear to maintain a near neutral internal pH by transporting protons across the membrane.
Neutrophile	Multiplies in the range of pH 5 to 8
Acidophile	Grows optimally at a pH below 5.5
Alkaliphile	Grows optimally at a pH above 8.5
Water Availability	Prokaryotes that can grow in high solute solutions increase their internal solute concentration by either pump-in into the cell or synthesizing certain small organic compounds.
Osmotolerant	Can grow in relatively high salt solutions, up to approximately 10% NaCl
Halophile	Requires sodium chloride some require concentrations of 20% or greater

Microbial nutritional and Growth

The growth of any bacterium depends not only on a suitable physical environment, but also an available source of chemicals to use as nutrients. From these, the cell must synthesize all of the cell components discussed in the previous chapter, including lipid membranes, cell walls, proteins, and nucleic acids. These components are made from building blocks such as fatty acids, sugars, amino acids, and nucleotides. In turn, each of these building blocks is composed of a variety of elements, including carbon and nitrogen.

Required Elements

The elements that make up cell constituents are called **major elements**. These include carbon, Oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, magnesium, calcium, and iron. They are the essential components of proteins, carbohydrates, lipids, and nucleic acids (table 3-1).

The source of carbon, the most abundant of the major elements, distinguishes different groups of prokaryotes. Those that use organic carbon are called heterotrophs (hetero means "different"). Medically important bacteria use an organic source of carbon such as glucose. Autotrophs use an organic source of carbon such as (auto means, self) utilize inorganic carbon in the form of carbon dioxide as their carbon source.

Some elements, termed **trace elements** are required by bacteria in very minute amount such as cobalt, zinc, copper molybdenum and manganese**.**

Growth Factors

Some bacteria cannot synthesize some of their cell constituents, such as amino acids, vitamins, purines, and pyrimidines, from the major elements. Consequently,

these organisms can only grow in environments where such compounds are available. Those low molecular weight compounds that must be provided to a particular bacterium are called **growth factors**.

Microorganisms display a wide spectrum in their growth factor requirements, reflecting differences in their biosynthetic capabilities. The fewer enzymes an organism has for the biosynthesis of small molecules such as amino acids, the more growth factors must be provided. For example, *E. coli* is quite versatile and does not require any growth factors. It grows in a medium containing only glucose and six different inorganic salts. In contrast, species of *Neisseria* require at least 40 additional ingredients, including 7 vitamins and all of the 20 amino acids. Bacteria such as *Neisseria* that require a range of growth factors are called **fastidious**.

Table 3-1: Representative Functions of the Major Elements

Chemical	Function
Carbon, oxygen, and hydrogen	Component of cellular constituents including amino acids, lipids, nucleic acids, and sugars.
Nitrogen	Component of amino acids and nucleic acids
Sulfur	Component of some amino acids
Phosphorus	Component of nucleic acids, membrane lipids, and ATP.
Potassium, magnesium, and calcium	Required for the functioning of certain enzymes; additional functions as well.
Iron	Part of certain enzymes.

Energy Sources

Organisms derive energy either from sunlight or from the oxidation of chemical compounds. Organisms that harvest the energy of sunlight are called phototrophs (*photo* means "light" and *troph* means "nourishment"). These include plants, algae,

and photo-synthetic bacteria. Organisms that obtain energy by oxidizing chemical compounds "chemical"). Mammalian cells, fungi, and many types of bacteria oxidize organic compounds such as sugars, amino acids, and fatty acid. Some prokaryotes can extract energy from seemingly unlikely sources such as hydrogen sulfide, hydrogen gas and other inorganic compounds, an ability that distinguishes them from eukaryotes. Microbiologists often group prokaryotes according to the energy and carbon sources they utilize (table 3-2)

1-Photoautotrophs

Photoautotrophs use the energy of sunlight and the carbon in the atmosphere to make the organic compounds required by many other organisms, including humans. Because of this, they are called primary producers. Cyanobacteria are important photoautotrophs that inhabit both freshwater and saltwater habitats. Many can fix nitrogen, providing another indispensable role in the biosphere.

.

2- Photoheterotrophs

Photoheterotrophs use the energy of sunlight and derive their carbon from organic compounds. Some are facultative in their nutritional capabilities. For example, some members of a group of bacteria called the **purple non-sulfur bacteria** can grow anaerobically using light as an energy source and organic compounds as a carbon source (photoheterotrophs). They can also grow aerobically in the dark using organic sources of carbon and energy (chemoheterotrophs).

3- Chemolithoautotrophs

Chemolithoautotrophs, nitrifying bacteria commonly chemoautotrophs, use inorganic compound for energy and derive their carbon from CO_2. These prokaryotes live in seemingly inhospitable environments such as sulfur hot springs, which are rich in reduced inorganic compounds such as hydrogen sulfide.

4-Chemoorganoheterotrophs

Chemoorganoheterotrophs, commonly referred as chemoheterotrophs, use organic to compounds for energy and as a carbon source. They are by far the most common group associated with humans and other animals. Some play beneficial roles such as providing a source of vitamin K in the gut, whereas others cause disease.

Table 3-2: Energy and Carbon Sources Used by Different Groups of Prokaryotes

Type	Energy Source	Carbon source
photoautotroph	Sunlight	CO_2
photoheterotroph	Sunlight	Organic compounds
chemolithoautotoph	Inorganic chemicals (H_2S, $NH_3NO_2^-$, Fe^{2+}, H_2S)	CO_2
chemoorganoheterotroph	Organic compounds Organic compounds (sugars, amino acids, etc.).	Organic compounds

Cultivating Prokaryotes in the Laboratory

To cultivate or culture microorganisms, a sample called an **inoculum** (plural: *inocula*) is introduced into a collection of nutrients called a **medium.** Microorganisms that grow from an inoculum are also called a **culture**; thus, the word **culture** can refer to the act of cultivating microorganisms or to the microorganisms that are cultivated.

Cultures can be grown in liquid media called **broths**, or on the surface of **solid** media.

Complex Media

A commonly used complex medium, nutrient broth, consists of only 5 grams of peptone and 3 grams of beef extract per liter of distilled water, if agar is added, then nutrient agar results.

Many medically important bacteria are fastidious, requiring a medium that is even richer than nutrient agar. One rich medium commonly used in clinical laboratories is **blood agar**. It contains red blood cells,, which supply a variety of nutrients including Hemin, in addition to other ingredients. A medium used to culture even more fastidious bacteria is **chocolate agar**, named for its appearance rather than the ingredients. Chocolate agar contains lysed red blood cells and additional nutrients.

Several biological supply companies manufacture hundreds of different types of media, each one specially formulated to permit the plentiful growth of one or several groups of organisms. Even with the availability of all of these different media, however, many organisms, including *Treponema pallidum*, the spirochete that causes syphilis, have yet to be successfully grown on culture media.

Chemically Defined

Chemically defined media are composed of mixtures of pure chemicals. They are generally not practical for use in most routine laboratory work, but they are invaluable when studying nutritional are requirements of bacteria. Glucose-salts, which support the growth of *E. coli*, contain only those chemicals listed in table 3-3.

To maintain the pH near neutrality, buffers are often added to the medium. A common buffer is a mixture of two salts of phosphoric acid-the sodium phosphates, Na_2HPO_4 and NaH_2PO_4. These salts limit pH changes, because they can combine chemically with the H^+ ions of strong acids and the can OH^- ions of strong bases to produce neutral compounds.

Table 3-3: ingredient in two representative types of media that support the growth of *E. coli*

Nutrient Broth (complex medium)	**Glucose-Salts (defined medium)**
Peptone Meat extract	Dipotassium phosphate Monopotassium phosphate Magnesium sulfate Ammonium sulfate Calcium chloride Iron sulfate Water

They are often included in a defined medium because some bacteria can produce enough acid as a by-product of their metabolism to inhibit their own growth. This typically is not as much of a problem in complex media because the amino acids and other natural components provide at least some buffering function.

Special Types of Culture Media

These media can be either complex or chemically defined depending on the needs of the microbiologist.

Selective Media

Selective media inhibit the growth of organisms other than the one being sought. For ex; **Thayer-Martin agar** is used to isolate *Neisseria gonorrhoeae* from clinical specimens. This is a variation of **chocolate agar** to which three or more antimicrobial drugs have been added. The antimicrobials inhibit fungi, Gram-positive bacteria, and Gram-negative rods. Because these drugs do not inhibit most strains of *N. gonorrhoeae*., they allow growth with little competition from these other organisms.

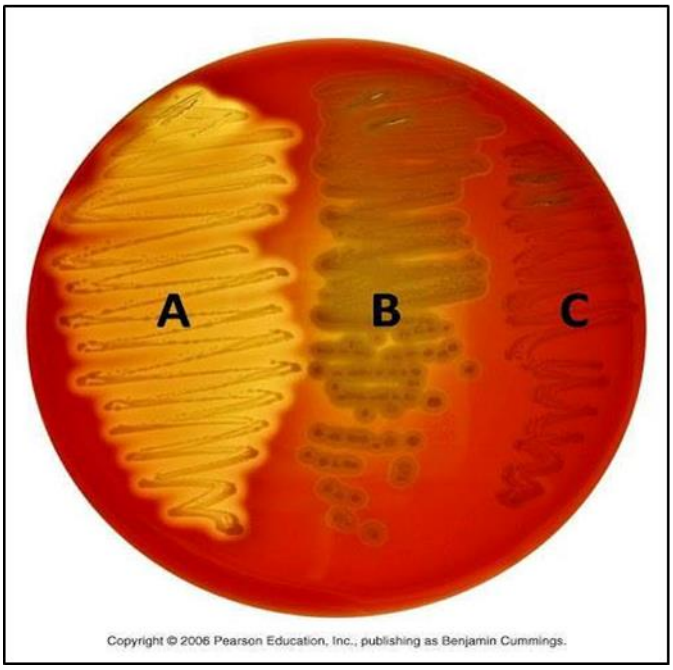
MacConkey agar is used to isolate Gram-negative rods that typically reside the intestine from various clinical specimens such as urine. This complex medium contains, in addition to peptones and other nutrients, two inhibitory compounds:

crystal violet, a dye, inhibits Gram-positive bacteria, and bile salts inhibit most non-intestinal bacteria.

Mannitol salt agar is considered as selective media? Why?

Differential Media

Differential media contain a substance that certain bacteria change in a recognizable way. For example, **blood agar**, in addition to being nutritious, is differential; it is used to detect bacteria that produce a hemolysin, a substance that lyses red blood cells. The lysis appears as a zone of clearing around the colony growing on the blood agar plate. The type of hemolysis is used as an identifying characteristic. For example, species of *Streptococcus* that reside harmlessly in the throat often cause a type of hemolysis called **alpha hemolysis**, which is characterized by a zone of greenish clearing around the colonies (Figure 3-1). In contrast, *Streptococcus pyogenes*, which causes sore throat, causes **beta hemolysis**, which is characterized by a clearer zone of hemolysis. Still other bacteria have no effect on red blood cells.



- **Figure 3-1: Hemolysis on blood agar.**

A) Complete hemolysis (β-hemolysis)

B) Partial hemolysis (α-hemolysis).

C) No hemolysis or undifferentiated hemolysis (γ-hemolysis).

MacConkey agar, which is selective, is also differential. In addition to peptones and other nutrients, it contains the sugar lactose and a pH indicator. Bacteria that ferment the sugar produce acid, which turns the pH indicator pink. Thus, those lactose-fermenting bacteria that can grow on MacConkey agar, such as *E. coli*, form pink colonies. Lactose-negative bacteria form tan or colorless colonies such as *Salmonella*.

Mannitol salt agar is consider as differential media? why?

Table 3-4 Characteristics of medium used to cultivate bacteria and its characteristic

Medium	Characteristic
Categories	Complex Composed of ingredient such as peptones and extracts, which may vary in their chemical composition
Chemically defined	Composed of precise mixtures of pure chemicals such as ammonium sulfate
Selective	Medium to which additional ingredients have been added that inhibit the growth of many organisms other than the one being sought.
Differential	Medium that contains an ingredient that can be changed by certain bacteria in a Differential recognizable way
Representative Types of Agar Media	
Blood agar	Complex medium used routinely in clinical labs. Not selective. Differential because colonies of hemolytic organisms are surrounded by a zone of clearing of the red blood cells.
Chocolate agar	Complex medium used to culture fastidious bacteria, particularly those found in clinical specimens. Not selective or different:
Glucose-salts	Chemically defined medium. Used in laboratory experiments to study nutritional requirements of bacteria. Not selective or differential.
MacConkey	Complex medium used to isolate Gram-negative rods that typically reside in the intestine. Selective because bile salt and dyes inhibit Gram-positive organisms agar and Gram-negative cocci. Differential because the pH indicator turns red when the sugar in the medium, lactose is fermented.
Nutrient agar	Complex medium used for routine laboratory work. Supports the growth of a variety of non-fastidious bacteria.
Thayer-Martin	Complex medium used to isolate Neisseria species, which are fastidious. Selective contains antibiotics most that inhibit organisms except Neisseria species.

Growth of bacterial population

Measurement of Bacterial Growth

Growth is an orderly increase in the quantity of cellular constituents. It depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In-most bacteria, growth involves increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division. This asexual process-of reproduction is called binary fission.

For unicellular organisms such as the bacteria, growth can be measured in terms-of two different parameters: changes in cell mass and changes in cell numbers. Methods

Methods far Measurement of cell Mass

Methods for measurement of the cell mass involve both direct and indirect techniques;

1. **Direct physical** measurement of dry weight, wet weight, or volume of cells after centrifugation.
2. **Direct chemical** measurement of some chemical component of the cells such as total N, total protein, or total DNA content.
3. **Indirect measurement of chemical activity** such as rate of O2 production or consumption, CO2 production or consumption, etc.
4. **Turbidity measurements** employ a variety of instruments to determine the amount of light suspension of cells. Particulate objects such as bacteria scatter light in proportion optical density (OD) of a suspension of cells is directly related to scattered by a or to their numbers. The turbidity cell mass or cell number, after construction and calibration of a **standard curve**. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria. Figure (4-1)

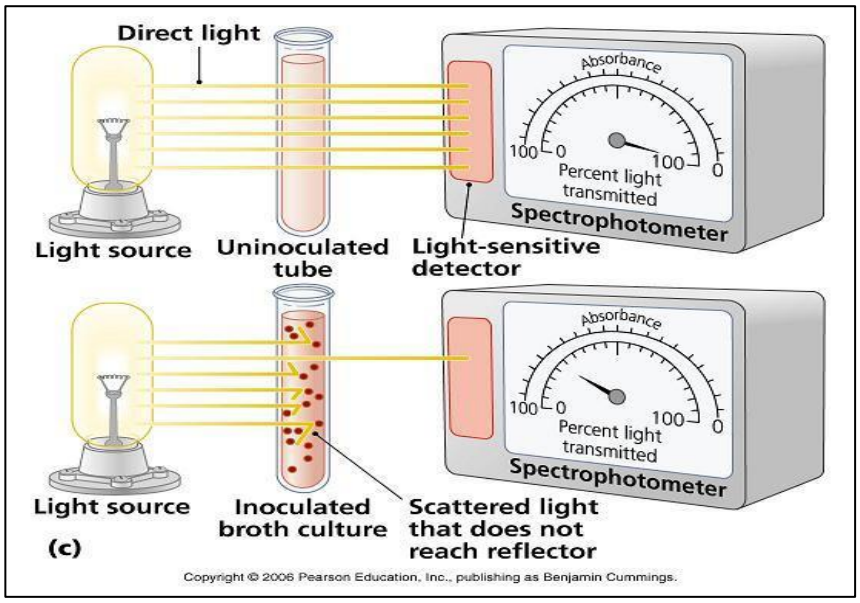


Figure (4-1): Measuring turbidity with a spectrophotometer. The percentage of light that reaches the detector is inversely proportional to the optical density. To use turbidity to estimate cell number, a one-time experiment must be done to determine the correlation between cell concentration and optical density of a culture.

Methods for Measurement of Cell Numbers

Measuring techniques involve direct counts, visually or instrumentally, and indirect viable cell counts.

1. **Direct microscopic counts** are possible using special slides known as counting chambers (**petroff-hauser counting chamber**) (fig. 4-2). Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted (>10^7 cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.
2. **Indirect viable cell counts**, also called **plate counts**, involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in a nontoxic diluent (e.g. water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a colony forming unit (cfu) and the number of cfu's is related to the viable number of bacteria in the sample. Scientists count the colonies on plates with 30-300 colonies and multiply the

number by the reciprocal of the dilution to estimate the number of bacteria per ml of the original culture. This method is called a viable plate count (Figure 4-3). When a plate has fewer than 30 colonies it is not used to estimate the number of bacteria in the original sample.

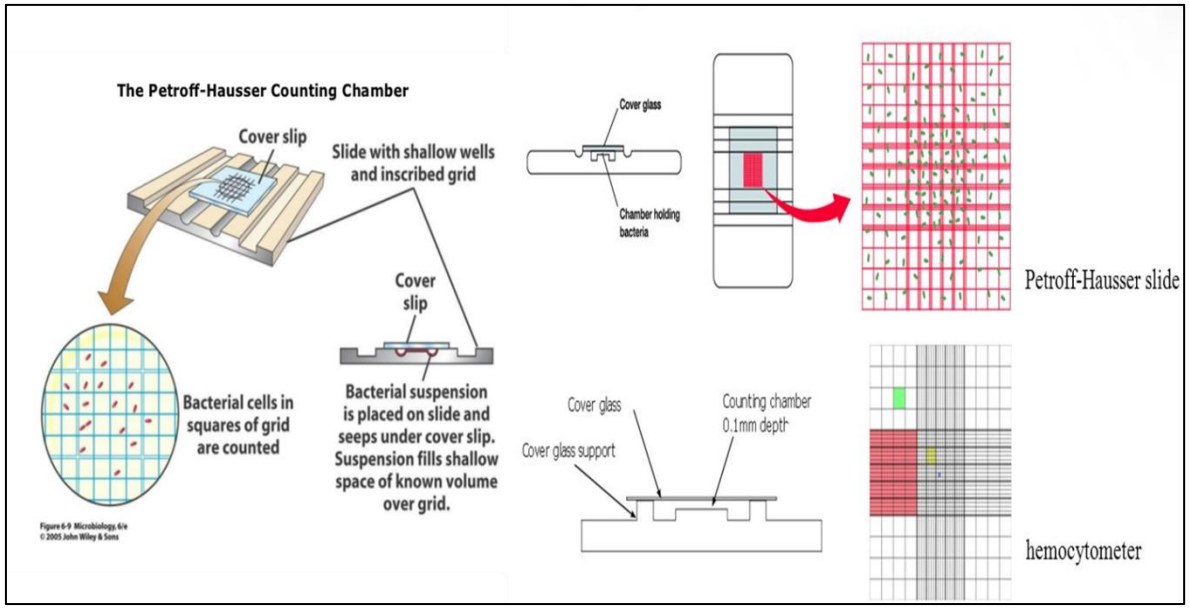


Figure 4-2: **petroff-hauser counting chamber for estimating microbial numbers**. Two views showing that the counter is a glass slide with an etched grid that is exactly 0.02 mm lower than the bottom of the cover slip. A bacterial suspension placed next to the cover slip through a pipette moves under the cover slip and over the grid by capillary action, each square millimeter, the grid has 25 large squares, each of which is divided into 16 small squares. Enlarged view of one large square containing 15 cells. The number of bacteria in several large squares is counted and averaged. The calculations involved in estimating the number of bacteria per milliter (cm).

Membrane filtration:

Viable plate counts allow scientists to estimate the number of microorganisms when the population is very large, but if the population density is very small-as is the case, for example, for fecal bacteria in a stream or lake-microbes are more accurately counted by membrane filtration (fig 4-4). In this method, a large sample (perhaps as large as several liters) is poured (or drawn under a vacuum) through a membrane filter with pores small enough to trap the cells. The membrane is then transferred onto a solid medium. In this case, the number of colonies is equal to the number of CFU_S in the original large sample.

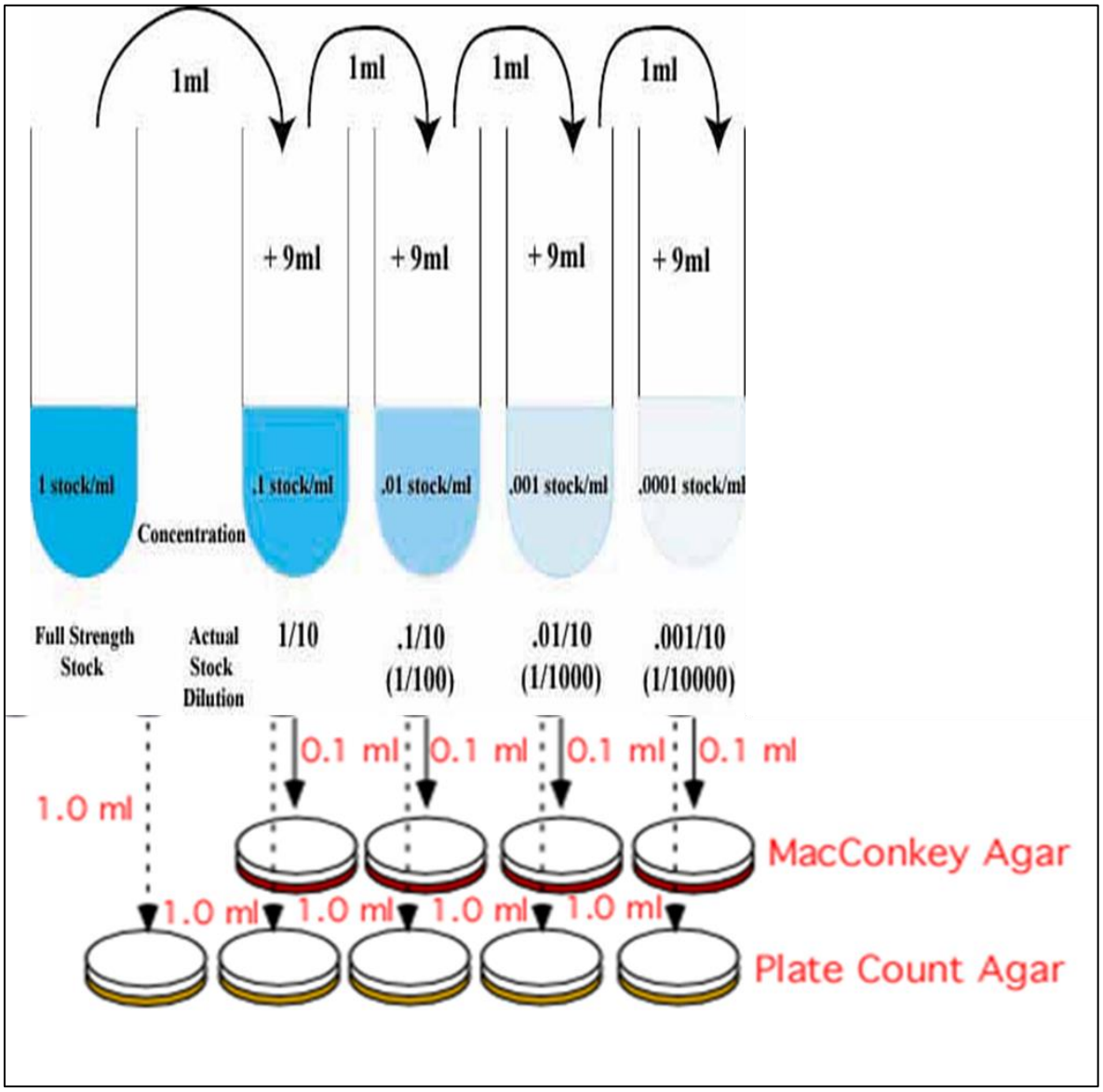


Figure 4-3: A viable plate count for estimating microbial population size, (a) Serial dilutions. A series of 10-fold dilutions is made, (b) Plating. A 0.1-ml sample from each dilution is poured onto a plate and spread with a sterile rod. Alternatively, 0.1 ml of each dilution can be mixed with melted agar medium and poured into plates. (c) Counting. Plates are examined after incubation. Some plates may contain so much growth that colonies are **too numerous to count (TNTC)**. The number of colonies is multiplied by the reciprocal of the dilution to estimate the concentration of bacteria in original culture-in this case, 65 colonies x 10,000 ^ 650,000 bacteria/ml.

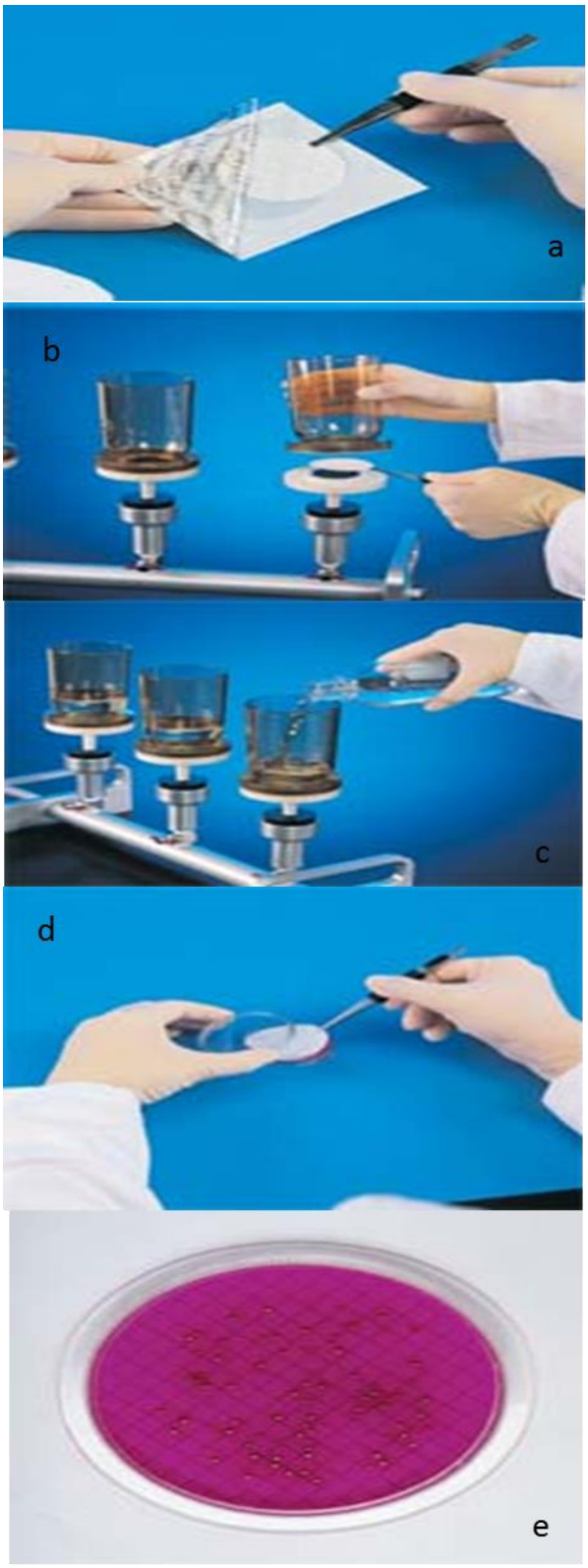


Figure 4-4: The use of Millipore filter-paper apparatus estimate microbial population size,

(a) Using a sterile filter paper to trap bacteria
(b) Putting the sterile filter paper on a sterilized Millipore filter paper apparatus.
(c) Loading the sample to be sterilized on the filter paper within filtration glass.
(d) After all the bacteria in a given volume of sample are trapped on a membrane filter, the filter is transferred onto an appropriate medium and incubated. The microbial population is estimated by multiplying the number of colonies counted by the volume of sample filtered, bacteria trapped on the surface of a membrane filter, colonies growing on a solid medium after being transferred from the membrane filter. Scientists use a super-imposed grid to help them count the colonies. If the colonies in (e) resulted from filtering 2.5 liters of stream water, what is the minimum number of bacteria per liter in the stream?

The microbial growth curve

In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 2°, 2^1, 2^2, 2^3.....2^n (where n - the number of generations). This is called exponential growth. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of lime, plotting the data will yield a typical bacterial growth curve (Figure 4-5).

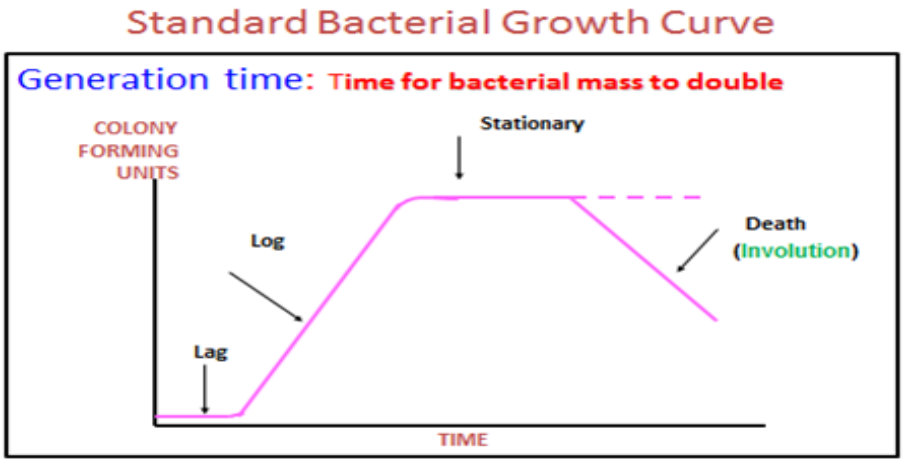


Figure 4-5: The typical bacterial growth curve. When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initial adjust to the new medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually) they show loss of viability (death phase). Note the parameters of the x and y axes. Growth is expressed as change in the number viable cells vs time. Generation times are calculated during the exponential phase of growth. Time measurements are in hours for bacteria with short generation times.

Four characteristic phases of the growth cycle are recognized

1-Lag Phase: Immediately after inoculation of the cells into a fresh medium, the population remains temporarily unchanged. Although there is no apparent cell

division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

2-Exponential (log) Phase: The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture expressed is generation time, also the doubling time of the bacterial population. Generation time (G) is defined as the time (t) per generation (n= number of generations). Hence, G=t/n is the expressed as equation from which calculations of generation time (below) derive.

3-Stationary Phase: Exponential growth cannot be continued forever in a batch culture (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors:

a. Exhaustion of available nutrients;
b. Accumulation of inhibitory metabolites or end products;
c. Exhaustion of space, in this case called a lack of "biological space".

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce secondary metabolites, such as antibiotic, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It's during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4-Death Phase: If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (note: if counting by turbidimetric "measurements or microscopic counts, the death phase cannot be

observed). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Growth Rate and Generation Time

Bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature. pH, etc.) define as a bacterium generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more (Table 4-1)

Table 4-1: Generation times for some common bacteria

Bacterium	Medium	Generation Time/minutes
Escherichia coli	Glucose-salts	17
Bacillus megateriumcoalerinn	Sucrose-salts	25
Streptococcus lava's	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium iaponicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

❖ **Only the colored columns requested from students**

Calculation of Generation Time

When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. If we start with one cell, when it divides, there are 2 cells in the first generation. 4 cells in the second generation, 8 cells in the third generation, and so on. The generation time is the time interval required for the cells for the cells (or population) to divide.

G=generation time (time for the cells to divide)

t= time interval in hours or minutes

B= number of bacteria at the beginning of a time interval

b= number of bacteria at the end of the time interval

n =number of generations (number of times the cell population doubles during the time interval)

b-B x 2" (This equation is an expression of growth by binary fission) G= t/n

G=t/n

Solve for n:

G=t/n

Solve for n

Logb=logB+nlog2

n= logb-logB/log2

n= (logb-logB)/0.301

n=1000/0.301(logb-logB)

n= 3.3logb/B

G=t/n

Example: What the generation-time of a bacterial population that increases from 10.000 cells to 10.000,000 cells in four hours of growth?

G=240 minutes /3.3 log $10/10^4$

G =240 minutes /3.3 x 3

G= 24 minutes

Continuous Culture

Bacteria can be maintained in a state of continuous exponential growth by using a chemostat (figure 4-5). This device continually drips fresh medium into a liquid culture contained in a growth chamber. With each drop that enters, an equivalent volume, containing cells, wastes, and spent medium, leaves through an outlet. By manipulating the concentration of nutrients in the medium and the rate at which it enters the growth chamber, a constant cell density and generation time of log phase cells can be maintained. This makes it possible to study a uniform population of cells over a altering the cellular long period of time. The effect of adding various supplements to the medium or altering the cellular environment on long-term cell growth can be determined.

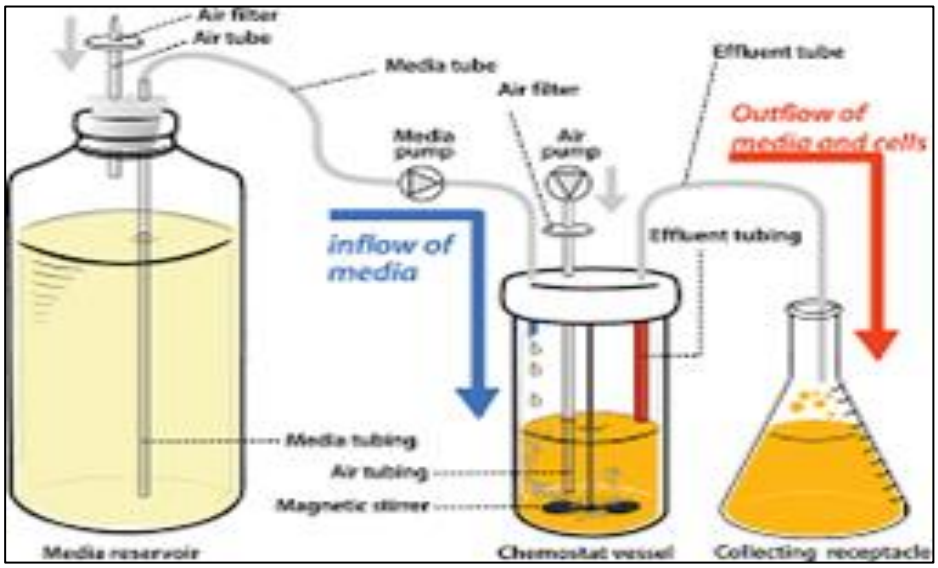


Figure 4-6: Chemostat, This device can maintain a culture in a steady state of exponential growth by allowing fresh-medium to drip in while an equivalent volume of spent medium, cells, and wastes leaves

Genetic Recombination and Transfer

Genetic recombination refers to the exchange between two DNA molecules segments that are composed of identical or nearly identical nucleotide sequences called homologous sequences.

Horizontal gene transfer among prokaryotes

Both prokaryotes and eukaryotes replicate their genomes and supply copies to their descendants. This is known as **vertical gene transfer** the passing of genes to the next generation. In addition, many prokaryotes can acquire genes from other microbes of the same generation, a process termed **horizontal gene transfer**. In horizontal gene transfer, a donor cell contributes part of its genome to a recipient cell, which may be a different species or even different genus from the donor. Typically, the recipient cell inserts part of the donor's DNA into its own-chromosome, becoming a recombinant cell. Cellular enzymes then usually degrade the remaining unincorporated DNA. Horizontal gene transfer is a rare event, typically occurring in less than 1 % of a population. Here we consider the three types of horizontal gene transfer: **transformation, transduction, and bacterial conjugation**.

Transformation

In transformation, a recipient cell takes up DNA from the environment, such DNA that might be released from dead organisms. Frederick Griffith (1879-1941) discovered this process in 1928 while attempting to develop a vaccine for pneumonia caused by *Streptococcus pneumoniae*, though the organism was called *Diplococcus pneumoniae* at that time. Griffith worked with two strains of *Streptococcus*. Cells of the first strain have a protective capsule that enables them to escape a body's defensive white blood cells; thus, these encapsulated cells cause deadly pneumonia when injected into mice (Figure 4-7a). (They are called strain **S** because they form

smooth colonies on an agar surface.) The application of heat kills these encapsulated cells and renders them harmless when injected into mice (Figure 4-7b). In contrast, cells of the second strain (called strain **R** because they form **rough** colonies) are mutants that cannot make a capsule. The unencapsulated cells of strain R do not cause disease (Figure 4-7c) because a mouse's defensive white blood cells quickly devour them.

Griffith discovered that when he injected both **heat-killed strain S and living strain R** into a mouse, the mouse died from pneumonia even though neither of the injected strains was harmful when administered alone (Figure 4-7d). Further, and most significantly, Griffith isolated numerous living, encapsulated cells from the dead mouse. He realized that harmless, unencapsulated strain R bacteria had been transformed into deadly, encapsulated strain S bacteria. Subsequent investigations showed that transformation also occurs *In vitro* (Figure 4-7e). The fact that the living encapsulated cells retrieved at the end of this experiment outnumbered the dead encapsulated cells injected at the beginning indicated that strain R cells were not merely appropriating capsules released from dead strain S cells. Instead, strain R cells had acquired the capability of producing their own capsules by assimilating capsule-coding genes released from strain S cells. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty extracted various chemicals from S cells and determined that the transforming agent was DNA. This discovery was one of the conclusive pieces of proof that DNA is the genetic material of cells.

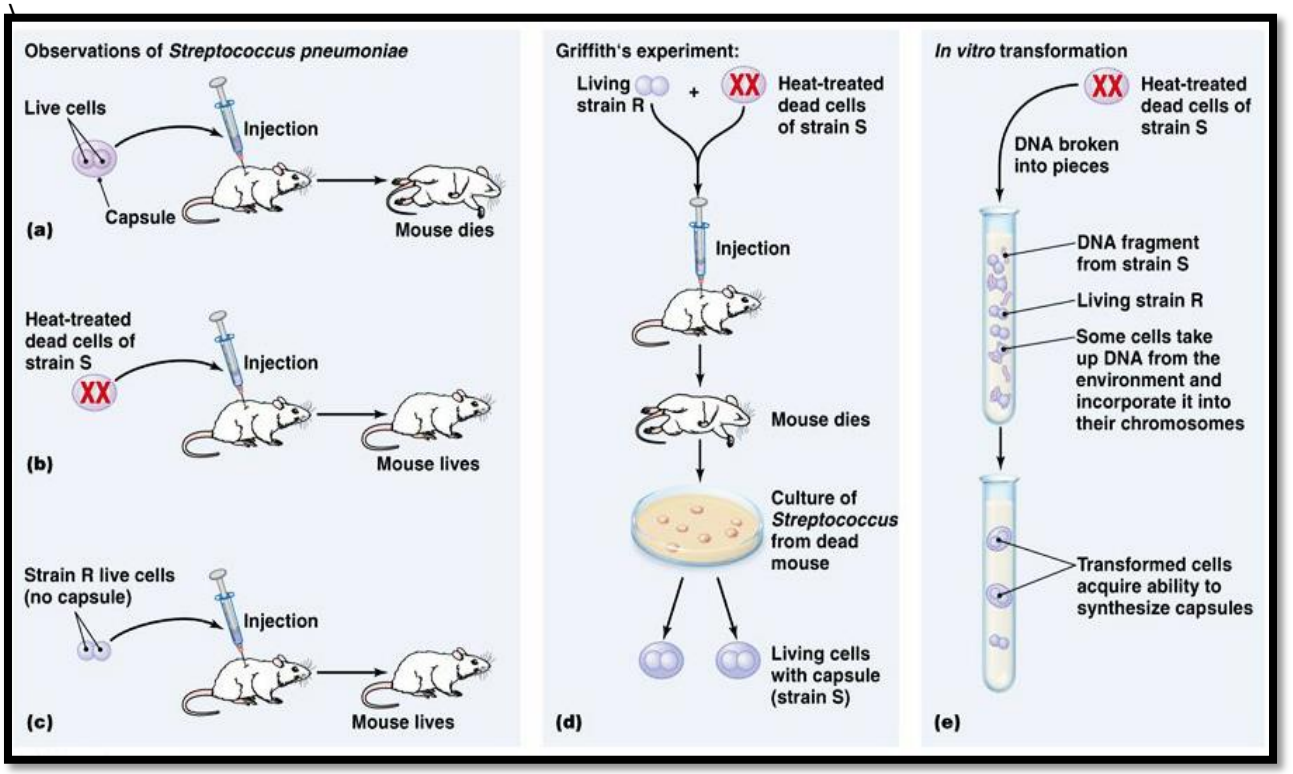


Figure 4-7: Transformation in *St. pneumoniae*. Griffith's observations revealed that (a) encapsulated strain S killed mice, (b) heating renders strain S harmless to mice, and (c) unencapsulated strain R did not harm mice. In Griffith's experiment (d), a mouse injected concurrently with killed strain S and live strain R (each harmless) the mouse was died and was found to contain numerous living, encapsulated bacteria, (e) A demonstration that transformation of R cells to S cells also occurs *In vitro*.

Transduction

A second method of horizontal gene transfer, called **transduction**, involves the transfer of DNA from one cell to another via a replicating virus. Transduction can occur either between prokaryotic cells or between eukaryotic cells; it is limited only by the availability of a virus capable of infecting both donor and recipient cells. Here we will consider transduction in bacteria.

A virus that infects bacteria is called a **bacteriophage or phage (faj)**. The process by which a phage participates in transduction is depicted in Figure 4-8. To replicate, a bacteriophage attaches to a bacterial host cell and injects its genome into the cell. Phage enzymes, translated by cellular ribosomes, degrade the cell's DNA.

The phage genome now controls the cell's functions and directs it to synthesize new phage DNA and phage proteins. Normally, phage proteins assemble around phage DNA to form new phage particles, but some phages mistakenly incorporate remaining fragments of bacterial DNA to form transducing phages. Eventually the host cell lyses, releasing daughter and transducing phages. Transduction occurs when a transducing phage injects donor DNA into a new host cell (the recipient). The recipient host cell incorporates the donated DNA into its chromosome by recombination.

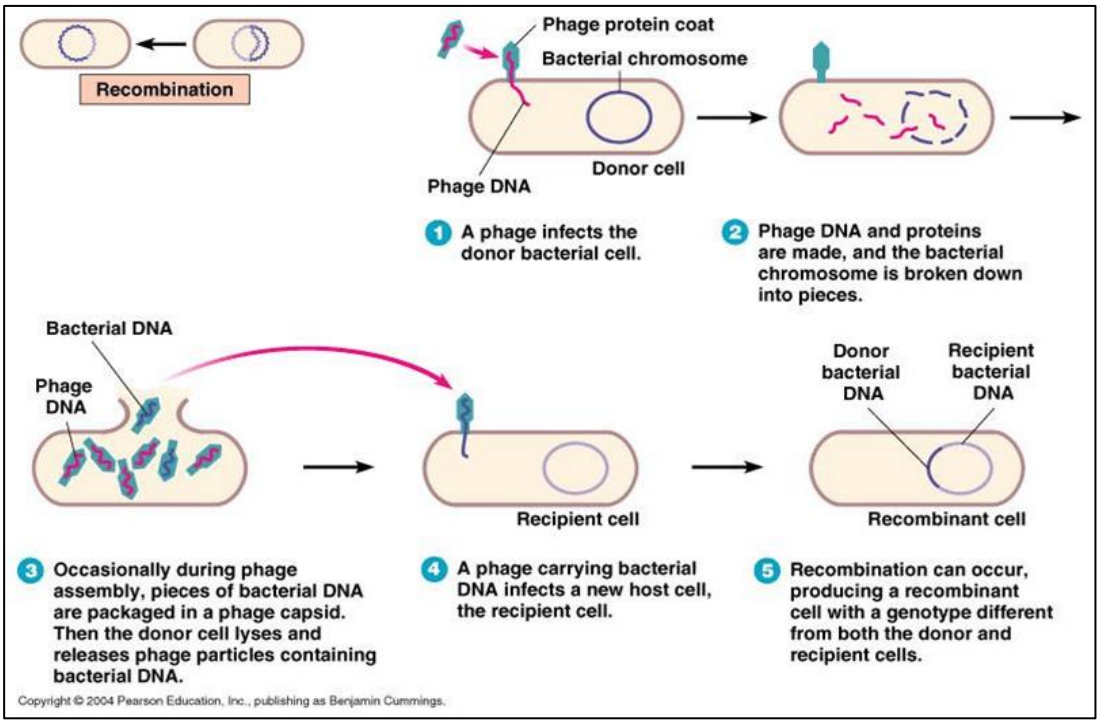


Figure 4-8: Transduction. After a virus called a bacteriophage (phage) attaches to a host bacterial cell, it injects it's genome into cell and direct the cell to synthesize new phage. During assembly of new phages, some host DNA may be incorporated, forming transducing, which subsequently carry donor DNA to a recipient host cell.

Bacterial Conjugation

A third method of genetic transfer in bacteria is **conjugation**. Unlike the donor cells in transformation and transduction, a donor cell in conjugation remains alive. Further, conjugation requires physical contact between donor and recipient cells. Scientists discovered conjugation between cells of *E. coli*, and it is best understood in

this species. Thus, the remainder of our discussion will focus on conjugation in this bacterium.

Conjugation is mediated by conjugation pili (pili, singular: pilus), also called **sex pili** which are proteinaceous, rod-like structures extending from the surface of a cell. The gene coding for conjugation pili is located on a plasmid called an F (fertility) plasmid or F factor. (Recall that a plasmid is a small, circular, extrachromosomal, molecule of DNA); Cells that contain an F plasmid are called F^+ cells, and they serve as donors during conjugation. Recipient cells are F^-; that is, they lack an F plasmid and therefore have no conjugation pili. The process of bacterial conjugation is illustrated in Figure 4-9. First, a sex pilus connects a donor cell (F^+) to a recipient cell (F^-) cell, and the pilus then draws the cells together. After the cells touch, they stabilize their contact, probably via the fusion of cell membranes. The donor cell then replicates one strand of its F plasmid and transfers it to the recipient. The F^- recipient then synthesizes a complementary strand of F plasmid DNA and thus becomes an F^+ cell.

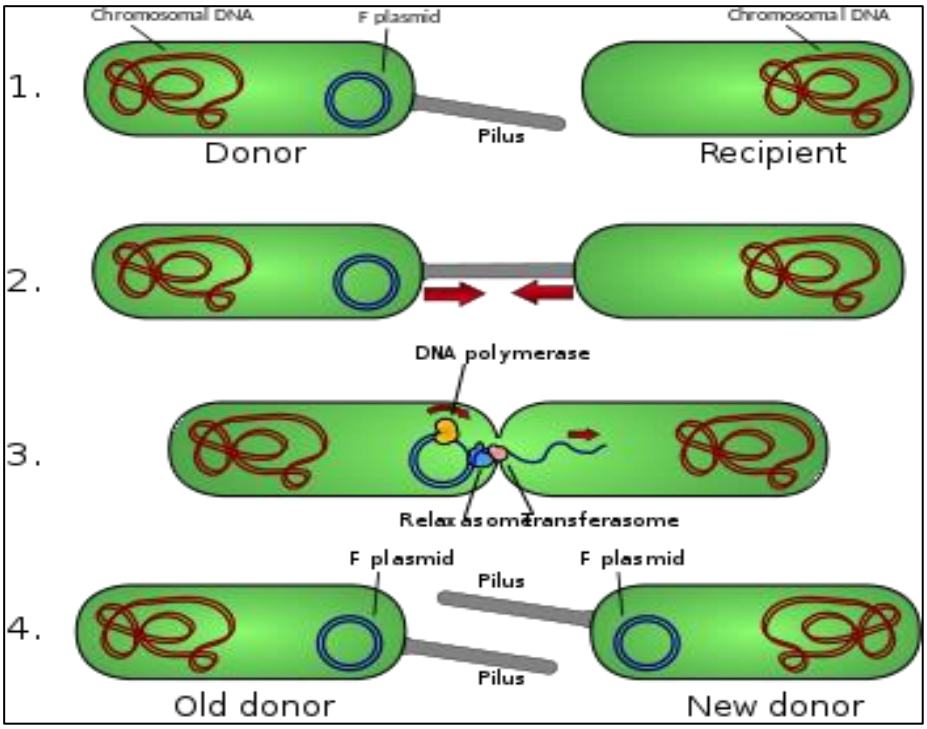


Figure4-9: Bacterial conjugation, four steps represent the process in which a conjugation pilus connecting two strains of *E. coli* cells mediates the transfer of DNA between them.

Control of Microbial growth

Approaches to control

1. The methods used to destroy or remove microorganisms and viruses can be physical, such as heat treatment, irradiation, and filtration, or chemical.

Principles of Control

2. **Sterilization** removes or destroys all microorganisms and viruses on or in a product.

3. **Disinfection** eliminates most or all disease-causing microorganisms or viruses on or in a material.

4. **Chemicals** used for disinfecting inanimate objects are called **disinfectants**; those formulated for use on skin are called **antiseptics**.

5. **Pasteurization** utilizes a brief heat treatment to reduce the number of spoilage organisms or kill disease-causing microbes.

6. **A decontaminated** item has been treated to reduce die number of disease-causing microbes to a level that is safe to handle.

7. **A sanitized**, item has a substantially reduced microbial population that meets accepted health standards.

Environmental Conditions

1.Factors such as pH or presence of fats and other organic materials strongly influence microbial death-rates,

2. The presence of dirt, grease, and organic compounds such as blood and other body fluids can interfere with heat penetration and die action of chemical disinfectants.

Using Heat to Destroy Microorganisms and Viruses

1. Heat can be used to destroy vegetative microorganisms and viruses, but temperatures above boiling are required to kill endospores.

Moist heat

1. **Moist heat**, such as boiling water and pressurized steam, destroys microorganisms by causing irreversible coagulation of their proteins.
2. **Pasteurization** utilizes a brief heat treatment to destroy, spoilage and disease-causing organisms; increasing the shelf life of products and protecting consumers.
3. **Pressure cookers and autoclaves** heat water in an enclosed vessel that causes the pressure in the vessel to increase beyond atmospheric pressure, increasing the temperature of steam, which kills endospores.
4. The most important aspect of the commercial canning process is to ensure that endospores of *Clostridium botnlinum* are destroyed.

Dry Heat

1. Direct flame and ovens generate dry heat, which destroys microorganisms by oxidizing cells to ashes or irreversibly denaturing their proteins.
2. Dry heat takes much longer than wet heat to kill microorganisms.

Removal of Microorganisms by Filtration

Filtration of Fluids

1. Depth filters have complex, tortuous passages; that retain microorganisms while letting the suspending fluid pass through the small holes.
2. Membrane filters are produced with graded pore sizes extending below the dimensions of the smallest known viruses.

Using radiation to destroy Microorganisms and Viruses

Gamma Irradiation

1. Gamma rays cause biological damage by producing superoxide and hydroxyl free radicals.
2. Irradiation can be used to sterilize heat-sensitive materials and to decrease the numbers of microorganisms in foods.
3. Irradiation has been approved by the FDA to control insects in fruits, vegetables-, and grains, to destroy the trichina parasite in pork, and to control *Salmonella* species and *E. coli* O157:H7 in meats.

Ultraviolet Radiation

1.Ultraviolet light damages the structure and function of nucleic acids by causing the formation of covalent bonds between adjacent thymine molecules in DNA, creating thymine dimers (figure5-1).

2.UV light is used to disinfect surfaces.

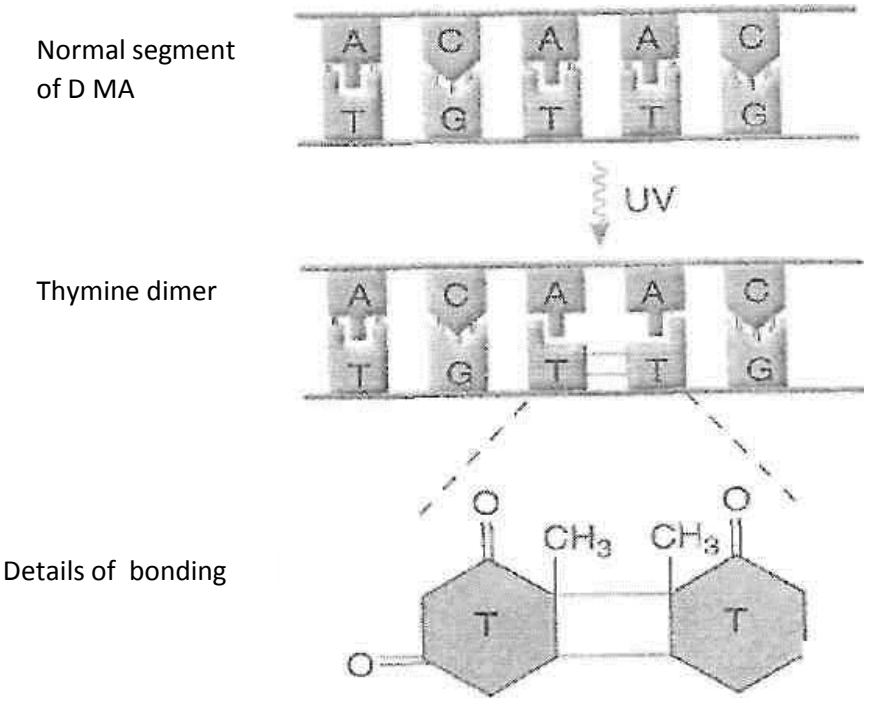


Fig 5-1: Formation of pyrimidine dimmers by the action of ultraviolet (UV) radiation. This shows what occurs when two adjacent thymine bases on one strand of DNA are induced by UV rays to bond laterally with each other. The result is a thymine dimer shown in greater detail. If they are not repaired, dimers can prevent that segment of DNA from being correctly replicated or transcribed. Massive dimerization is lethal to cells.

Chemical Agents in Microbial Control

Antimicrobial chemicals occur in the liquid, gaseous, or even solid state. They serve as disinfectants, antiseptics, sterilants (chemicals that sterilize), degermers, or preservatives (chemicals that inhibit the deterioration of substances). For the sake of convenience (and sometimes safety) many solid or gaseous antimicrobial chemicals are dissolved in water, alcohol, or a mixture of the two to produce a liquid solution. Solutions containing pure water as the solvent are termed **aqueous**, whereas those dissolved in pure alcohol or water-alcohol mixtures are termed **tinctures**.

Choosing a Microbicidal Chemical

The choice and appropriate use of antimicrobial chemical agents is of constant concern in medicine and dentistry. Although actual clinical practices of chemical decontamination vary widely, some desirable qualities in a germicide have been identified, including:

- Rapid action in low concentration
- Solubility in water or alcohol and long-term stability
- Broad-spectrum microbicidal action without being toxic to human and animal tissues.
- Penetration of inanimate surfaces to sustain a cumulative or persistent Action.
- Resistance to becoming inactivated by organic matter,

Glasses of Germicidal chemicals

Germicides are represented in a number of chemical families. Each type has characteristics that make it more or less appropriate for specific uses.

Table 5-1: chemical used in sterilization and disinfection and preservation of nonfood substances.

Chemical (examples)	Characteristics
Alcohols (ethanol and isopropanol)	Aqueous solutions are used as antiseptics for disinfecting some instruments and surfaces. Alcohols leave no residue, are easy to obtain, and are inexpensive. They evaporate quickly, which limits their contact time.
Aldehydes (formaldehyde and glutaraldehyde)	Formalin is used in vaccine preparation. Glutaraldehyde is more effective than formaldehyde against endospores and is commonly used as a sterilant. Aldehydes inactivate proteins and nucleic acids. They are irritating to the respiratory system, skin, and eyes.
Biguanides (chlorhexidine)	Chlorhexidine is widely used as an antiseptic in soaps and lotions, and more recently, impregnated into catheters and surgical mesh.
Ethylene Oxide Gas	Commonly used as a sterilant. It easily penetrates hard-to-reach places and fabrics and does not damage moisture-sensitive material. It is explosive, toxic, and potentially carcinogenic.
Halogens (chlorine and iodine)	Chlorine is used to disinfect inanimate objects, surfaces, drinking water, and wastewater. Organic compounds and other impurities neutralize its activity .Tincture of iodine and iodophores can be used as disinfectants or antiseptics. Chlorine and iodine are both oxidizing agents.
Metals **(silver)**	Silver sulfadiazine is used in topical dressings to prevent infection of bums. Silver nitrate drops can be used to prevent gonococcal eye infections of newborns. Most other metal compounds are too toxic to be used medically.

Chemotherapeutic agents (synthetic antibiotics): antimicrobial agents of synthetic origin useful in the treatment of microbial or viral disease. Examples are sulfonilamides, isoniazid, ethambutol, AZT, nalidixic acid and chloramphenicol.

antimicrobial agents produced by microorganisms that kill or-inhibit other microorganisms.

Antibiotics: are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Most of these microorganisms form some type of a spore or other dormant cell, and there is thought to be some relationship (besides temporal) between antibiotic production and the processes of sporulation. Among the molds, the notable - antibiotic producers are *Penicillium* and *Cephalosporium*, which are the main source of the beta-lactam Antibiotics (penicillin and its relatives). In the bacteria, the Actinomycetes, notably *Streptomyces* species, produce a variety of types of antibiotics including the aminoglycosides (e.g: streptomycin), macrolides (e.g: erythromycin), and the tetracyclines. Endospore-forming *Bacillus* species produce **polypeptide** antibiotics such as polymyxin and Bactiracin.

Characteristics of the Ideal Antimicrobial Drug

- Selectively toxic to the microbe but nontoxic to host cells.
- Microbicidal rather than microbistatic.
- Relatively soluble; functions even when highly diluted in body fluids
- Remains potent long enough to act and is not broken down or excreted prematurely
- Doesn't lead to the development of antimicrobial resistance
- Complements or assists the activities of the host's defenses
- Remains active in tissues and body fluids
- Does not disrupt the host's health by causing allergies or predisposing the host to other infections.

Mechanisms of Drug Action

There are five major components of cells known to be useful targets in an actively dividing cell (figure 5-2). These are the cell wall and membrane, the genetic material, protein synthesis, and metabolic pathways. We will cover the effects of drugs on theses cell components, based on this outline and:

1. Inhibition of cell wall synthesis
2. Breakdown of the cell membrane structure or function
3. Inhibition of structures and functions of DNA and RNA
4. Inhibition of protein synthesis and
5. Blocks on key metabolic pathways.

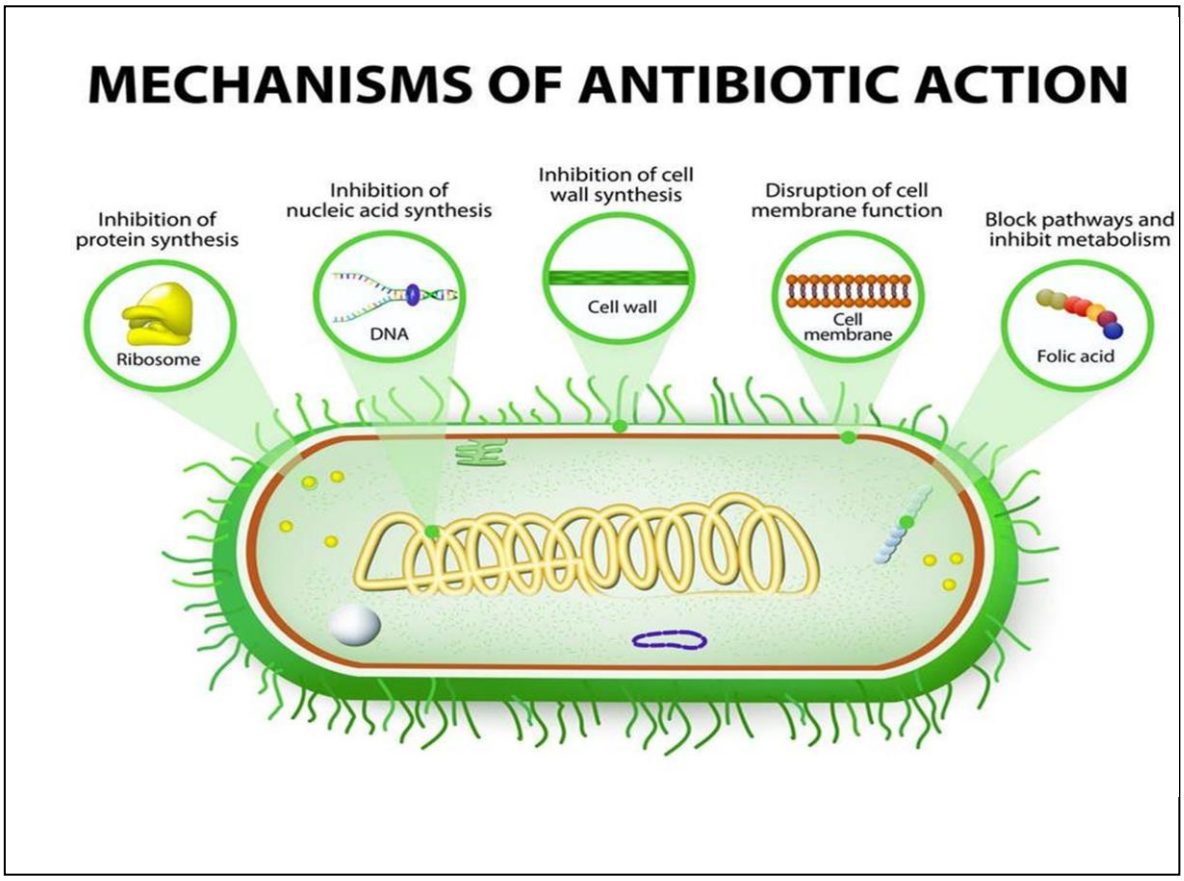


Figure 5-2: Antibiotics site of action. As shown above, there are five site in the bacterial cell could be a target for an antibiotics.

The Spectrum of an Antimicrobial Drug

One result of drugs acting upon a particular microbial structure or function is that each drug has a particular range of activity. This has been termed the drug's *spectrum.* Traditionally, narrow-spectrum drugs are effective on a small range of cell types. This is usually because they target a specific component that is found in only certain bacteria. For instance, bacitracin blocks the elongation of the peptidoglycan in gram-positive bacteria but has no effect on gram-negatives. Drugs that are effective against a wider range of microbes are termed medium or broad spectrum, depending on the particular drug. For example, the medium-spectrum drug ampicillin is effective on both gram-positive and gram-negative bacteria, but not all of them. Broad-spectrum drugs, like tetracycline, have the greatest range of activity. They work on most gram-negative and gram-positive bacteria, rickettsias, and mycoplasmas. The broader-spectrum drugs exert their effect on cell components such as ribosomes, which are found in most of the pathogens.

1- Antimicrobial Drugs That Affect the Bacterial Cell Wall

The cell walls of most bacteria contain a rigid girdle of peptidoglycan, which protects the cell against rupture from hypotonic environments. Active cells must constantly synthesize new peptidoglycan and transport it to its proper place in the cell envelope. Drugs such as penicillins and cephalosporins react with one or more of the enzymes required to complete this process, causing the cell to develop weak points at growth sites and to become osmotically fragile. Antibiotics that produce this effect are considered bactericidal, because the weakened cell is subject to lysis. It is essential to note that most of these antibiotics are active only in young, growing cells, because old, inactive, or dormant cells do not synthesize peptidoglycan.

Penicillins and cephalosporins bind and block peptidases that cross-link

the glycan molecules, thereby interrupting the completion of the cell wall. Penicillins that do not penetrate the outer membrane are less effective against gram-negative bacteria. Broad-spectrum penicillins (carbenicillin) and cephalosporins (ceftriaxone) can pass into the cell walls of gram-negative species. Cycloserine inhibits the formation of the basic peptidoglycan subunits, and vancomycin hinders the elongation of the peptidoglycan.

2- Antimicrobial Drugs That Disrupt cell Membrane Function

A cell with a damaged membrane invariably dies from disruption in metabolism or lysis. It does not even have to be actively dividing to be destroyed. The antibiotic classes that damage cell membranes usually have specificity for particular microbial groups, based on differences in the types of lipids in their cell membranes Polymyxins interact with membrane phospholipids and cause leakage of proteins and nitrogen bases, particularly in gram-negative bacteria. The polyene antifungal antibiotics (amphotericin B and nystatin) form complexes with the sterols on fungal membranes, which cause abnormal openings and seepage of small ions. Unfortunately, this selectivity is not exact, and the universal presence of membranes in microbial and animal cells alike means that most of these antibiotics can be quite toxic to human.

3-Antimicrobial Drugs That Affect Nucleic Acid Synthesis

Antimicrobial drugs interfere with nucleic acid synthesis by blocking synthesis of nucleotides, inhibiting replication or stopping transcription. Because functioning DNA and RNA are required for proper translation as well, the effects on protein metabolism can be far-reaching.

Other antimicrobials inhibit DNA synthesis. Chloroquine (an antimalarial drug) binds and cross-links the double helix. The newer broad-spectrum quinolones inhibit DNA unwinding enzymes or helicases, thereby

stopping DNA transcription. Antiviral drugs that are analogs of purines and pyrimidines, including azidothymidine (AZT) and acyclovir, insert in the viral nucleic acid and block further replication.

4-Antimicrobial Drugs That Block Protein Synthesis

Most drugs that inhibit translation, or protein synthesis, react with the ribosome-mRNA complex. Although human cells also have ribosomes, the ribosomes of eucaryotes are different in size and structure from those of procaryotes, so these antimicrobials usually have a selective action against bacteria. One potential therapeutic consequence of drugs that bind to the procaryotic ribosome is the damage they can do to eucaryotic mitochondria, which contain a procaryotic type of ribosome Two possible targets of ribosomal inhibition are the 305 subunit and the 50S subunit. Aminoglycosides (strepto- mycin, gentamicin, for example) insert on sites on the 305 subunit and cause the misreading of the mRNA, leading to abnormal proteins. Tetracyclines block the attachment of tRNA on the A acceptor site and effectively stop further synthesis.

5- **Antimicrobial Drugs That Affect Metabolic Pathways**

Sulfonamides and trimethoprim are drugs that act by mimicking the normal substrate of an enzyme in a process called **competitive inhibition.** Sulfonamides and trimethoprim interfere with folate metabolism by blocking enzymes required for the synthesis of tetrahydrofolate, which is needed by bacterial cells for the synthesis of folic acid "and the eventual production of DNA and RNA and amino acids: Trimethoprim and sulfonamides are often given simultaneously to achieve a synergistic effect.

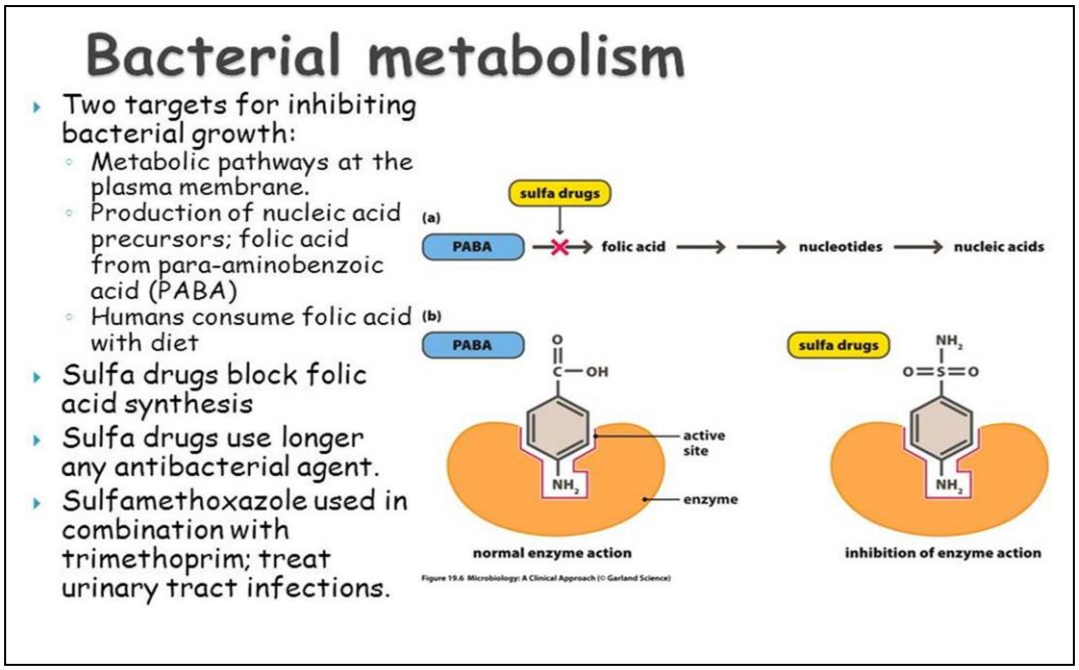


Figure 5-3: Antibiotic blocking the folic acid synthesis pathway.

Bacterial resistance to antibiotics: The most important pathogens to emerge in multiple drug resistant forms so far have been *Mycobacterium tuberculosis* and *Staphylococcus aureus* Bacterial resistance to an antimicrobial agent may be due to some innate property of the organism or it may due to acquisition of some genetic trait as described below.

Inherent (Natural) Resistance: - Bacteria may be inherently resistant to an antibiotic. For example, a streptomycete may have some natural gene that is responsible for resistance to its own antibiotic; or a Gram-negative bacterium has an outer membrane that establishes a permeability barrier against the antibiotic; or an organism lacks a transport system for the antibiotic; or it lacks the target or reaction that is hit by the antibiotic.

Acquired Resistance:- Bacteria can develop resistance to antibiotics, e.g. bacterial populations previously-sensitive to antibiotics become resistant. This type of resistance results from changes in the bacterial genome. Acquired resistance is driven by two genetic processes in bacteria: (1) mutation and

selection (sometimes referred to as vertical evolution); (2) exchange of genes between strains and species (sometimes called horizontal evolution or horizontal gene transmission).

Survey of Procaryotic Groups with Unusual Characteristics

The bacterial world is so diverse that we cannot do complete justice to it in this introductory chapter. This variety extends into all areas of bacterial biology, including nutrition, mode of life, and behavior. Certain types of bacteria exhibit such unusual qualities that they deserve special mention. In this minisurvey, we will consider some medically important groups and some more remarkable representatives of bacteria living free in the environment that are ecologically important. Many of the bacteria mentioned here do not have the morphology typical of bacteria discussed previously, and in a few cases, they are vividly different.

Free-Living Nonpathogenic Bacteria

Photosynthetic Bacteria

The two general types of photosynthetic bacteria are those that produce oxygen during photosynthesis and those that produce some other substance, such as sulfur granules or sulfates.

Cyanobacteria: Blue-Green Bacteria

The cyanobacteria were called blue-green algae for many years and were grouped with the eucaryotic algae. However, further study verified that they are indeed bacteria with a gram-negative cell wall and general procaryotic structure. These bacteria range in size from 1µm to 10 µm, and they can be unicellular or can occur in colonial or filamentous groupings**.**

This group is sometimes called the blue-green bacteria in reference to their content of phyco-cyanin pigment that tints some members a shade of blue. Cyanobacteria are very widely distributed in nature. Some members are so pollution-resistant that they serve as biological indicators of polluted water.

Gliding, Fruiting Bacteria

The gliding bacteria are a mixed collection of gram-negative bacteria that live in water and soil. The name is derived from the tendency of members to glide over moist surfaces. The gliding property evidently involves rotation of filaments or fibers just under the outer membrane of the cell wall. They do not have flagella. Several morphological forms exist, tree-shaped fruiting bodies. Probably the most intriguing and exceptional members of this group are the slime bacteria, or *Myxobacteria*.

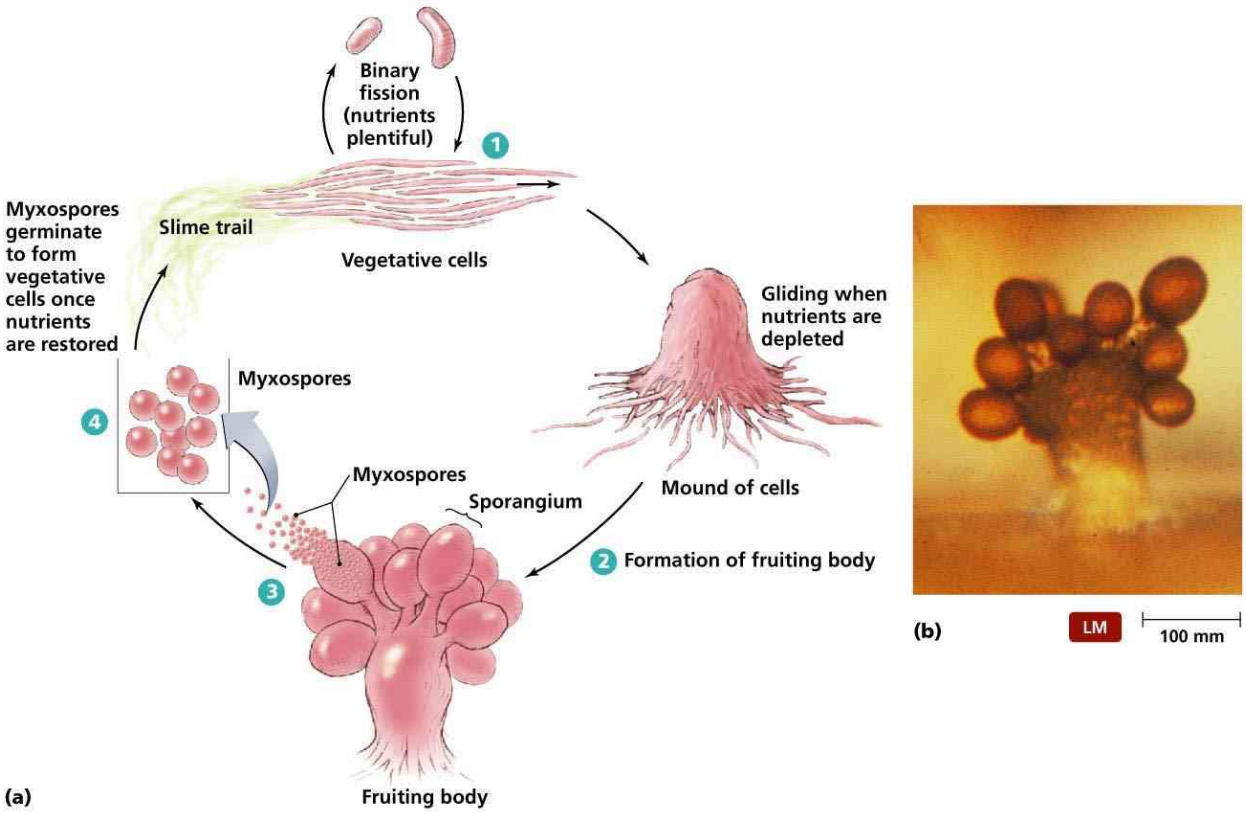


Figure 6-1: life cycle of fruiting body (*Myxobacteria*)

Unusual Forms of Medically Significant Bacteria

Most bacteria are free-living or parasitic forms that can metabolize and reproduce by independent means. Two groups of bacteria-the rickettsias and chlamydias-have adapted to life inside their host cells, where they arc considered obligate intracellular parasites.

Rickettsias

Rickettsias are distinctive, very tiny, gram-negative bacteria**.** Although they have a somewhat typical bacterial morphology, they are atypical in their life cycle and other adaptations. Most are pathogens that alternate between a mammalian host and blood-sucking arthropods, such as fleas, lice, or ticks. Several important human diseases are caused by rickettsias. Among these are **Rocky Mountain spotted fever**, caused by *Rickettsia rickettsii* (transmitted by ticks), and **endemic typhus**, caused by *Rickettsia typhi* (transmitted by lice).

Spirochetes are a phylogenetically distinct group of Bacteria which have a unique cell morphology and mode of motility. Spirochetes are very thin, flexible, spiral- shaped procaryotes that move by means of structures called axial filaments or endoflagella. The flagellar filaments are contained within a sheath between the cell wall peptidoglycan and an outer membrane. Most spirochetes are free living (in muds and sediments), or live in associations with animals (e.g. in the oral cavity or GI tract). A few are pathogens of animals (e.g. leptospirosis in dogs, syphilis in humans and **Lyme Disease** (LD) in dogs and human.

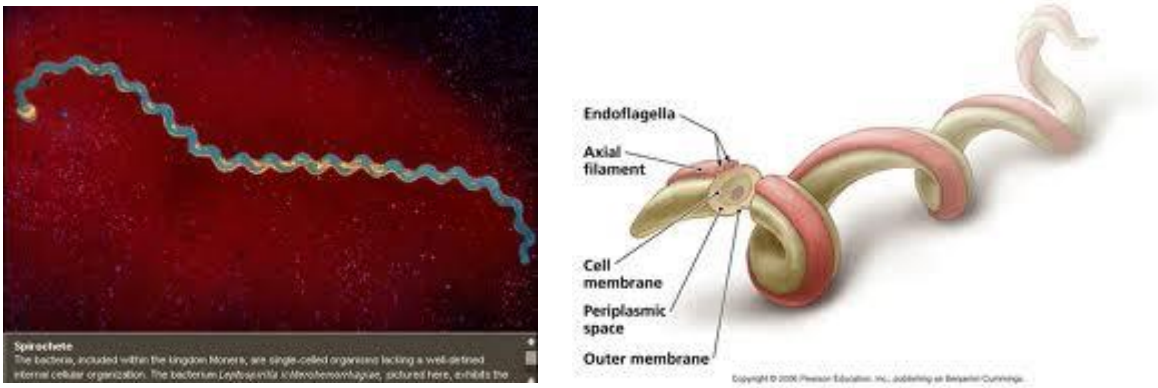


Figure 6-2: spirochete and the structure of endoflagellum

Table 6-1: Comparison of the Two Spiral - Shaped Bacteria

	Overall appearance	Mode of locomotion	Number of helical turns	Gram reaction	Example of important types
Spirilla	Rigid helix spirilla	Polar flagella ; cells Swim by rotating around like corkscrews ; do not flex: 1 to several flagella can be in tufts	Varies from 1 to 20	Gram-negative	Most are harmless ; one species , *Spirillum minor* , causes rat bite fever
Spirochetes	Flexiable helix	Periplasmic flagella within sheath ; cells flex ; can swim by rotation or by creeping on surfaces: Curved or spiral forms : 2 to 100 periplasmic flagella	Varies from 3 to 70	Gram-negative	*Treponema pallidum* , cause of syphilis ; *Borrelia* and *Leptospira* , important pathogens

Other spiral shaped and Curved Bacteria

The main thing that unifies this group of bacteria is their spiral or vibrioid (curved) shape,. Bacteria referred to as "spirilla" are Gram-negative aerobic heterotrophic bacteria with a helical or spiral shape. Their Unlike spirochetes, they have a rigid cell wall and are motile by means of ordinary polar flagella. Spirilla are inhabitants of microaerophilic aquatic environments. Spirilla are thought to play a significant role in recycling of organic matter, particularly in aquatic environments.

Two pathogens of humans are found among the spiral *Campylobacter jejuni* is an important cause of bacterial diarrhea, especially in children. The bacterium is transmitted via contaminated food, usually undercooked poultry or shellfish, or untreated drinking water.

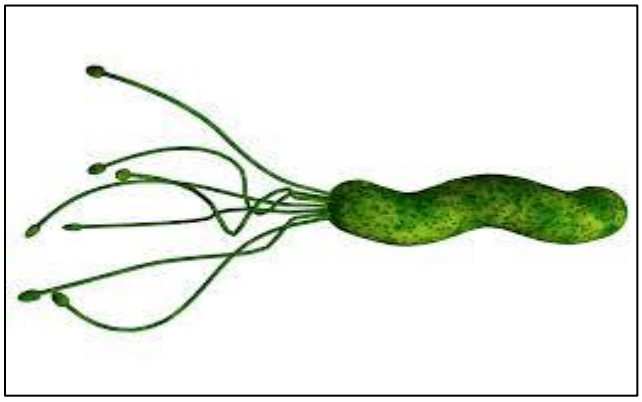


Figure 6-3: Shape of H. *pylori*

Helicobacter pylori is able to colonize the gastric mucosal cells lining of the stomach of humans and it has been well established as the cause of peptic ulcers.

Bacteria with a curved rod or comma shape are referred to as "vibrios". Like the spiral forms, vibrios are very common bacteria in aquatic environments. In Bergey's Manual (2001). **Vibrionaceae** is a family on the level with Enterobacteriaceae. Vibrios are facultative like enterics, but they have polar flagella, and oxidase-positive. In aquatic habitats they overlap with the Pseudomonadaceae in their ecology, although ***Pseudomonas*** species favor **fresh water** and vibrios prefer **salt water**. The genus ***Vibrio*** contains an important pathogen of humans, *Vibrio cholera,* the cause of Asiatic cholera. Cholera is an intestinal disease with a pathology related to diarrheal diseases caused by the enteric bacteria. Five species of marine vibrios exhibit the property of **bioluminescence,** the ability to emit light of a blue-green color. These bacteria may be found as saprophytes of dead fish or as symbionts of living fish and invertebrates in marine environments

The small vibrioid bacterium, *Bdellovibrio,* is a tiny curved rod that is a parasite of other Gram-negative bacteria, including E. coli. It preys on other bacteria by entering into the periplasmic space and obtaining nutrients from the cytoplasm of its host cell while undergoing an odd type of reproductive cycle.

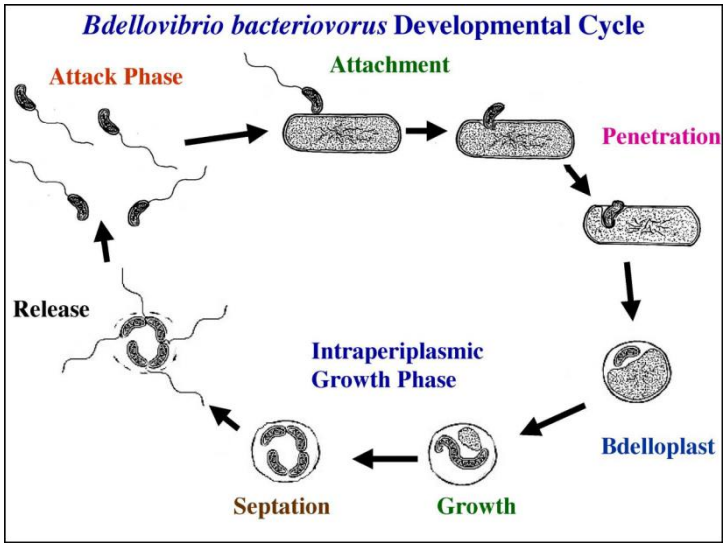


Figure 6-5: Life cycle of *Bdellovibrio*

Pseudomonads.

"Pseudomonad" is an informal term for bacteria which morphologically and physiologically resemble members of the genus Pseudomonas. They are unified only as Gram-negative rods with a strictly-respiratory mode of metabolism. The morphology and habitat of many pseudomonads sufficiently overlaps with the enterics so that microbiologists must quickly learn how to differentiate these two types of Gram-negative motile rods. Pseudomonads move by polar flagella; enterics such as *E. coli* swim by means of peritrichous flagella. Enterics ferment sugars such as glucose; pseudomonads generally do not ferment sugars. Pseudomonads are typically .oxidase- positive

Most pseudomonads are free-living organisms in soil and water; they play an important role in decomposition, biodegradation, and the C and N cycles. One pseudomonas species is an important of humans, ***Pseudomonas aeruginosa***.

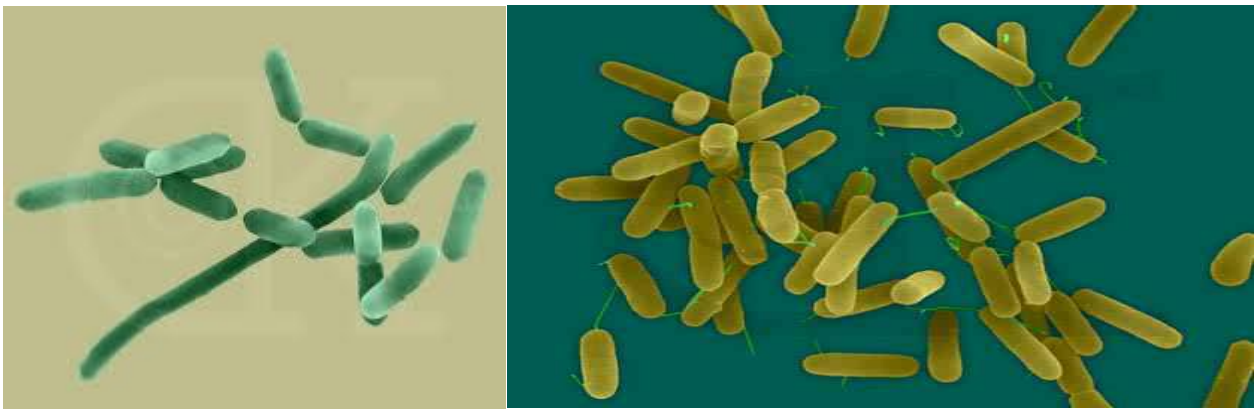
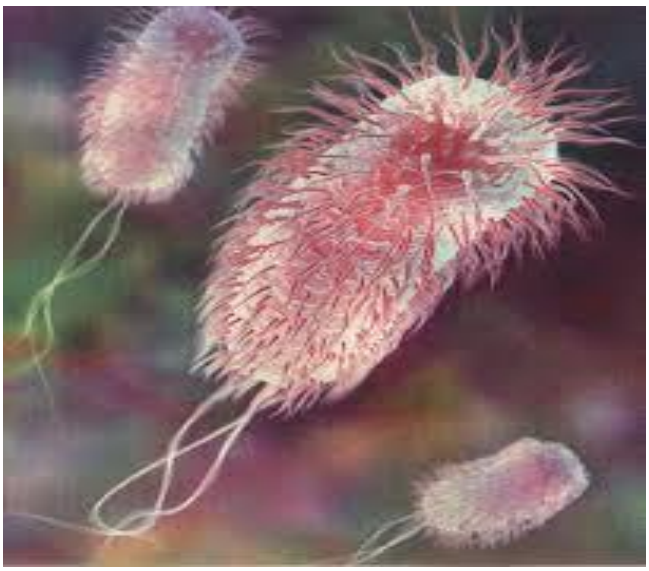


Figure 6-6: The shape of *Pseudomonas* spp.

Enteric bacteria:

The Enteric Bacteria are Gram-negative rods with facultative anaerobic metabolism that live in the intestinal tracts of animals. This group consists of *Escherichia coli* and its relatives, the members of the family Enterobacteriaceae.. Generally, a distinction can be made on the ability to ferment glucose: *Escherichia coli* is, of course, the type species of the enterics. *E. coli* is such a regular inhabitant of the intestine of humans that it is used by public health authorities as an indicator of fecal pollution of drinking water supplies, swimming beaches, foods, etc. *E. coli* is the most studied of all organisms in biology because of its occurrence, and the ease and speed of growing the bacteria in the laboratory. It has been used in hundreds of thousands of experiments in cell biology, physiology, and genetics, and was among the first cells for which the entire chromosomal DNA base sequence was determined. A few strains of *E. coli* are pathogenic (one is notorious, strain 0157:H7, that keeps turning up in raw hamburger headed for a fast-food restaurants). Pathogenic strains of *E. coli* cause intestinal tract infections (usually acute and uncomplicated, except in the very young), uncomplicated urinary tract infections.



The pyogenic cocci

Spherical bacteria which cause various suppurative (pus-producing) infections in animals. It include the Gram-positive cocci *Staphylococcus aureus, Streptococcus pyogenes* and, and the Gram-negative cocci, *Neisseria gonorrhoeae* and *N. meningitidis*. These bacteria are leading pathogens of humans. It is estimated that they produce at least one third of all the bacterial infections of humans, including sore throat, pneumonia, food poisoning, various skin diseases and severe types of septic shock, gonorrhea and meningitis

Two species of *Staphylococcus* live in association with humans: *Staphylococcus epidermidis* which lives normally on the skin and mucous membranes, and *Staphylococcus aureus* which may occur normally at various locales, but in particular on the nasal membranes (nares). *S. epidermidis* is rarely a pathogen and probably benefits its host by producing acids on the skin that retard the growth of dermatophytic fungi. Different strains of *S. aureus* differ in the range of diseases they can cause, including boils and pimples, wound infections. *S. aureus* is the leading cause of nosocomial (hospital-acquired) infections by Gram-positive bacteria.

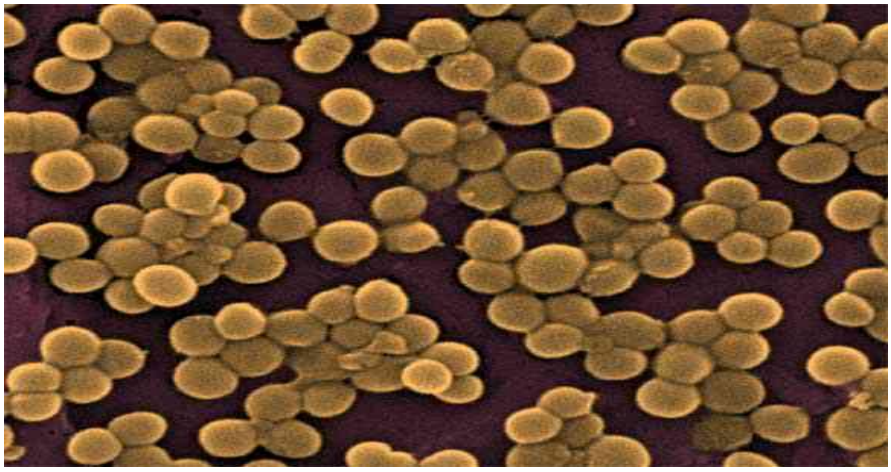


Figure 6-7: Spherical bacteria

Streptococcus pypgenes, more specifically the Beta-hemolytic Group A Streptococci, like *S. aureus*, causes an array of suppurative diseases and toxinoses (diseases due to the production of a bacterial toxin), it is the main streptococcal pathogen for man, most often causing tonsillitis or strep throat.

The Neisseriaceae comprises a family of Gram-negative. The neisseriae are small, Gram-negative cocci usually seen in pairs with flattened adjacent sides. Two species are primary pathogens of humans, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, the bacterial causes of gonorrhea and meningococcal meningitis. *Neisseria gonorrhoeae* is the second leading cause of sexually-transmitted Sometimes. The bacterium is able to colonize and infect the newborn eye resulting neonatal ophthalmia, which may produce blindness.

Figure 6-8: The shape of Niesseria appears like two adjust kidney

Lactic acid bacteria :

Gram-positive, nonsporeforming rods and cocci which produce lactic acid as a sole or major end product of fermentation. They are important in the food industry as fermentation organisms in the production of cheese, yogurt, buttermilk, sour cream, pickles, sauerkraut, sausage and other foods. Certain

oral lactic acid bacteria are responsible for the formation of dental plaque and the initiation of dental caries (cavities).

Endospore-forming bacteria produce a unique resting cell called an endospore. They are Gram-positive and usually rod-shaped (described previously).

Actinomycetes and related bacteria are a large group of Gram-positive bacteria that usually grow by filament formation, or at least show a tendency towards structures called spores, but they are not the same as endospores. Actinomycetes such as *Streptomyces* have a world-wide distribution in soils. They are important in aerobic decomposition of organic compounds and have an important role in biodegradation and the carbon cycle. Actinomycetes are the main producers of antibiotics in industrial settings, being the source of most tetracyclines, macrolides (e.g. erythromycin), and aminoglycosides (e.g. streptomycin, gentamicin, etc.). Two bacteria in this diverse group are important pathogens of humans: *Mycobacterium tuberculosis* is the cause of tuberculosis; *Corynebacterium diphtheriae* is the cause of diphtheria.

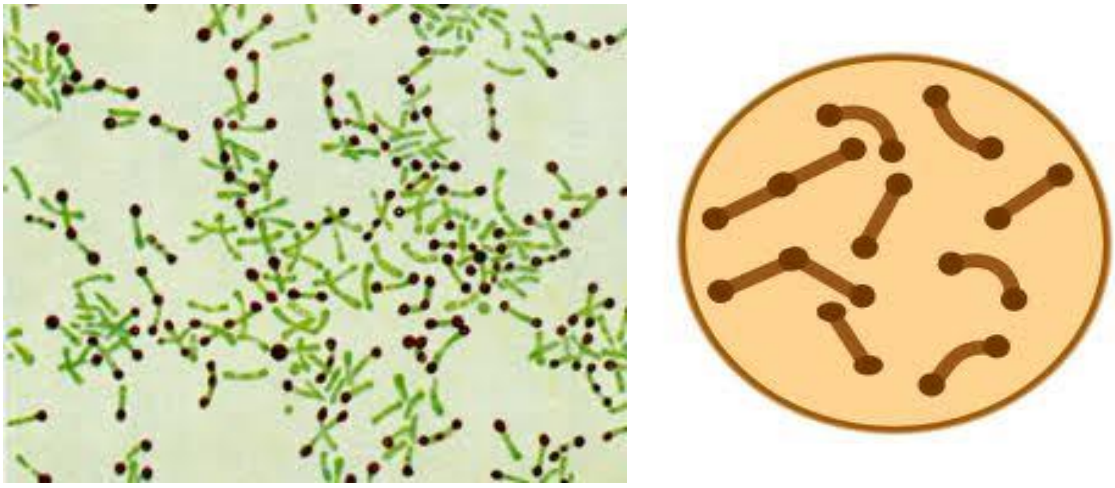


Figure 6-9: Actinomycetes as appear undermicroscope

Mycoplasmas

These facultative or obligate anaerobes lack cell walls, which means they stain pink when Gram stained. Indeed, until their nucleic acid sequences proved their similarity to Gram-positive organisms, mycoplasmas were classified in a phylum of Gram-negative microbes

Mycoplasmas are able to survive without cell walls because they colonize osmotically protected habitats such as animal and human both, and because they have tough cytoplasmic membranes, many of which contain lipids called **sterols** that give the membranes strength and rigidity. Because they lack cell walls, they are **pleomorphic**. They were named "mycoplasmas" because their filamentous forms resemble the filaments of fungi.

When growing on soil media most species form a distinctive "fried egg" appearance because cells in the center colony grow into the agar while those around the perimeter only spread across the surface.

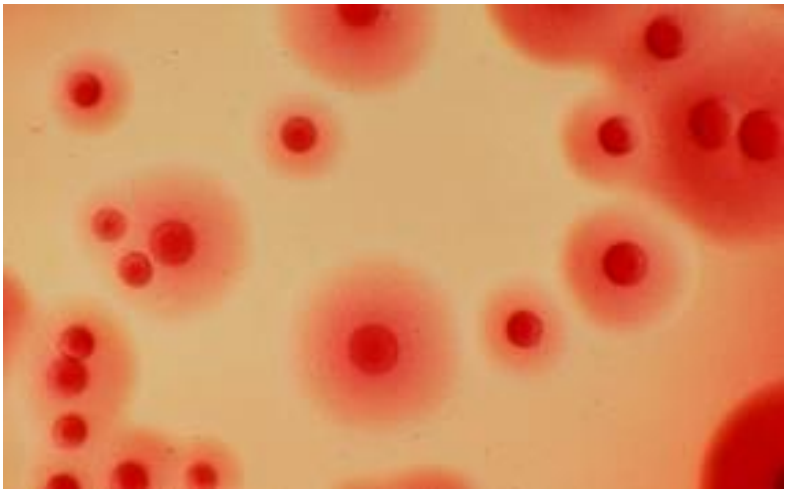


Figure 6-10: Mycoplasma colony on agar media with appearance like fried egg

Archaea: The Other Procaryotes

The discovery and characterization of novel procaryotic cells that have unusual anatomy, physiology, and genetics changed our views of microbial taxonomy and classification. These single-celled, simple organisms, called **archaea,** are now considered a third cell type in a separate super-kingdom (the Domain Archaea). They are procaryotic in general structure and they do share many bacterial, characteristics. But evidence is accumulating that they are actually more closely related to Domain Eukarya than to bacteria. For example, archaea and eucaryotes share a number of ribosomal RNA sequences that are not found in bacteria, and their protein synthesis and ribosomal subunit structures are similar. Outlines selected points of comparison of the three domains.

Among the ways that the archaea differ significantly from other cell types are that certain genetic sequences are found only in their rRNA, and that they have unique membrane lipids and cell wall construction. It is clear that the archaea are the most primitive of all life forms and are probably related to the first cells that originated on the earth 4 billion years ago.

+Table 6-2: Comparison of Three Cellular Domains

Characteristic	Bacteria	Archaea	Eukarya
Cell type	Procaryotic	Procaryotic	Eucaryotic
Chromosomes	Single, or few, circular	Single, circular	Several, linear
Types of ribosomes	70S	70S but structure is similar to 80S	80S
Contains unique ribosomal	+	+	+
RNA signature sequences			
Number of sequences shared with Eukarya	One	Three	(All)
Protein synthesis similar to Eukarya	-	+	
Presence of peptidoglycan in cell wall	+	-	-
Cell membrane lipids	Fatty acids with ester linkages	Long-chain, branched hydrocarbons with ether linkages	Fatty acids with ester linkages
Sterols in membrane	-(some exceptions)	-	+

Members of the group called *Methanogens* can convert CO_2 and H_2 into methane gas (CH_4) through unusual and complex pathways. These archaea are common inhabitants of anaerobic mud and the bottom sediments of lakes and oceans. The gas they produce collects in swamps and may become a source of fuel. Methane may also contribute to the "greenhouse effect,"' which maintains the earth's temperature and can contribute to global warming. Not all methanogens live in extreme environments. They are common members of the normal flora of the oral cavity and large intestine of humans.

Other types of archaea-the extreme halophiles-require salt to grow and may have such a high salt tolerance that they can multipl in sodium chloride solutions (36% NaCl) that would destroy most cells. They exist in the saltest places on the earth-island seas, salt lakes, salt mines, and salted fish. They are not particularly common in the ocean because the salt content is not high enough. Many of the "halobacteria" use a red pigment to synthesize ATP in the presence of light. These pigments are responsible for "red herrings," the color of the Red Sea, and the red color of salt ponds.

Archaea adapted to growth at very low temperatures are called psychrophilic (loving cold temperatures); those growing at very high temperatures are hyperthermophilic (loving high temperatures). Hyperthermophiles flourish at temperatures between 80° and 121°C and cannot grow at 50°C. They live in volcanic waters and soils and submarine vents and are also often salt- and acid-tolerant as well.

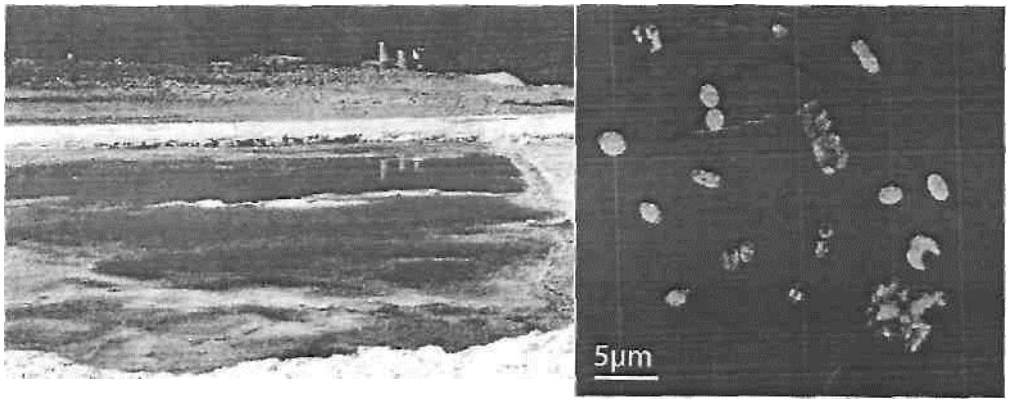


Fig. 6-11 :

Halophiles around the world, (a) A solar evaporation pond in Owens Lake, California, is extremely high in salt and mineral content. The archea that dominate in this warm, saline habitat produce brilliant red pigments with which they absorb light to drive cell synthesis, (b) A sample taken from a saltern in Australia viewed by fluorescent microscopy (1,000x). Note the range of cell shapes (cocci, rods, and square) found in this community