The Science of Microbiology: Introduction

Microbiology is the study of microscopic organisms, which are defined as any living organism that is either a single cell (unicellular), a cell cluster, or has no cells at all (acellular). This includes eukaryotes, such as fungi and protists, and prokaryotes. Viruses and prions, though not strictly classed as living organisms, are also studied. Microbiology typically includes the study of the immune system, or immunology. Generally, immune systems interact with pathogenic microbes; these two disciplines often intersect which is why many colleges offer a paired degree such as "Microbiology and Immunology".Microbiology is a broad term which includes virology, mycology, parasitology, bacteriology, immunology and other branches. A microbiologist is a specialist in microbiology and these related topics.

Microbiological procedures usually must be aseptic, and use a variety of tools such as light microscopes with a combination of stains and dyes.The most commonly used stains are called basic dyes, and are composed of positively charged molecules. Two types of basic dyes are simple stains and differential stains. Simple stains consist of one dye and identify the shape and multicell arrangement of bacteria. Methylene blue, carbolfuchsin, safranin, and crystal violet are some of the most commonly used stains. Differential stains on the other hand, use two or more dyes and help us to distinguish between two or more organisms or two or different parts of the organism. Types of differential stains are gram, Ziehl-Neelsen acid fast, negative, flagella, and endospore. Specific constraints apply to particular fields of microbiology, such as parasitology, which heavily utilizes the light microscopy, whereas microscopy's utility in bacteriology is limited due to the similarity is many cells physiology. Indeed, most means of differentiating bacteria is based on growth or biochemical reactions. Virology has very little need for light microscopes, relying on almost entirely molecular means. Mycology relies on all technologies the most evenly, from macroscopy to molecular techniques.

Microbiology is actively researched, and the field is advancing continuously. It is estimated that only about one percent of the microorganisms present in a given environmental sample are culturable and Although microbes were directly observed over three hundred years ago, the precise determination, quantitation and description of its functions is far to be complete, given the overwhelming diversity detected by genetic and culture-independent means.

Branches

The branches of microbiology can be classified into pure and applied sciences. Microbiology can be also classified based on taxonomy, in the cases of bacteriology, mycology, protozoology, and phycology. There is considerable overlap between the specific branches of microbiology with each other and with other disciplines.

Pure microbiology

Taxonomic arrangement

- Bacteriology: The study of bacteria.
- Mycology: The study of fungi.
- Protozoology: The study of protozoa.
- Phycology (or algology): The study of algae.
- Parasitology: The study of parasites.
- Immunology: The study of the immune system.
- Virology: The study of the viruses.
- Nematology:The study of the nematodes

Integrative arrangement

- Microbial cytology: The study of microscopic and submicroscopic details of microorganisms.
- Microbial physiology: The study of how the microbial cell functions biochemically. Includes the study of microbial growth, microbial metabolism and microbial cell structure.
- Microbial ecology: The relationship between microorganisms and their environment.
- Microbial genetics: The study of how genes are organized and regulated in microbes in relation to their cellular functions. Closely related to the field of molecular biology.
- Cellular microbiology: A discipline bridging microbiology and cell biology.
- Evolutionary microbiology: The study of the evolution of microbes. This field can be subdivided into:
    - Microbial taxonomy: The naming and classification of microorganisms.
    - Microbial systematics: The study of the diversity and genetic relationship of microorganisms.
- Generation microbiology: The study of those microorganisms that have the same characters as their parents.
- Systems microbiology: A discipline bridging systems biology and microbiology.
- Molecular microbiology: The study of the molecular principles of the physiological processes in microorganisms.

Other

- Nano microbiology: The study of those microgasims
- Exo microbiology (or Astro microbiology): The study of microorganisms in outer space.

Applied microbiology

- Medical microbiology: The study of the pathogenic microbes and the role of microbes in human illness. Includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology.
- Pharmaceutical microbiology: The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins,vaccines, and other pharmaceutical products and that cause pharmaceutical contamination and spoil.
- Industrial microbiology: The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and wastewater treatment. Closely linked to the biotechnology industry. This field also includes brewing, an important application of microbiology.
- Microbial biotechnology: The manipulation of microorganisms at the genetic and molecular level to generate useful products.
- Food microbiology and Dairy microbiology: The study of microorganisms causing food spoilage and foodborne illness. Using microorganisms to produce foods, for example by fermentation.
- Agricultural microbiology: The study of agriculturally relevant microorganisms. This field can be further classified into the following:
    - Plant microbiology and Plant pathology: The study of the interactions between microorganisms and plants and plant pathogens.
    - Soil microbiology: The study of those microorganisms that are found in soil.
- Veterinary microbiology: The study of the role in microbes in veterinary medicine or animal taxonomy.
- Environmental microbiology: The study of the function and diversity of microbes in their natural environments. This involves the characterization of key bacterial habitats such as the

rhizosphere and phyllosphere, soil and groundwater ecosystems, open oceans or extreme environments (extremophiles). This field includes other branches of microbiology such as:

  - Microbial ecology
  - Microbially-mediated nutrient cycling
  - Geomicrobiology
  - Microbial diversity
  - Bioremediation
- Water microbiology (or Aquatic microbiology): The study of those microorganisms that are found in water.
- Aeromicrobiology (or Air microbiology): The study of airborne microorganisms.
- Epidemiology: The study of the incidence, spread, and control of disease.

General Properties of Microorganisms

The Microbial World

Microorganisms are creatures that are not directly visible to the unaided eye (nacked eye). All these creatures are living in nature. Part of these is living in a saprophytic form on soil, water, vegetation and so on. Some of these saprophytic microorganisms may invade the body of human or animal causing important diseases (**Parasitism**). Another part of these creatures are living in or on the human or animal body (**normal flora**). This type of relationship called **mutualism** which confers benefits to both partners. Some of these normally existing microorganisms become harmful to the host and this relationship called **parasitism** during which the host provides the primary benefits to the parasite.

A. Microorganisms

- belong to the Protista biologic kingdom.
- include some eukaryotes and prokaryotes, viruses, viroids, and prions.

- are classified according to their structure, chemical composition, and biosynthetic and genetic organization.

B. Eukaryotic cells

- contain organelles and a nucleus bounded by a nuclear membrane.
- contain complex phospholipids, sphingolipids, histones, and sterols.
- lack a cell wall (plant cells and fungi have a cell wall).
- have multiple diploid chromosomes and nucleosomes.
- have relatively long-lived mRNA formed from the processing of precursor mRNA, which contains exons and introns.
- have 80S ribosomes and uncoupled transcription and translation.
    - Protozoa (kingdom Protista)
    - are classified into seven phyla; three of these phyla (Sarcomastigophora, Apicomplexa, Ciliophora) contain medically important species that are human parasites.
- Fungi (kingdom Fungi)
    - are eukaryotic cells with a complex carbohydrate cell wall.
    - have ergosterol as the dominant membrane sterol.
    - may be monomorphic, existing as single-celled yeast or multicellular, filamentous mold.
    - may be dimorphic, existing as yeasts or molds, depending on temperature and nutrition.
    - may have both asexual and sexual reproduction capabilities. (Deuteromycetes, or Fungi Imperfecti, have no known sexual stages.).
- Algae: all organisms that produce O2 as a product of photosynthesis, all algae contain hlorophyll in their chloroplast. Algae may be unicellular or multicellular.
- Slime molds**:** They are characterized by the presence of an amoeboid multinucleated mass called plasmodium as a stage in their life cycle. The plasmodium of the slime mold is

analogous to mycelia in fungi. The growth of slime molds depend on nutrients provided bacteria or plant cells. The reproduction is through plasmodia.

C. Prokaryotic cells

- have no organelles, no membrane-enclosed nucleus, and no histones; in rare cases, they contain complex phospholipids, sphingolipids, and sterols.
- have 70S ribosomes.
- have a cell wall composed of peptidoglycan-containing muramic acid.
- are haploid with a single chromosome.
- have short-lived, unprocessed mRNA.
- have coupled transcription and translation.
    - Typical bacteria
        - have normal peptidoglycan.
        - may be normal flora or may be pathogenic in humans.
        - do not have a sexual growth cycle; however, some can produce asexual spores.
    - Mycoplasmas
        - are the smallest and simplest of the bacteria that are self-replicating.
        - lack a cell wall.
        - are the only prokaryotes that contain sterols.
    - Rickettsia organisms
        - are obligate intracellular bacteria that are incapable of self-replication.
        - depend on the host cell for adenosine triphosphate (ATP) production.
    - Chlamydiae
        - are bacteria-like obligate intracellular pathogens with a complex growth cycle involving intracellular and extracellular forms.

- depend on the host cell for ATP production.

D. Viruses

- are not cells and are not visible with the light microscope.
- are obligate intracellular parasites.
- contain no organelles or biosynthetic machinery, except for a few enzymes.
- contain either RNA or DNA as genetic material.
- are called bacteriophages (or phages) if they have a bacterial host.

E. Viroids

- are not cells and are not visible with the light microscope.
- are obligate intracellular parasites.
- are single-stranded, covalently closed, circular RNA molecules that exist as base-paired, rod-like structures.
- cause plant diseases but have not been proved to cause human disease, although the RNA of the hepatitis D virus is viroid-like.

F. Prions

- are infectious particles associated with subacute, progressive, degenerative diseases of the central nervous system (e.g., Creutzfeldt-Jakob disease).
- Co-purify with a specific glycoprotein (PrP) that has a molecular weight of 27 – 30 kDa.
- are resistant to nucleases but are inactivated by proteases and other agents that inactivate proteins.
- are altered conformations of a normal cellular protein that can auto-catalytically form more copies of itself.

Eukaryotes and prokaryotes are true microorganisms because:

1. They contain all the enzymes required for their multiplication.

2. They possess the biological requirements necessary for the production of metabolic energy.

Bacterial Structure

Bacteria: are small unicellular microorganisms, widely distributed in the nature either as free living, normal flora on or in human or animal body (like enteric bacteria in human intestine) or as a parasites infect human or animals causing important diseases. Bacterial cells consist of nucleoid (analogous to nucleus in eukaryotes) in the cytoplasm which is surrounded by cell envelop. Classification of bacteria may depend on structural, physiologic, biochemical or genetic criteria, of these are:

1. Spore formation: spores are specialized cell structure that may allow survival of bacteria in unsuitable environments.

2. Fermentation of carbohydrate.

3. Gram staining: is an effective criterion that divide bacteria into gram positive and gram negative bacteria.

4. Genetic criteria.

Bacteria have different ways for generating metabolic energy:

1. **Photosynthesis:** Photosynthesis is similar to respiration. in photosynthesis the reductant & oxidant are created photochemically by light energy absorbed by pigments in the membrane.

2. **Respiration:** Is a chemical reduction of an oxidant (electron acceptor)by a reductant (electron donor) The reductant may be organic or inorganic. Oxygen (O2) often employed as an oxidant.

3. **Fermentation:** Fermentation is characterized by substrate phosphorylation (an enzymatic process in which a pyrophosphate bond is donated directly to ADP by a phosphorylated metabolic intermediate . The formation of ATP in fermentation is not coupled to the transfer of electrons. For example the fermentation of glucose ($C_6H_{12}O_6$) yields a net gain of two pyrophosphate bonds in ATP & produces two molecules of lactic acid ($C_3H_6O_3$).

A. Bacterial shape

- can usually be determined with appropriate staining and a light microscope.
- is usually round (coccus), rod-like (bacillus), or spiral with most species; cocci and bacilli often grow in doublets (diplococci) or chains (streptococci). Cocci that grow in clusters are called staphylococci.
- may be pleomorphic with some species, such as Bacteroides.
- is used, along with other properties, to identify bacteria.
- is determined by the mechanism of cell wall assembly.
- may be altered by antibiotics that affect cell wall biosynthesis (e.g., penicillin).

B. Bacterial nucleus

- is not surrounded by a nuclear membrane, nor does it contain a mitotic apparatus.
- is generally called a nucleoid or nuclear body.
- consists of polyamine and magnesium ions bound to negatively charged, circular, supercoiled, double-stranded DNA; small amounts of RNA; RNA polymerase; and other proteins.
- **nucleoid:** The prokaryotic nucleoid, the equivalent of eukaryotic nucleus. The nuclear membrane & mitotic apparatus are absent. The nuclear region is filled with DNA fibrils. Bacterial DNA consist of a single continuous circular molecule (single haploid chromosome). The number of nucleoids & hence the number of chromosomes depend on the growth

conditions (rapidly growing bacteria contain more nucleoids than slowly growing bacteria). The DNA is associated at one end with an invagination of the cytoplasmic membrane called mesosome. This attachment thought to play a role in the separation of the two sister chromosomes following chromosomal replication.

C. Bacterial cytoplasm

- contains ribosomes and various types of nutritional storage granules.
- contains no organelles.
- **Cytoplasmic structures** : Prokaryotic cell lack plastids such as mitochondria and chloroplasts; the electron transport systems are localized instead in the cytoplasmic membrane.
- Bacteria often store reserve materials in the form of insoluble granules called the inclusion bodies always function in the storage of energy or as reservoir of structural building blocks.
- Many bacteria accumulate granules of polyphosphate, which are reserves of inorganic phosphate (**polyphosphate granules**) that can be used in the synthesis of nucleic acid & phospholipid synthesis called metachromatic granules (e.g. corynebacterium).
- Certain specialized bacteria contain protein-bound vesicles, these include **carboxysomes**, which contain carboxylase the key enzyme of CO2 fixation. **Magnetosomes** (membrane bound granules of iron compounds) that allow bacteria to exhibit magnetotaxis (migration or orientation of bacterial cell with respect to earth's magnetic field.

D. Bacterial ribosomes

- have a sedimentation coefficient of 70S and are composed of 30S and 50S subunits containing 16S, and 23S and 5S RNA, respectively.
- are the sites of action of many antibiotics that inhibit protein biosynthesis.
- have proteins and RNAs that differ from those of their eukaryotic counterparts.

- form the basis for the selective toxicity of antibacterial protein synthesis inhibiting agents, which affect 70S ribosomes (e.g., erythromycin) but not 80S ribosomes.
- are membrane-bound if engaged in protein biosynthesis.

.E. Cell (cytoplasmic) membrane

- is a typical phospholipid bilayer.
- contains the cytochromes and enzymes involved in electron transport and oxidative phosphorylation.
- contains carrier lipids and enzymes involved in cell wall biosynthesis.
- contains enzymes involved in phospholipid synthesis and DNA replication.
- contains chemoreceptors.
- is responsible for selective permeability and active transport, which are facilitated by membrane-bound permeases, binding proteins, and various transport systems.
- is the site of action of certain antibiotics, such as polymyxin.

F. Mesosomes

- are convoluted invaginations of the plasma membrane.
- function in DNA replication and cell division as well as in secretion.
- are termed septal mesosomes if they occur at the septum (cross-wall) or lateral mesosomes if they are nonseptal.

G. Plasmids

- are small, circular, nonchromosomal, double-stranded DNA molecules.
- are capable of self-replication.
- are most frequently extrachromosomal, but may become integrated into bacterial DNA.

- contain genes that confer protective properties, such as antibiotic resistance, virulence factors, or their own transmissibility to other bacteria.

H. Transposons

- are small pieces of DNA that move between the DNA of bacteriophages.
- are bacteria or plasmids not capable of self-replication.
- code for antibiotic resistance enzymes, metabolic enzymes, or toxins.
- may alter expression of neighboring genes or cause mutations to genes into which they are inserted.

I. Cell envelope

- is composed of the macromolecular layers that surround the bacterium.
- always includes a cell membrane and a peptidoglycan layer.
- includes an outer membrane layer in gram-negative bacteria.
- may include a capsule, a glycocalyx layer, or both.
- contains antigens that frequently induce a specific antibody response.

Cell envelope including :-

The layer surround the prokaryotic cell are referred collectively as cell envelope. The structure and composition of cell envelope is differing between G positive & G negative bacteria.Many bacteria, both G positive & G negative eubacteria & archebacteria, possess a subunit layer of protein or glycoprotein (s-layer) as the outermost component of the cell envelope. The S-layer glycoprotein of G positive bacteria are structurally similar to the lipopolysaccharide of G negative bacteria. The functions of these S-layers are:

1. Protect the cell from wall-degrading enzymes.

2. Invasion of bacteriophages

3. It plays a role in maintenance of bacterial shape.

4. Involved in the cell adhesion to host epithelial surfaces.

G positive cell envelope:

It composed of, the cytoplasmic membrane, a thick peptidoglycan layer (15-80 nm in diameter); some bacteria have an outer layer either capsule or S-layer.

G negative cell envelope:

This is a highly complex, multilayer structure. The cytoplasmic membrane is called the **inner membrane** surrounded by single sheet of **peptidoglycan** (2 nm in diameter) to which is anchored a complex layer called **outer membrane**. The outmost capsule or S-layer may also be present. The space between the inner & outer membrane is called **periplasmic space**

1.Cell wall

- refers to that portion of the cell envelope that is external to the cytoplasmic membrane and internal to the capsule or glycocalyx.
- confers osmotic protection and gram-staining characteristics.
- is composed of peptidoglycan, teichoic and teichuronic acids, and polysaccharides in gram-positive bacteria.
- is composed of peptidoglycan, lipoprotein, and an outer phospholipid membrane that contains lipopolysaccharide in gram-negative bacteria.
- contains penicillin-binding proteins.

2-Peptidoglycan

- is also called mucopeptide or murein and is unique to prokaryotes.
- is found in all bacterial cell walls, except Mycoplasma.
- is a complex polymer that consists of a backbone, which is composed of alternating N-acetylglucosamine and N-acetylmuramic acid and a set of identical tetrapeptide side chains, which are attached to the N-acetylmuramic acid and that are frequently linked to adjacent tetrapeptide side chains by identical peptide cross-bridges or by direct peptide bonds.
- contains the glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine, which is cleaved by the bacteriolytic enzyme lysozyme (found in mucus, saliva, and tears).
- may contain diaminopimelic acid, an amino acid unique to prokaryotic cell walls.
- is the site of action of certain antibiotics, such as penicillin and the cephalosporins.
- comprises up to 50% of the cell wall of gram-positive bacteria but only 2%â€“10% of the cell wall of gram-negative bacteria.

3-Teichoic and teichuronic acids

- are water-soluble polymers, containing a ribitol or glycerol residue linked by phosphor diester bonds.
- are found in gram-positive cell walls or membranes.
- are chemically bonded to peptidoglycan (wall teichoic acid) or membrane glycolipid (lipoteichoic acid), particularly in mesosomes.
- contain important bacterial surface antigenic determinants, and lipoteichoic acid helps anchor the wall to the membrane.
- may account for 50% of the dry weight of a gram-positive cell wall.

4-Lipoprotein

- cross-links the peptidoglycan and outer membrane in gram-negative bacteria.
- is linked to diaminopimelic acid residues of peptidoglycan tetrapeptide side chains by a peptide bond; the lipid portion is non-covalently inserted into the outer membrane.

5-Periplasmic space

- is found in gram-negative cells.
- refers to the area between the cell membrane and the outer membrane.
- contains hydrated peptidoglycan, penicillin-binding proteins, hydrolytic enzymes (including β-lactamases), specific carrier molecules, and oligosaccharides.

6- Outer membrane

- is found in gram-negative cells.
- is a phospholipid bilayer in which the phospholipids of the outer portion are replaced by lipopolysaccharides.
- protects cells from many things, including harmful enzymes and some antibiotics, and prevents leakage of periplasmic proteins.
- contains embedded proteins, including matrix porins (nonspecific pores), some nonpore proteins (phospholipases and proteases), and transport proteins for small molecules.

7- Lipopolysaccharide

- is found in the outer leaflet of the outer membrane of gram-negative cells.

- consists of lipid A, several long-chain fatty acids attached to phosphorylated glucosamine disaccharide units, and a polysaccharide composed of a core and terminal repeating units.
- is negatively charged and noncovalently cross-bridged by divalent cations.
- is also called endotoxin; the toxicity is associated with the lipid A.
- contains major surface antigenic determinants, including O antigen found in the polysaccharide component.

8- Bayer's junction

- is found in gram-negative cells.
- is the region of the wall where the inner leaflet of the outer membrane is contiguous with the outer leaflet of the cell membrane.

J. External layers including :-

1-Capsule

- is a well-defined structure of polysaccharide surrounding a bacterial cell and is external to the cell wall. The one exception to the polysaccharide structure is the poly-D-glutamic acid capsule of Bacillus anthracis.
- protects the bacteria from phagocytosis and plays a role in bacterial adherence.

2-Glycocalyx

- refers to a loose network of polysaccharide fibrils that surrounds some bacterial cell walls.
- is sometimes called a slime layer.
- is associated with adhesive properties of the bacterial cell.

- is synthesized by surface enzymes.
- contains prominent antigenic sites.

K. Appendages including :

1-Flagella

- are protein appendages for locomotion.
- consist of a basal body, hook, and a long filament composed of a polymerized protein called flagellin.
- may be located in only one area of a cell (polar) or over the entire bacterial cell surface (peritrichous).
- contain prominent antigenic determinants.

2-Pili (fimbriae)

- are rigid surface appendages composed mainly of a protein called pilin.
- exist in two classes: ordinary pili (adhesins), involved in bacterial adherence, and sex pili, involved in attachment of donor and recipient bacteria in conjugation.
- are, in the case of ordinary pili, the colonization antigens or virulence factors associated with some bacterial species, such as Streptococcus pyogenes and Neisseria gonorrhoeae.
- may confer antiphagocytic properties, like the M protein of S pyogenes.

L. Endospores

- are formed as a survival response to certain adverse nutritional conditions, such as depletion of a certain resource.
- are metabolically inactive bacterial cells that are highly resistant to desiccation, heat, and various chemicals.

- possess a core that contains many cell components, a spore wall, a cortex, a coat, and an exosporium.
- contain calcium dipicolinate, which aids in heat resistance in the core.
- germinate under favorable nutritional conditions after an activation process that involves damage to the spore coat.
- are helpful in identifying some species of bacteria (e.g., Bacillus and Clostridium).
- are not reproductive structures.

M. Gas vesicles

- Gas vesicles, which bounded by a membrane of protein that is impermeable for water and solutes, but permeable for gas. These are the component of gas vacuoles.
- Bacteria contain proteins resembling both actin and non-actin cytoskeletal proteins of eukaryotic cells that play a cytoskeletal role. They helps to determine the cell shape, segregate chromosomes, localize protein within the cell & regulate cell division.

Eukaryotic cell organelles

Eukaryotic nucleus:

The nucleus in eukaryotic cells is bounded by membrane that is continuous with the endoplasmic reticulum. This membrane exhibit selective permeability due to pores that permit exchange of large molecules such as proteins & mRNAs into & out of the nucleus. Small molecules can diffuse freely in & out of the nucleus. The chromosomes o eukaryotic cell contain linear DNA molecules arranged in a double helix. It is associated with basic proteins called histones that bind to DNA by ionic interactions.

Cytoplasmic structures:

The cytoplasm of eukaryotic cells is characterized by the presence of endoplasmic reticulum , vacuoles, self-reproducing plastids, and an elaborate cytoskeleton composed of microtubules, microfilaments &intermediate filaments.

The endoplasmic reticulum is a network of membrane-bound channels. In some regions, the membrane are coated with ribosomes. Protein synthesized on these ribosomes pass through the membrane into the channels of the endoplasmic reticulum, through which it can be transported to other parts of the cell. A related structure, the Golgi apparatus, pinches off vesicles that can fuse with the cell membrane, releasing the enclosed proteins into the surrounding medium.

Plastids

The plastids include **mitochondria**, which contain in their membranes the respiratory electron transport system, and **chloroplasts** (in photosynthetic organisms). The plastids contain their own DNA which code for some of their constituent proteins and tRNA.

Anaerobic eukaryotes are amitochondriate, but some(e.g. microaerophilic flagellae *Trichomonas vaginallis*) posses a membrane bound organelle, the hydrogenosome. The hydrogenosome is defined by its unusual function; under anaerobic conditions it produces hydrogen gas from the oxidation of pyruvate or malate.

The cytoskeleton includes arrays of actin **microtubules**, which play a role in Cytoplasmic membrane function & cell shape as well as forming the mitotic spindle & flagellar components. Arrays of actin and myosin-containing **microfilaments,** which provide the mechanism of ameboid motility. The **intermediate filaments**, whose function to structure cytoplasm & to resist stresses externally applied to the cell

Surface layers:

The cytoplasm is enclosed within a plasma membrane composed of protein & phospholipids, similar to prokaryotic cell membrane. Most animal cells have o other surface layers; however, plant cells have an outer cell wall composed of cellulose. Many eukaryotic microorganisms also have an outer cell wall, which may be composed of a polysaccharide such as cellulose or chitin or may be inorganic.

Motility organelles:

Many eukaryotic microorganisms have organelles called flagella or cilia that move with a wave-like motion to propel the cell through water. Eukaryotic flagella emanate from the polar region of the cell, whereas cilia, which are shorter than flagella, surround the cell. Both flagella & cilia of eukaryotic cells have the same basic structure & biochemical composition. Both composed of a series of microtubules, hollow protein cylinder composed of a protein called tubulin, surrounded by a membrane. It consist of a 9 peripheral pairs of microtubules surrounding 2 single central microtubules

Bacterial Growth

A. General characteristics of bacterial growth

- refers to an increase in bacterial cell numbers (multiplication), which results from a programmed increase in the biomass of the bacteria.
- results from bacterial reproduction due to binary fission, which may be characterized by a parameter called generation time (i.e., the average time required for cell numbers to double).
- may be determined by measuring cell concentration (turbidity measurements or cell counting) or biomass density (dry weight or protein determinations).
- usually occurs asynchronously (i.e., all cells do not divide at precisely the same moment).

B. Cell concentration

- may be measured by viable cell counts involving serial dilutions of sample followed by a determination of colony-forming units on an agar surface.
- may be determined by particle cell counting or turbidimetric density measurements (includes both viable and nonviable cells).

C. Bacterial growth curve .

- requires inoculation of bacteria from a saturated culture into fresh liquid media.
- is unique for a particular nutritional environment.
- is frequently illustrated in a plot of logarithmic number of bacteria versus time; the generation time is determined by observing the time necessary for the cells to double in number during the log phase of growth.
- consists of four phases:
    - Lag phase : metabolite-depleted cells adapt to new environment.
    - Exponential or log phase: cell biomass is synthesized at a constant rate. Cells in this stage are generally more susceptible to antibiotics.
    - Stationary phase :cells exhaust essential nutrients or accumulate toxic products.
    - Death or decline: cells may die due to toxic products.

The lag phase:

- The lag phase represent a period during which the cells, depleted of metabolites of enzymes as a result of unfavorable conditions that existed at the end of their previous growth, adapt to their new environment. Enzymes & metabolites are formed & accumulate until they are present in concentrations that permit growth to resume. The duration of lag phase is depending on the similarity between the previous & new culture media.

The Exponential phase:

During this phase, the cells are in a steady state. New cells materials are being synthesized at a constant rate & catalytic & the masses increase in exponential manner. This continues until one of two things happens:

1. One or more nutrients in the medium become exhausted.

2. Accumulation of toxic metabolic products & inhibit growth.

For aerobic organisms, the nutrients in the medium become limited are usually oxygen. When the cell concentration exceeds about 1x 10^7/ ml the growth rate will decease unless oxygen is forced in the medium. When the bacterial concentration reaches 4-5x 10^9/ml, the rate of oxygen diffusion cannot meet the demand even in the aerated medium, and thus growth is slowed.

The maximum stationary phase:

The exhaustion of nutrients or the accumulation of toxic products finally causes growth to stop completely. When this occurs, the total cell count slowly increases although the viable count stays constant, i.e. the death rate is balanced by the formation of new cells through growth & division.

The decline or death phase:

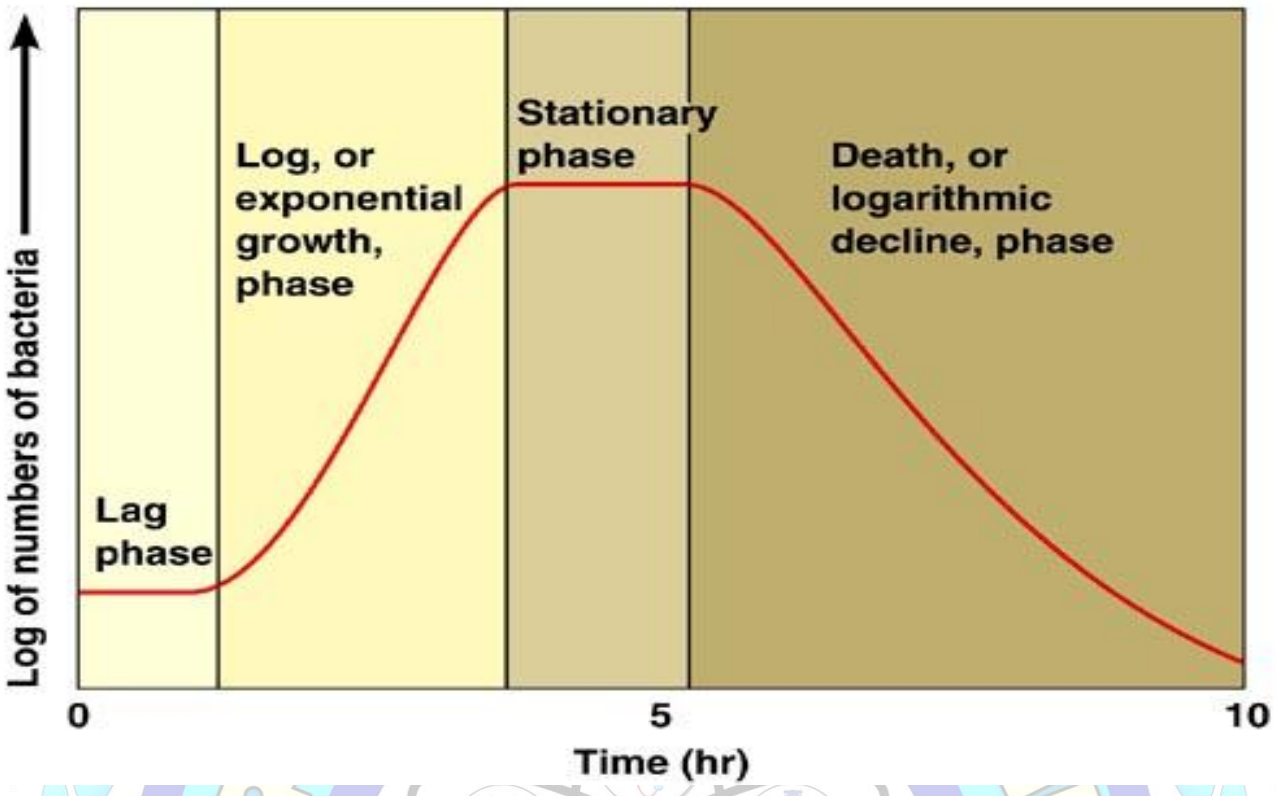
After a period of time in the stationary phase, which varies with the organism & with the culture conditions, the death rate increases until reaches a steady level. A small number of survivors may persist for months. This persistence may reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die & lyses.

Maintenance of cells in the exponential phase:

Cells can be maintained in exponential phase by transferring them repeatedly into fresh medium of identical composition while they are still growing exponentially.

The meaning of death:

For microbial cells, death means the irreversible loss of the ability to reproduce (growth & division). The test of death is the culture of cells on solid media; a cell is considered dead if it fails to give rise to a colony on any medium. Then the reliability of the test depends upon choice of medium & conditions.



phase	Growth rate
Lag phase	Zero
Acceleration phase	Increased
Exponential phase	Constant
Retardation phase	Decreasing
Maximum stationary phase	Zero
Decline phase	Death

D. Chemostat

- is a device that maintains a bacterial culture in a specific phase of growth or at a specific cell concentration.
- is most frequently used to maintain a bacterial culture in the exponential growth phase.
- is based on the principle that toxic products and cells are removed at the same rate as fresh nutrients are added and new cells are synthesized.
- operates best if one nutrient limits bacterial growth.

E. Synchronous growth

- refers to a situation in which all the bacteria in a culture divide at the same moment.

may be achieved by several methods, including thymidine starvation (thymidine-requiring bacteria), alternate cycles of low and optimal incubation temperatures, spore germination, selective filtration of old (large) and young (small) cells, or â€œtrapped cellâ€ filtration

Bacterial Cultivation

A. General characteristics of bacterial cultivation

- refers to the propagation of bacteria.
- involves specific pH, gaseous, and temperature preferences of bacteria.
- is performed in either liquid (broth) or solid (agar) growth medium.
- requires an environment that contains:
    - A carbon source
    - A nitrogen source
    - An energy source

- Inorganic salts
- Growth factors
- Electron donors and acceptors

B. Superoxide dismutase

- is an enzyme in aerobes and facultative and aerotolerant anaerobes that allows them to grow in the presence of the superoxide free radical (O_2^-).
- carries out the reaction $2O_2^- + 2H^+$ â†’ $H_2O_2 + O_2$.
- produces hydrogen peroxide (H_2O_2), which is toxic to cells but is destroyed by catalase or is oxidized by a peroxidase enzyme.

C. Oxygen requirements

- **Obligate aerobes**
  - refer to bacteria that require oxygen for growth.
  - contain the enzyme superoxide dismutase, which protects them from the toxic O_2^-.
  -
- **Obligate anaerobes**
  - are killed by the O_2^-; they grow maximally at a PO_2 concentration of less than 0.5% - 3%.
  - lack superoxide dismutase, catalase, and cytochrome c oxidase (enzymes that destroy toxic products of oxygen metabolism).
  - require a substance other than oxygen as a hydrogen acceptor during the generation of metabolic energy.
  - use fermentation pathways with distinctive metabolic products.
  - outnumber aerobes 1000:1 in the gut and 100:1 in the mouth.

- comprise 99% of the total fecal flora (10^{11}/g of stool in the large bowel).
- usually cause polymicrobial infections (i.e., infections involving more than one genus or species).
- are foul smelling.
- are generally not communicable or transmissible, except as endospores, such as Clostridium difficile.
- generally are found proximal to mucosal surfaces; when this barrier is broken, anaerobes can escape into tissues.
- **Mucosal surfaces can be disrupted by:**

1-Gastrointestinal obstruction or surgery.

2-Diverticulitis

3- Bronchial obstruction

4- Tumor growth

5- Ulceration of the intestinal tract by chemotherapeutic agents

- **Facultative anaerobes**
  - grow in the presence or absence of oxygen.
  - shift from a fermentative to a respiratory metabolism in the presence of air.
  - display the Pasteur effect, in which the energy needs of the cell are met by consuming less glucose under a respiratory metabolism than under a fermentative metabolism.
  - include most pathogenic bacteria.

- **Aero-tolerant anaerobes**
    - resemble facultative bacteria but have a fermentative metabolism both with and without an oxygen environment.

D. Nutritional requirements

- **Heterotrophs**
    - require preformed organic compounds (e.g., sugar, amino acids) for growth.
- **Autotrophs**
    - do not require preformed organic compounds for growth because they can synthesize them from inorganic compounds and carbon dioxide.

E. Growth media

- **Minimal essential growth medium**
    - contains only the primary precursor compounds essential for growth.
    - demands that a bacterium synthesize most of the organic compounds required for its growth.
    - dictates a relatively slow generation time.
- **Complex growth medium**
    - contains most of the organic compound building blocks (e.g., sugars, amino acids, nucleotides) necessary for growth.
    - dictates a faster generation time for a bacterium relative to its generation time in minimal essential growth medium.
    - is necessary for the growth of fastidious bacteria.

- **Differential growth medium**
    - contains a combination of nutrients and pH indicators to allow visual distinction of bacteria that grow on or in it.
    - is frequently a solid medium on which colonies of particular bacterial species have a distinctive color.
- **Selective growth medium**
    - contains compounds that prevent the growth of some bacteria while allowing the growth of other bacteria.
    - uses certain dyes or sugars, high salt concentration, or pH to achieve selectivity.

Bacterial Metabolism

A. General characteristics

- **Bacterial metabolism**
    - is the sum of anabolic processes (synthesis of cellular constituents requiring energy) and catabolic processes (breakdown of cellular constituents with concomitant release of waste products and energy-rich compounds).
    - is heterotrophic for pathogenic bacteria.
    - varies depending on the nutritional environment.
- **Bacterial transport systems**
    - involve membrane-associated binding or transport proteins for sugars and amino acids.
    - frequently require energy to concentrate substrates inside the cell.
    - are usually inducible for nutrients that are catabolized; glucose, which is constitutive, is an exception.
    - frequently use phosphotransferase systems when sugars are transported.

B. Carbohydrate metabolism

- **Fermentation**
  - is a method by which some bacteria obtain metabolic energy.
  - is characterized by a substrate phosphorylation.
  - involves the formation of ATP not coupled to electron transfer.
  - requires an organic electron acceptor, such as pyruvate.
  - results in the synthesis of specific metabolic end products that may aid in the identification of bacterial species.
- **Respiration**
  - refers to the method of obtaining metabolic energy that involves an oxidative phosphorylation.
  - involves the formation of ATP during electron transfer and the reduction of gaseous oxygen in aerobic respiration.
  - involves a cell membrane electron transport chain composed of cytochrome enzymes, lipid cofactors, and coupling factors.

C. Regulation

- **Regulation of enzyme activity**
  - may occur because enzymes are allosteric proteins, susceptible to binding of effector molecules that influence their activity.
  - may occur by feedback inhibition involving the end product.
  - may involve substrate-binding enhancement (cooperatively) of catalytic activity.

- **Regulation of enzyme synthesis**
    - may involve allosteric regulatory proteins that activate (activators) or inhibit (repressors) gene transcription.
    - may involve end product feedback repression of biosynthetic pathway enzymes.
    - may involve substrate induction of catabolic enzymes.
    - may involve attenuation control sequences in enzyme mRNA.
    - may involve the process of catabolite repression, which is under positive control of the catabolite activator protein.
- **Pasteur effect**
    - occurs in facultative bacteria.
    - is caused by oxygen blocking the fermentative capacity of the bacteria.
    - means that the energy needs are met by using less glucose during aerobic growth.

Cell Wall Synthesis

- involves the cytoplasmic synthesis of peptidoglycan subunits, which are translocated by a membrane lipid carrier and cross-linked to existing cell wall by enzymes associated with the plasma membrane of gram-positive bacteria or found in the periplasmic region of gram-negative bacteria.
- involves the covalent linkage of teichoic acid to N-acetylmuramic acid residues in gram-positive cells.
- is affected by many antibiotics, including penicillins, cephalosporins, and carbapenems.
- includes the addition of three components (lipoprotein, outer membrane, lipopolysaccharide), whose constituents or subunits are synthesized on or in the cytoplasmic membrane and assembled outside of it in gram-negative cells.

Sterilization and Disinfection

A. Terminology

- Sterility : total absence of viable microorganisms as assessed by no growth on any medium
- Bactericidal : kills bacteria
- Bacteriostatic : inhibits growth of bacteria
- Sterilization: removal or killing of all microorganisms
- Disinfection : removal or killing of disease-causing microorganisms
- Sepsis : infection
- Aseptic: without infection
- Antisepsis : any procedure that inhibits the growth and multiplication of microorganisms.
- Antibiotics: Naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentration.
- Antibacterial: synthetic derivatives of antibiotics.

B. Kinetics of killing

- is affected by menstruum or medium, the concentration of organisms and antimicrobial agents, temperature, pH, and the presence of endospores.
- can be exponential (logarithmic).
- can result in a killing curve that becomes asymptotic, requiring extra considerations in killing final numbers, especially if the population is heterogeneous relative to sensitivity.

C. Methods of control include

Moist heat (autoclaving at 121°C for 15 minutes at a steam pressure of 15 pounds per square inch kills microorganisms, including endospores)(Autoclaving is a steam & heat sterilization

by 121^{0}C and 15 lb/sq inch for 20-30 minutes. Autoclaving is very efficient in killing all bacteria & their spores & thus routinely used for sterilization of culture media.)

- Dry heat and incineration (both methods oxidize proteins, killing bacteria)
- Ultraviolet radiation (blocks DNA replication)(Radiation: Uv light & ionizing radiation have various applications as sterilizing agents)
- Hot air sterilization: is used to sterilize that must remain dry by using 160-170 ^{0}c for 1 hour.
- Chemicals includes :

a- Phenol : is used as a disinfectant standard that is expressed as a phenol coefficient, which compares the rate of the minimal sterilizing concentration of phenol to that of the test compound for a particular organism.

b- Chlorhexidine : is a diphenyl cationic analogue that is a useful topical disinfectant.

c- Iodine : is bactericidal in a 2% solution of aqueous alcohol containing potassium iodide ,acts as an oxidizing agent and combines irreversibly with proteins and can cause hypersensitivity reactions.

d- Chlorine : inactivates bacteria and most viruses by oxidizing free sulfhydryl groups.

e- Formaldehyde : is used as a disinfectant in aqueous solution (37%).

f- Ethylene oxide: is an alkylating agent that is especially useful for sterilizing heat-sensitive hospital instruments , requires exposure times of 4-6 hours, followed by aeration to remove absorbed gas.

g- Alcohol : requires concentrations of 70% - 95% to kill bacteria given sufficient time, Isopropyl alcohol (90% - 95%) is the major form in use in hospitals.

h- Peroxygen: such as hydrogen peroxide (H2O2) has broad-spectrum activity against viruses, bacteria, yeasts & bacterial spores. Sporocidal activity requires higher concentrations (10-30%) of H2O2 & longer contact time.

Antimicrobial Chemotherapy

A. General characteristics of antimicrobial chemotherapy

- is based on the principle of selective toxicity, which implies that a compound is harmful to a microorganism but innocuous to its host.
- involves drugs that:
    - Are anti-metabolites
    - Inhibit cell wall biosynthesis
    - Inhibit protein synthesis
    - Inhibit nucleic acid synthesis
    - Alter or inhibit cell membrane permeability or transport
        - includes both bacteriostatic (inhibit growth) and bactericidal (kill) drugs.
        - may be characterized as broad spectrum (effective against a wide variety of bacterial species) or narrow spectrum (effective against one or a few bacterial species).
        - may use synergistic combinations of bacteriostatic drugs (e.g., trimethoprim and sulfonamide).
        - incorporates both drugâ€“parasite relationships (e.g., location of bacteria and drug distribution) and alterations of hostâ€“parasite relationships (e.g., immune response and microbial flora) to be effective.

B. Drug antimicrobial activity

- is usually determined by dilution or diffusion tests.
- is quantitated by determining the minimal inhibitory concentration.
- may differ in vitro and in vivo.
- is affected by pH, drug stability, microbial environment, number of microorganisms present, length of incubation with drug, and metabolic activity of microorganisms.
- may be modified for a specific bacterium if genetic or nongenetic drug resistance develops.

C. Drug resistance

- **Non-genetic mechanisms of drug resistance**
  - may involve loss of specific target structures, such as cell wall by L forms of bacteria.
  - may result from metabolic inactivity of microorganisms.
- **Genetic mechanisms of drug resistance**
  - may result from either chromosomal or extrachromosomal (plasmid) resistance.
  - may involve a chromosomal mutation that alters the structure of the cellular target (e.g., penicillin-binding protein) of the drug or the permeability of the drug.
  - may result from the introduction of a plasmid (R factor or R plasmid) that codes for enzymes (²ج-lactamase) that degrade the drug or modify it (acetyltransferase), or from the introduction of proteins that pump it from the cell in an energy-dependent fashion.
- **R factor or R plasmid**
  - contains insertion sequences and transposons.
  - may acquire additional resistance genes by plasmid fusion or from transposons.
  - may consist of two plasmids, the resistance transfer factor (RTF), which codes for replication and transfer, and the r or resistance determinant, which contains genes for replication and resistance.

- can be transmitted from species to species.
- is responsible for the rapid development of multiple drug-resistant bacteria over the past 30 years.

Toxins

A. Definition

- Toxins are broadly defined as microbial products that damage host cells or host tissues.

B. Classification

- Toxins are generally classified into two groups: exotoxins and endotoxins.

C. Mechanism of action

- **Many toxins possess an A and a B polypeptide fragment.**
  - The A (active) subunit enters the cell and exerts its toxic effect.
  - The B (binding) subunit is responsible for initial attachment of the toxin to the specific target tissue.
  - Antitoxin interacts only with the B subunit to block its attachment; once toxin is bound, the antitoxin is ineffective.

D. Toxins composed of A and B polypeptides

- Pseudomonas aeruginos exotoxin A that also inhibits protein synthesis via the tRNA EF-2.
- Shigella dysenteriae shiga neurotoxin that inhibits synthesis via the 60S ribosomal unit by RNase action on 28S ribosomal RNA.
- Escherichia coli heat-labile enterotoxin similar to choleragen that also stimulates adenylate cyclase to overproduce cyclic AMP and induce loss of fluids and electrolytes.

- Bordetella pertussis ADP-ribosylation of a G protein, which increases adenylate cyclase activity by preventing its inactivation.
- Clostridium tetani tetanospasm exotoxin that acts on synaptosomes; gangliosides bind the toxin and block the release of glycine, which obliterates the inhibitory reflex response of nerves, causing uncontrolled spastic impulses (hyperreflexia of skeletal muscles).

E. Toxins composed of a single polypeptide

- Clostridium perfringens toxin that is an enzyme phospholipase C; it disrupts cellular and mitochondrial membranes.
- E coli heat-stable enterotoxin that stimulates guanylate cyclase to overproduce cyclic guanosine monophosphate, which impairs chloride and sodium absorption.
- Staphylococcus aureus exfoliative toxin that disrupts the stratum granulosum in the epidermis.

Bacteriophages

A. General characteristics of bacteriophages

- are bacterial viruses that are frequently called phages.
- are obligate intracellular parasites.
- are host-specific infectious agents for bacteria.
- are called bacteriophage virions when they are complete (genetic material and capsid) infectious particles.
- contain protein and RNA or DNA as major components.

B. Morphologic classes of bacteriophages

- **Polyhedral phages**

- are usually composed of an outer polyhedral-shaped protein coat (capsid) that surrounds the nucleic acid.
- may contain a lipid bilayer between two protein capsid layers (PM-2 phage).
- have either circular double-stranded or single-stranded DNA or linear single-stranded RNA as their genetic material, although one phage that has three pieces of double-stranded RNA has been described.

- **Filamentous phages**
  - have a filamentous protein capsid that surrounds a circular single-stranded DNA genome .
  - are male bacteria specific in that infection occurs through the pili, which are only present on male bacteria.
  - do not lyses their host cells during the replication process.
- **Complex phages**
  - have a protein polyhedral head containing linear double-stranded DNA and a protein tail and other appendages.
  - include the T and lambda phages of E coli.

C. Genetic classes of bacteriophages

- **RNA phages**
  - refer to all phages with RNA as their genetic material.
  - are specific for bacteria with male pili (male-specific phages).
  - contain single-stranded RNA which can act as polycistronic mRNA.
- **DNA phages**
  - refer to all phages with DNA as their genetic material.
  - contain nucleic acid bases that are frequently glucosylated or methylated.

- may contain some unusual nucleic acid bases, such as 5-hydroxymethyl cytosine or 5-hydroxymethyl uracil.
- are classified as virulent or temperate, depending on whether their pattern of replication is strictly lytic (virulent) or alternates between lytic and lysogenic (temperate).

Bacterial Genetics

Genetics: is the science that defines & analyzes heredity & variation. It aims to understanding the structure & functions of microbial genome, its gene products & their role in infection & disease. The unit of heredity is gene.

Gene : is a segment or portion of DNA that carries in its nucleotide sequence information for a specific biochemical or physiological property, through coding for a single polypeptide sequence.

Phenotype : refers to the observable properties (or characters) of an organism which are produced by interaction of genotype with the environment, i.e. the effect of both genes and environment. These include the structural and physiological properties of a cell or an organism (e.g. the eye color in human, resistance to an antibiotic in bacterium).

Genotype : refers to the genetic constitution of an organism

Genome : is the sum of the genes of an organism, or the totality of genetic information in an organism.

Microbial genetic :is based largely upon observation of growth. Phenotypic variation has been observed on the basis of gene capacity to permit growth under condition of selection, e.g. a bacterium containing a gene that confers resistance to ampicillin can be distinguished from that lacking the gene by its growth in the presence of that antibiotic (selective agent).

Gene expression : refers to a gene product that can be observed under appropriate condition at the level of phenotype.

Restriction enzymes : that cleave DNA at specific sites giving rise to restriction fragments.

Plasmids : are small genetic elements capable of independent replication in bacteria and yeast.

Introduction of DNA restriction fragment into a plasmid allow the fragment to be amplified many times. Amplification of specific region of DNA also can be achieved with bacterial enzymes using the **polymerase chain reaction (PCR)** or other enzyme-based methods for nucleic acid amplification (e.g. transcription-mediated amplification). DNA inserted into plasmid can be placed under control of high-expression bacterial **promoters** that allow encoded protein to be expressed at high levels. Thus bacterial genetics fostered the development of **genetic engineering**, a technology that leads to tremendous advances in the field of medicine (e.g. production of human insulin).

I. Organization of Genetic Information and General Concepts

A. Deoxyribonucleic acid (DNA)

- stores genetic information as a sequence of nucleotide bases (adenine, thymine, guanosine, cytosine).
- is generally double stranded, composed of complementary base pairs (A=T or G≡C) joined by hydrogen bonds.

B. Ribonucleic acid (RNA)

- transcribes and translates DNA-bound genetic instructions for protein synthesis.
- is generally single stranded.
- substitutes uracil for the thymine base used by DNA; the complementary base pairs for RNA are A=U or G≡C.
- is found in three types:
    - Messenger RNA (mRNA)
        - is the template that carries DNA gene sequences to ribosomes, the site of protein synthesis.
    - Ribosomal RNA (rRNA)

- is a structural component of ribosomes.
- acts as a substrate for protein synthesis.

- Transfer RNA (tRNA)
  - carries specific amino acids to the triplet-encoded, mRNA-borne message that translates the message into the amino acid structure of proteins.

Genetic information is stored as a sequence of bases in DNA and some RNA. Most DNA molecules are double-stranded with complementary bases (each strand made up of a deoxyribose phosphate backbone and series of purine and pyrimidine bases; the pyrimidine bases are thymine (T) & cytosine (C) and the purine bases are adenine (A) and guanine (G). Each of the 4 bases is bound to phospho-deoxyribose to form a nucleotide. Adenine always pairs with thymine and the guanine pairs with cytocine by hydrogen bonding in the center of the molecule. The complementarity of the bases enables one strand (template strand) to provide the information for coping or expression of information in the other strand (coding strand). The base pair are stacked within the center of the DNA double helix and they determine its genetic information. The most general function of RNA is communication of DNA gene sequences in the form of messenger RNA (mRNA) to ribosomes. The ribosomes which contain ribosomal RNA (rRNA) and protein, translate the message into the primary structure of proteins via aminoacyl transfer RNA(tRNA). RNA molecule range in size from small tRNA (contain fewer than 100 bases) to mRNA which may carry genetic messages extending to several thousand bases.

II. Comparison of Prokaryotic and Eukaryotic Genomes

A. Eukaryotic genomes

- **Structure**
  - Except in some fungi, eukaryotes are diploid with two homologous copies of each chromosome.

- Virtually all genetic information is contained in two or more linear chromosomes located in a membrane-bound nucleus.
- Unlike prokaryotes and viruses, eukaryotic genomes contain introns (DNA sequences not translated into gene products) and redundant genetic information.
- Certain eukaryotic organelles (mitochondria, chloroplasts) contain a self-replicating, circular, double-stranded DNA molecule (plasmid) relating to their intracellular function.

- **Replication**
  - begins at several points along the linear DNA molecule.
  - is regulated by specific gene inducer or repressor substances.
  - involves a specialized structure, the spindle, that pulls newly formed chromosomes into separate nuclei during mitosis.

B. Prokaryotic genomes

- Structure

a- Most prokaryotes are haploid (single chromosome).

b- Genes essential for bacterial growth are carried on a single, circular chromosome encoding generally several thousand genes; they are not enclosed in a membrane-circumscribed nucleus.

c- Many bacteria contain additional, specialized genes on smaller extrachromosomal plasmids. Prokaryotic plasmids exist in transmissible and nontransmissible forms and may be integrated into the bacterial chromosome.

d- Specialized information may also be carried on transposons, moveable genetic elements that cannot self-replicate. Transposons contain insertion sequences and can transfer their

information by inserting themselves into other loci in the same or other genetic elements (e.g., plasmids, chromosomes, viral DNA).

- **Replication**

a- Replicons

- is a general term for double-stranded DNA circles (chromosomes, plasmids) capable of self-replication. Plasmid replication is independent of chromosome replication.
- replicate bidirectionally (5 PO_4 to 3 OH) from a fixed origin.
- The replicon attaches to a projection of the cell membrane (mesosome), which acts as the replication origin site, and one of the DNA strands is broken.
- The 5 end of the broken strand attaches to a new membrane site.
- Elongation of the cell membrane via localized membrane synthesis pulls the broken strand through the mesosomal attachment site, where replication takes place.
- Replication is completed, and the free ends of the new replicon are joined.

b- Transposons

- are replicated, along with the code of the host, after insertion into a replicon.

C. Viral genomes

- **Structure**
  - Genetic information may be coded as DNA or RNA and in double-stranded or single-stranded form.
  - The viral genome may contain exotic bases.
- **Replication**
  - takes place only after successful infection of an appropriate host.

- proceeds when the injected viral genome subverts normal replicative processes of the host, producing new virus particles.

- **Bacteriophage types**
  - may be discerned by their mode of propagation.
  - Lytic phages quickly produce many copies of themselves as they kill the host.
  - Temperate phages can lie seemingly dormant in the host (prophage state), timing replication of prophage genetic material to replication of the host cell. Various activation signals trigger the prophage to enter a lytic cycle, resulting in host death and the release of new phages.

III. Gene Transfer Between Organisms

- maintains genetic variability in microbes through the exchange and recombination of allelic forms of genes.
- is most efficient between cells of the same species.
- may also occur as the crossing over of homologous chromosomes or by nonhomologous means (e.g., movement of plasmids or transposons, insertion of viral genes).
- can result in the acquisition of new characteristics (e.g., antigens, toxins, antibiotic resistance).
- occurs via three mechanisms: conjugation, transduction, and transformation .

A. Conjugation

- is a one-way transfer of genetic material (usually plasmids) from donor to recipient by means of physical contact.
- typically involves three types of plasmids:
  - **F^+ cell**

- possesses a fertility (F) plasmid, mediating the creation of a sex pilus necessary for conjugal transfer of the F plasmid to the recipient.
- can integrate into chromosomal DNA, creating high-frequency recombination donors from which chromosomal DNA is readily transferred.

- **R factors**
  - contain genes conferring drug resistance. Frequently, the resistance genes are carried on transposons.
  - express resistance phenotype through natural selection.
- **F^+ cell and R factors**
  - are recombinant fertility or resistance plasmids in which limited regions of chromosomal DNA can be replicated and transferred by conjugation independently of the chromosome.

B. Transduction

- is phage-mediated transfer of host DNA sequences.
- can be performed by temperate phages and, under special conditions, by lytic phages.
- **occurs in two forms:**
  - In generalized transduction, the phage randomly packages host DNA in a bacteriophage coat and may transfer any gene. The transducing particle contains only host DNA.
  - In specialized transduction, the lysogenic phage favors the transfer of host DNA segments near the site of prophage integration. Specialized transducing phages contain both viral and host genes.

C. Transformation .

- is the direct uptake and recombination of naked DNA fragments through the cell wall by competent bacteria. Natural occurrence of this process is uncommon.
- is sometimes mediated by surface competence factors (DNA receptor enzymes) produced only at a specific point in the bacterial growth cycle.
- can sometimes be forced by treatment with calcium chloride and temperature shock.
- is used in recombinant DNA research and commercially to introduce human genes via vectors into bacteria for rapid and large-scale production of human gene products.

IV. Gene Expression

A. Processes affecting expression

- **Transcription**
  - is the transfer of DNA-bound protein synthesis instructions to mRNA.
  - is mediated in bacteria by RNA polymerase.
  - is initiated by the binding of sigma factor, a subunit of RNA polymerase, to the promoter region of the DNA molecule.
  - involves the unwinding of a short sequence of DNA bases and alignment of complementary ribonucleotide bases onto the DNA template.
  - occurs in a 5 PO_4 to 3 OH direction.
- **Translation**
  - occurs at the ribosomes.
  - is accomplished by the tRNA-mediated linkage of amino acids, in accordance with the triplet-encoded mRNA transcript.
  - is the assembly of polypeptide chains from the mRNA transcript.

B. Regulation of expression

- occurs primarily during transcription.
- is determined partly by the ability of the DNA promoter region to bind with sigma factor.
- can be facilitated or blocked by regulator proteins binding to operator sequences near the promoter.
- typically affects an operon, a group of genes under the control of one operator controlled by the action of regulatory proteins.

- Negative control
  - is inhibition of transcription by the binding of a repressor protein.
  - is exemplified by:
    - The lac operon
      - controls expression of three structural genes for lactose metabolism via a repressor protein.
      - Transcription is induced by the presence of lactose (allolactose), which binds to the repressor protein and frees the lac operator.
    - The trp operon
      - controls tryptophan synthesis.
      - Synthesis of tryptophan is halted by the binding of a repressor protein (tryptophan complex) to the trp operator when excess tryptophan is available.
- Positive control
  - is the initiation of transcription in response to the binding of an activator protein.
  - Expression of the ara operon proceeds only when arabinose binds to a special protein, forming an activator compound necessary for the transcription of the ara operon.

- Cyclic adenosine monophosphate (cyclic AMP) binding protein, when bound to a specific DNA sequence near the promoter, enhances the expression of many genes associated with fermentation. Cyclic AMP enhances RNA polymerase activity.

V. Mutation

- occurs approximately once for any gene in every 1 million cells.
- is an induced or spontaneous heritable alteration of the DNA sequence.
- introduces variability into the gene pool and changes in the phenotype.
- may be caused by various mutagens, including ultraviolet light, acridine dyes, base analogues, and nitrous acid.

A. Mutation types

- **Nucleotide substitutions**
  - arise from mutagenic activity or the mispairing of complementary bases during DNA replication.
  - often do not significantly disrupt the function of gene products.
- **Frame-shift mutations**
  - result from the insertion or deletion of one or two base pairs, disrupting the phase of the triplet-encoded DNA message.
- **Deletions**
  - are usually large excisions of DNA, dramatically altering the sequence of coded proteins.
  - may also result in frameshift mutations.

- **Insertions**
    - change genes and their products by integration of new DNA via transposons.

B. Results of mutation

- **Missense mutations**
    - result in the substitution of one amino acid for another.
    - may be without phenotypic effect (silent mutation).
- **Nonsense mutations**
    - terminate protein synthesis and result in truncated gene products.
    - usually result in inactive protein products.

C. Reversions

- Function lost to mutation may be regained in two ways:
    - Genotypic (true) reversion
- is restoration at the site of DNA alteration.
    - Phenotypic (suppression) reversion
- is restoration of an activity lost to mutation, often by a mutation at a second site (suppressor mutation).

DNA replication

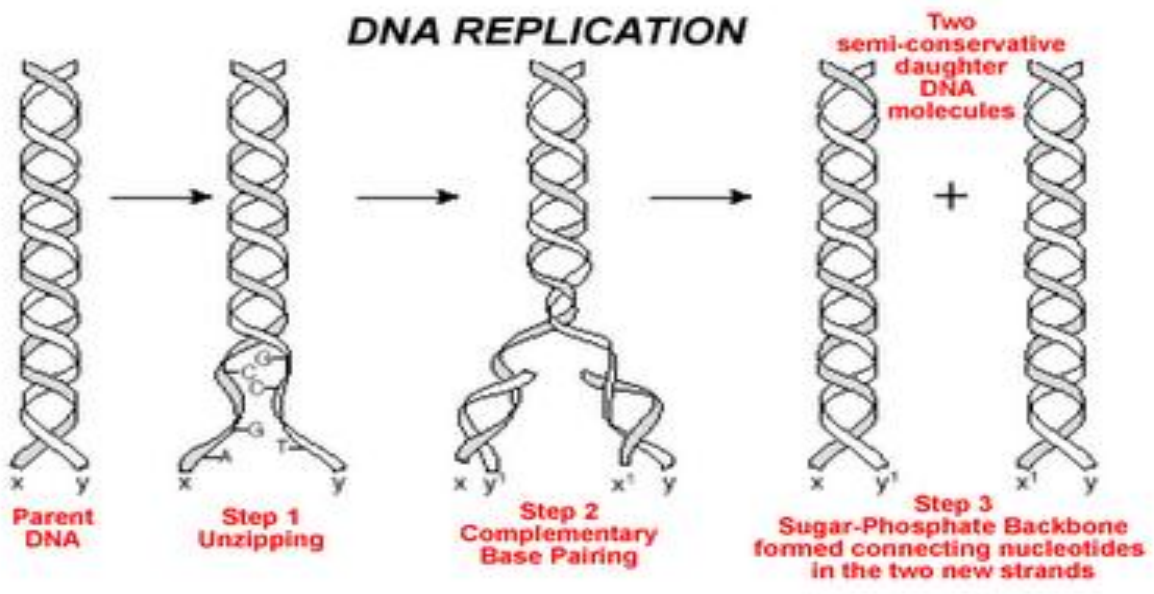
Ds-DNA is synthesized by **semiconservative replication**:

1. The parental duplex unwinds

2. Each strand serves as a template for DNA replication

3. New strand are synthesized as a complementary to the preexisting strand.

4. Each newly synthesized daughter molecule contains one parental & one newly synthesized strand.



Mechanisms of recombination:

The recombination may be :

Homologous : When there is close similarity in the sequence of donor & recipient DNA. It is always involves exchange between genes that share common ancestry.

Non-homologous :that result of enzyme-catalyzed recombination between dissimilar DNA sequences. These enzymes are encoded by integrated DNA.

Polymerase chain reaction

The **polymerase chain reaction** (**PCR**) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

PCR principles and procedure

PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size.

A basic PCR set up requires several components and reagents. These components include:

- *DNA template* that contains the DNA region (target) to be amplified.
- Two *primers* that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- *Taq polymerase* or another DNA polymerase with a temperature optimum at around 70 °C.
- *Deoxynucleoside triphosphates* (dNTPs; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.
- *Buffer solution*, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- *Divalent cations*, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis.
- *Monovalent cation* potassium ions.

The PCR is commonly carried out in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction .

Procedure

Typically, PCR consists of a series of 20-40 repeated temperature changes, called cycles, with each cycle commonly consisting of 2-3 discrete temperature steps, usually three. The cycling is often preceded by a single temperature step (called *hold*) at a high temperature (>90°C), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers.

- *Initialization step*: This step consists of heating the reaction to a temperature of 94–96 °C (or 98 °C if extremely thermostable polymerases are used), which is held for 1–9 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.
- *Denaturation step*: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
- *Annealing step*: The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5 degrees Celsius below the Tm of the primers used. Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA synthesis.
- *Extension/elongation step*: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C, and commonly a

temperature of 72 °C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

- *Final elongation*: This single step is occasionally performed at a temperature of 70–74 °C for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.
- *Final hold*: This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.

Immunity

is a biological term that describes a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion or is defined as an enhanced state of responsiveness to a specific substance, induced by prior contact with that substance.

Immunity classify as :

- Natural immunity
  - is present from birth and is nonspecific.
  - consists of various barriers to external insults; for example, skin, mucous membranes, macrophages, monocytes, neutrophils, eosinophils, and the contents of these cells.
- Acquired immunity
  - is expressed after exposure to a given substance and is specific.

- involves specific receptors on lymphocytes and the participation of macrophages for its expression.
- consists of:
- Humoral immunity, mediated by antibodies
- Cell-mediated immunity, mediated by lymphocytes

B. Immune system : is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumor cells.

Immune system consists of:

- Cellular and molecular components derived from the central and peripheral lymphoid organs.
- Central lymphoid organs
  - consist of the bone marrow and thymus.
  - are the location of maturation of lymphoid cells.
- Peripheral lymphoid organs
  - consist of the spleen, lymph nodes and lymphatic channels, tonsils, adenoids, Peyer's patches, and appendix.
  - are the location of reactivity of lymphoid cells.
- Cells of the immune system
  - include the white blood cells (approximately 4000 - 11000/mm^3 of blood), which are composed of:
  - Granulocytes(50% - 70%) of white blood cells
  - Lymphocytes(20% - 45%) of white blood cells
  - Monocytes and macrophages(3% - 8%)of white blood cells
- Molecules of the immune system

- Antibodies (immunoglobulins) are protein products of certain lymphocytes with a precise specificity for a particular antigen.
- Lymphokines are secreted lymphocyte products that play a role in the activation of the immune response.

C. Development of the immune system

- involves the maturation of pluripotential stem cells in the bone marrow or thymus into B cells and T cells, respectively.
- includes the generation of specific receptors on the cell surface of B cells and T cells.
- Pluripotential stem-cell sources
  - Embryonic yolk sac
  - Fetal liver
  - Adult bone marrow
- B cells
  - mature in the bursa (hence the name of B cells) of Fabricius in birds and in the fetal liver and adult bone marrow in humans (bursal equivalents).
  - are involved in the generation of humoral immunity.
  - have specific receptors (immunoglobulins) on their surface for antigen recognition.
  - mature into antibody-producing plasma cells.
  - are sessile and located predominantly in the germinal centers of the lymph nodes and spleen.
- T cells
  - mature in the thymus.
  - are involved in helping of B cells become antibody-producing plasma cells.
  - have specific receptors (T-cell receptors) on their surface for antigen recognition.

- are involved in cell-mediated immunity.
- participate in suppression of the immune response.
- are the predominant (95%) lymphocytes in the circulation.
- are found in the para-cortical and inter-follicular areas of the lymph nodes and spleen.

D. Physiology of immunity

- involves the following series of events that culminate in B-cell or T-cell activation (or both) and response to the introduction of a foreign entity into the circulation:
  - Processing of the foreign entity by a macrophage or B cell
  - Recognition of this foreign entity by specific, preformed receptors on certain B cells and T cells
  - Proliferation of these B cells and T cells, as stimulated by soluble signals (interleukins) between macrophages, B cells, and T cells
  - Blast transformation and a series of mitotic divisions leading to the generation (from B cells) of plasma cells that produce immunoglobulins and (from T cells) of sensitized T cells and all capable of interacting with the original foreign stimulus

Antigens (Immunogens)

A. Characteristics

- Immunogenicity: is the capacity to stimulate production of specific, protective humoral or cellular immunity.
- Specific reactivity :is the capacity to be recognized by the antibodies and T cells produced.
- Foreignness: is the recognition of a body as nonself (foreign proteins are excellent antigens).
- Size : must be at least approximately 10 kilodaltons (kd) to be recognized.
- Shape : tertiary and quaternary structure determines the extent of antigenicity.

B. Definitions

- **Epitope**
  - is the restricted portion of an antigen molecule that determines the specificity of the reaction with an antibody.
  - is the antibody-binding site on an antigen for a specific antibody.
  - generally contains four to six amino acid or sugar residues.
  -
- **Hapten**
  - is a small foreign molecule that is not immunogenic by itself but can bind to an antibody molecule already formed to it.
  - can be immunogenic if coupled to a sufficiently large carrier molecule.

Antibodies (Immunoglobulins)

Characteristics of antibodies

- are a heterogeneous group of proteins that contain carbohydrate.
- have sedimentation coefficients ranging from 7S to 19S.
- are found predominantly in the gamma globulin fraction of serum.
- consist of polypeptide chains linked by disulfide bonds, such that each antibody contains a minimum of two identical heavy (H) chains and two identical light (L) chains.
- have inter-chain disulfide bonds holding the chains together (i.e., L to H and H to H).
- have antigen-binding capacity defined by their specific H and L chains.

Structure of antibody molecules

- **H chains**
  - are polypeptide chains of 440 - 550 amino acid residues in length.

- have intra-chain domains of approximately 110 amino acid residues, formed by intra-chain disulfide bonds.
- have an amino-terminal variable domain, followed by three to four constant domains.
- are structurally different for each of the defined classes of antibody [mu , gamma , alpha , delta and epsilon].

- **L chains**
  - are polypeptide chains of approximately 220 amino acid residues in length.
  - have intrachain domains of approximately 110 amino acid residues, formed by intrachain disulfide bonds.
  - have an amino-terminal variable domain and a carboxy-terminal constant domain.
  - have two structurally distinct classes: kappa chains and lambda chains.

Immunoglobulin classes

The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains.

1. IgG - Gamma heavy chains. 2. IgM - Mu heavy chains . 3. IgA - Alpha heavy chains

4. IgD - Delta heavy chains .5. IgE - Epsilon heavy chains

Properties of immunoglobulins

A. IgG

1. All IgG's are monomers (7S immunoglobulin).

2 . IgG is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.

3. IgG is the major Ig in serum - 75% of serum Ig is IgG

4. IgG is the major Ig in extra vascular spaces

5. Placental transfer - IgG is the only class of Ig that crosses the placenta.

6. Fixes complement

7 . Binding to cells - Macrophages, monocytes, PMNs and some lymphocytes have Fc receptors for the Fc region of IgG.

B. IgM

1. IgM normally exists as a pentamer (19S immunoglobulin) .

2. IgM is the third most common serum Ig.

3. IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.

4. As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms.

6. IgM binds to some cells via Fc receptors.

C. IgA

1.Serum IgA is a monomer but IgA found in secretions is a dimer.

2. IgA is the 2nd most common serum Ig.

3. IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.

4. Normally IgA does not fix complement, unless aggregated.

5. IgA can binding to some cells - PMN's and some lymphocytes

D. IgD

1. IgD exists only as a monomer.

2. IgD is found in low levels in serum; its role in serum uncertain.

3. IgD is primarily found on B cell surfaces where it functions as a receptor for antigen.

4. IgD does not bind complement.

E. IgE

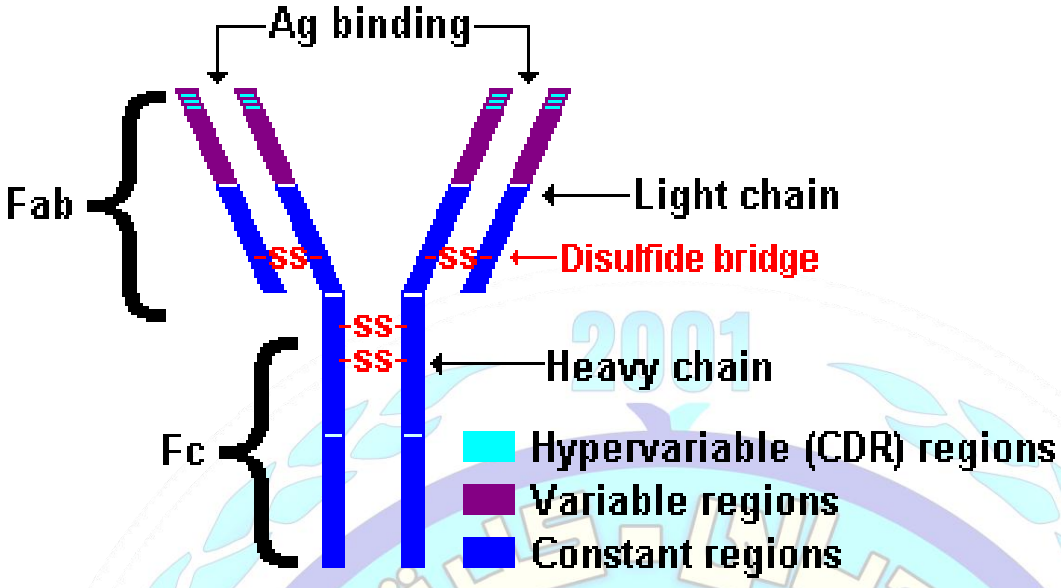
1. IgE exists as a monomer and has an extra domain in the constant region.

2. IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.

3. Involved in allergic reactions

4. IgE also plays a role in parasitic helminth diseases.

5. IgE does not fix complement.



The Complement System

A. Complement-mediated cell cytotoxicity

- causes lysis of a target cell.
- may be initiated by antibody fixation to a cell-surface antigen.
- may be caused by antigen-antibody complex formation.
- occurs by activation of either:
- Classic complement cascade mediated by IgG, IgG2, IgG3, or IgM antibody
- Alternative pathway initiated by certain antigens (i.e., lipopolysaccharide, endotoxin, zymosan) or antigen antibody complexes

B. Complement components

- is a collective term for a group of heterogeneous proteins involved in a sequential activation, culminating in target cell lysis.
- are not immunoglobulins.
- are present in normal serum.

- do not increase as a result of antigen stimulation.
- are manufactured early in ontogeny (first trimester).
- are made in macrophages and liver (except C1, which is made and assembled in gastrointestinal epithelium).
- are heat labile.
- are defined by number (C1, C2, C3) and letter (factor B, factor D) designations.

Hypersensitivity Reactions

- are categorized according to the Gell and Coombs classification.

A. Type I hypersensitivity (anaphylaxis)

- occurs in atopic persons.
- occurs in response to environmental antigens (e.g., allergens) or administered antigens (e.g., penicillin).
- is mediated by IgE (reaginic) antibody bound to the surface of mast cells or basophils.
- may be localized or systemic.
- IgE in immediate hypersensitivity
    - is produced in response to environmental antigens.
    - binds by the Fc portion of IgE to mast cells or basophils.
    - causes release of vasoactive and chemotactic factors from mast cells upon cross-linking of antigen on the surface.
    - can be measured in toto by use of RIST.
    - can also be measured for specific idiotypes by RAST.
- Products released by mast cells upon stimulation of surface IgE
    - Vasoactive mediators

- Histamine causes smooth muscle contraction in bronchioles and small blood vessels and increased permeability of capillaries; molecular weight is 111 daltons.
- Platelet-activating factor (PAF) a activates platelets.

- Chemotactic factors
  - Eosinophil chemotactic factor of anaphylaxis (ECF-A) causes influx of eosinophils; molecular weight is 2 kd.
  - Neutrophil chemotactic factor has a high molecular weight (660 kd); is chemotactic for neutrophils.

B. Type II hypersensitivity cytotoxic reactions

- involve the production of antibody to specific cell-surface epitopes, which cause destruction of the cell.
- Antibody to cell-surface antigen
  - can cause reduction in cell-surface charges.
  - can cause opsonic adherence via the Fc region of antibody to neutrophils, macrophages, and K cells (the cells responsible for ADCC); enhances cell phagocytosis and promotes cell death.
  - can activate complement to cause cell lysis.
- Examples of type II hypersensitivity reactions
  - Transfusion reactions ABO incompatibility involving IgM antibodies against A or B alloantigens
  - Rh incompatibility IgG antibodies against the D antigen on fetal red blood cells

- Hemolytic anemia antibody to red blood cell epitopes
- Myasthenia gravis antibody to muscle acetylcholine receptors

C. Type III hypersensitivity immune complex reactions

- involve soluble antigen that becomes bound antigen antibody complexes, which, especially in antigen excess, can cause a series of events that lead to pathologic expression, edema, neutrophil infiltrate, and lesions in blood vessels and kidney glomeruli.

- Consequences of antigen antibody complex formation
  - Platelet aggregation, leading to formation of microthrombi and release of vasoactive amines
  - Activation of complement and release of anaphylatoxins (causing histamine release) and chemotactic factors (for neutrophils)
  - Clotting factor XII activation, leading to fibrin, plasmin, and kinin formation
- Examples of type III hypersensitivity reactions
  - Arthus reaction immunization of rabbits with horse serum .
  - Farmer's lung antibody to inhaled aspergillus mold
  - Rheumatoid arthritis rheumatoid factor (IgM) against the Fc portion of self-IgG

D. Type IV hypersensitivity delayed-type hypersensitivity

- is differentiated from immediate-type hypersensitivity reactions (types I, II, and III).
- is an example of cell-mediated immunity; types I, II, and III are mediated by antibody and are examples of humoral immunity.

- Sequence of events in a type IV reaction

- An appropriate antigen (tuberculin, purified protein derivative of Mycobacterium tuberculosis, tumor cell, transplanted cell, virally transformed cell) is processed by macrophages; epitopes of antigen are expressed on the macrophage surface via class II HLAs; macrophages produce IL-1.
- T_H cells react to antigen epitope and class II antigens via TCR and CD4, respectively.
- T_H cells are also stimulated by IL-1 from macrophages.
- T_H cells produce IL-2, and IL-2 receptors become fully activated and release lymphokines, having an effect on T cells and macrophages.

- Lymphokines
  - that affect macrophages
  - that affect $CD8^+$ cells include IL-2, which activates them to become fully cytotoxic.
  - that are produced by $CD4^+$ and $CD8^+$ cells include TNF, osteoclast-activating factor (OAF), and histamine-releasing factor (HRF).
- $CD8^+$ cytotoxic cells
  - react to viral and tumor antigens and class I HLAs via TCR and CD8 molecules, respectively.
  - are further stimulated by IL-2 from T_H cells.
  - produce IL-2 themselves.
  - produce IFN-ϒ.

Bacteriology

Bacteriology is the study of bacteria. This subdivision of microbiology involves the identification, classification, and characterization of bacterial species. A person who studies bacteriology is a *bacteriologist*.

Staphylococci

The staphylococci are gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems.

The genus *Staphylococcus* has at least 35 species. The three main species of clinical importance are *Staphylococcus aureus, Staphylococcus epidermidis,* and *Staphylococcus saprophyticus. Staphylococcus aureus* is **coagulase-positive,** which differentiates it from the other species. *S aureus* is a major pathogen for humans. Almost every person will have some type of *S aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections. The coagulase-negative staphylococci are normal human flora and sometimes cause infection, often associated with implanted appliances and devices, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by **coagulase-negative** staphylococci are due to *S epidermidis;* infections due to *Staphylococcus lugdunensis, Staphylococcus warneri, Staphylococcus hominis,* and other species are less common. *S saprophyticus* is a relatively

common cause of urinary tract infections in young women. Other species are important in veterinary medicine.

Streptococci

The streptococci are gram-positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. Some are members of the normal human flora; others are associated with important human diseases attributable in part to infection by streptococci, in part to sensitization to them. Streptococci elaborate a variety of extracellular substances and enzymes.The streptococci are a large and heterogeneous group of bacteria and no one system suffices to classify them. Yet, understanding the classification is key to understanding their medical importance.

The classification of streptococci into major categories has been based on a series of observations over many years: (1) colony morphology and hemolytic reactions on blood agar; (2) serologic specificity of the cell wall group-specific substance and other cell wall or capsular antigens; (3) biochemical reactions and resistance to physical and chemical factors; and (4) ecologic features.

Hemolysis

Many streptococci are able to hemolyze red blood cells in vitro in varying degrees.Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β **hemolysis.** Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α **-hemolysis.** Other streptococci are non-hemolytic (sometimes called gamma hemolysis).

Capsular Polysaccharides

The antigenic specificity of the capsular polysaccharides is used to classify *S pneumoniae* into over 90 types and to type the group B streptococci (*S agalactiae*).

Biochemical Reactions

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents. Biochemical tests are most often used to classify streptococci after the colony growth and hemolytic characteristics have been observed. Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G. For example, the viridans streptococci are α-hemolytic or nonhemolytic and do not react with the antibodies commonly used for the Lancefield classification. Speciation of the viridans streptococci requires a battery of biochemical tests.

Many species of streptococci, including *S pyogenes* (group A), *S agalactiae* (group B), and the enterococci (group D), are characterized by combinations of features: colony growth characteristics, hemolysis patterns on blood agar (α -hemolysis, β-hemolysis, or no hemolysis), antigenic composition of group-specific cell wall substances, and biochemical reactions. *S pneumoniae* (pneumococcus) types are further classified by the antigenic composition of the capsular polysaccharides. The viridans streptococci can be α -hemolytic or nonhemolytic and are generally speciated by biochemical reactions.

Enterobacteriaceae

The Enterobacteriaceae are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (*Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus,* and others). Some enteric organisms, eg, *Escherichia coli,* are part of the normal flora and incidentally cause disease, while others, the salmonellae and shigellae, are regularly pathogenic for humans. The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. Enterobacteriaceae, enteric gram-negative rods, and enteric bacteria are the terms used in this chapter, but these bacteria may also be called coliforms.

Classification

The Enterobacteriaceae are the most common group of gram-negative rods cultured in the clinical laboratory and along with staphylococci and streptococci are among the most common bacteria that cause disease. The taxonomy of the Enterobacteriaceae is complex and rapidly changing since the introduction of techniques that measure evolutionary distance, such as nucleic acid hybridization and sequencing. More than 25 genera and 110 species or groups have been defined; however, the clinically significant Enterobacteriaceae comprise 20–25 species, and other species are encountered infrequently. In this chapter, taxonomic refinements will be minimized, and the names commonly employed in the medical literature will generally be used. The family Enterobacteriaceae have the following characteristics: They are gram-negative rods, either motile with peritrichous flagella or nonmotile; they grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on MacConkey's agar; grow aerobically and anaerobically (are facultative anaerobes); ferment rather than oxidize glucose, often with gas production; are catalase-positive, oxidase-negative, and reduce nitrate to nitrite; and have a 39–59% G + C DNA content.

Morphology and Identification

Typical Organisms

The Enterobacteriaceae are short gram-negative rods. Typical morphology is seen in growth on solid media in vitro, but morphology is highly variable in clinical specimens. Capsules are large and regular in klebsiella, less so in enterobacter, and uncommon in the other species.

Culture

E coli and most of the other enteric bacteria form circular, convex, smooth colonies with distinct edges. Enterobacter colonies are similar but somewhat more mucoid. Klebsiella colonies are large and very

mucoid and tend to coalesce with prolonged incubation. The salmonellae and shigellae produce colonies similar to *E coli* but do not ferment lactose. Some strains of *E coli* produce hemolysis on blood agar.

Growth Characteristics

Carbohydrate fermentation patterns and the activity of amino acid decarboxylases and other enzymes are used in biochemical differentiation . Some tests, eg, the production of indole from tryptophan, are commonly used in rapid identification systems, while others, eg, the Voges-Proskauer reaction (production of acetylmethylcarbinol from dextrose), are used less often. Culture on "differential" media that contain special dyes and carbohydrates (eg, eosin-methylene blue [EMB], MacConkey's, or deoxycholate medium) distinguishes lactose-fermenting (colored) from non-lactose-fermenting colonies (nonpigmented) and may allow rapid presumptive identification of enteric bacteria .

Many complex media have been devised to help in identification of the enteric bacteria. One such medium is triple sugar iron (TSI) agar, which is often used to help differentiate salmonellae and shigellae from other enteric gram-negative rods in stool cultures. The medium contains 0.1% glucose, 1% sucrose, 1% lactose, ferrous sulfate (for detection of H_2S production), tissue extracts (protein growth substrate), and a pH indicator (phenol red). It is poured into a test tube to produce a slant with a deep butt and is inoculated by stabbing bacterial growth into the butt. If only glucose is fermented, the slant and the butt initially turn yellow from the small amount of acid produced; as the fermentation products are subsequently oxidized to CO_2 and H_2O and released from the slant and as oxidative decarboxylation of proteins continues with formation of amines, the slant turns alkaline (red). If lactose or sucrose is fermented, so much acid is produced that the slant and butt remain yellow (acid). Salmonellae and shigellae typically yield an alkaline slant and an acid butt. Although proteus, providencia, and morganella produce an alkaline slant and acid butt, they can be identified by their

rapid formation of red color in Christensen's urea medium. Organisms producing acid on the slant and acid and gas (bubbles) in the butt are other enteric bacteria.

Escherichia

E coli typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. An isolate from urine can be quickly identified as *E coli* by its hemolysis on blood agar, typical colonial morphology with an iridescent "sheen" on differential media such as EMB agar, and a positive spot indole test. Over 90% of *E coli* isolates are positive for β-glucuronidase using the substrate 4-methylumbelliferyl β--glucuronide (MUG). Isolates from anatomic sites other than urine, with characteristic properties (above plus negative oxidase tests) often can be confirmed as *E coli* with a positive MUG test.

Klebsiella-Enterobacter-Serratia Group

Klebsiella species exhibit mucoid growth, large polysaccharide capsules, and lack of motility, and they usually give positive tests for lysine decarboxylase and citrate. Most *Enterobacter* species give positive tests for motility, citrate, and ornithine decarboxylase and produce gas from glucose. *Enterobacter aerogenes* has small capsules. Serratia produces DNase, lipase, and gelatinase. Klebsiella, enterobacter, and serratia usually give positive Voges-Proskauer reactions.

Proteus-Morganella-Providencia Group

The members of this group deaminate phenylalanine, are motile, grow on potassium cyanide medium (KCN), and ferment xylose. *Proteus* species move very actively by means of peritrichous flagella, resulting in "swarming" on solid media unless the swarming is inhibited by chemicals, eg, phenylethyl alcohol or CLED (cystine-lactose-electrolyte-deficient) medium. *Proteus* species and *Morganella morganii* are urease-positive, while *Providencia* species usually are urease-negative. The proteus-

providencia group ferments lactose very slowly or not at all. *Proteus mirabilis* is more susceptible to antimicrobial drugs, including penicillins, than other members of the group.

Citrobacter

These bacteria typically are citrate-positive and differ from the salmonellae in that they do not decarboxylate lysine. They ferment lactose very slowly if at all.

Shigella

Shigellae are nonmotile and usually do not ferment lactose but do ferment other carbohydrates, producing acid but not gas. They do not produce H_2S. The four *Shigella* species are closely related to *E coli.* Many share common antigens with one another and with other enteric bacteria (eg, *Hafnia alvei* and *Plesiomonas shigelloides*).

Salmonella

Salmonellae are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most salmonellae produce H_2S. They are often pathogenic for humans or animals when ingested. Arizona is included in the salmonella group.

Antigenic Structure

Enterobacteriaceae have a complex antigenic structure. They are classified by more than 150 different heat-stable somatic O (lipopolysaccharide) antigens, more than 100 heat-labile K (capsular) antigens, and more than 50 H (flagellar) antigens

O antigens are the most external part of the cell wall lipopolysaccharide and consist of repeating units of polysaccharide. Some O-specific polysaccharides contain unique sugars. O antigens are resistant to

heat and alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM.

K antigens are external to O antigens on some but not all Enterobacteriaceae. Some are polysaccharides, including the K antigens of *E coli;* others are proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence (eg, *E coli* strains producing K1 antigen are prominent in neonatal meningitis, and K antigens of *E coli* cause attachment of the bacteria to epithelial cells prior to gastrointestinal or urinary tract invasion).

H antigens are located on flagella and are denatured or removed by heat or alcohol. They are preserved by treating motile bacterial variants with formalin. Such H antigens agglutinate with anti-H antibodies, mainly IgG. The determinants in H antigens are a function of the amino acid sequence in flagellar protein (flagellin).

Pseudomonad Group

The pseudomonads are gram-negative, motile, aerobic rods some of which produce water-soluble pigments. Pseudomonads occur widely in soil, water, plants, and animals. *Pseudomonas aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. Other pseudomonads infrequently cause disease. The classification of pseudomonads is based on rRNA/DNA homology and common culture characteristics.

Pseudomonas aeruginosa

P aeruginosa is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defenses.

Morphology and Identification

Typical Organisms

P aeruginosa is motile and rod-shaped, measuring about 0.6 x 2 µm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains.

Culture

P aeruginosa is an obligate aerobe that grows readily on many types of culture media, sometimes producing a sweet or grape-like or corn taco-like odor. Some strains hemolyze blood. *P aeruginosa* forms smooth round colonies with a fluorescent greenish color. It often produces the nonfluorescent bluish pigment **pyocyanin,** which diffuses into the agar. Other *Pseudomonas* species do not produce pyocyanin. Many strains of *P aeruginosa* also produce the fluorescent pigment **pyoverdin,** which gives a greenish color to the agar. Some strains produce the dark red pigment **pyorubin** or the black pigment **pyomelanin.***P aeruginosa* in a culture can produce multiple colony types. *P aeruginosa* from different colony types may also have different biochemical and enzymatic activities and different antimicrobial susceptibility patterns. Sometimes it is not clear if the colony types represent different strains of *P aeruginosa* or are variants of the same strain. Cultures from patients with cystic fibrosis often yield *P aeruginosa* organisms that form mucoid colonies as a result of overproduction of alginate, an exopolysaccharide. In cystic fibrosis patients, the exopolysaccharide appears to provide the matrix for the organisms to live in a biofilm (see Chapter 9).

Growth Characteristics

P aeruginosa grows well at 37–42 ℃; its growth at 42 °C helps differentiate it from other *Pseudomonas* species in the fluorescent group. It is **oxidase-positive.** It does not ferment carbohydrates, but many strains oxidize glucose. Identification is usually based on colonial

morphology, oxidase positivity, the presence of characteristic pigments, and growth at 42 °C. Differentiation of *P aeruginosa* from other pseudomonads on the basis of biochemical activity requires testing with a large battery of substrates.

Pathogenesis

P aeruginosa is pathogenic only when introduced into areas devoid of normal defenses, eg, when mucous membranes and skin are disrupted by direct tissue damage; when intravenous or urinary catheters are used; or when neutropenia is present, as in cancer chemotherapy. The bacterium attaches to and colonizes the mucous membranes or skin, invades locally, and produces systemic disease. These processes are promoted by the pili, enzymes, and toxins described above. Lipopolysaccharide plays a direct role in causing fever, shock, oliguria, leukocytosis and leukopenia, disseminated intravascular coagulation, and adult respiratory distress syndrome. *P aeruginosa* and other pseudomonads are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed.

Clinical Findings

P aeruginosa produces infection of wounds and burns, giving rise to blue-green pus; meningitis, when introduced by lumbar puncture; and urinary tract infection, when introduced by catheters and instruments or in irrigating solutions. Involvement of the respiratory tract, especially from contaminated respirators, results in necrotizing pneumonia. The bacterium is often found in mild otitis externa in swimmers. It may cause invasive (malignant) otitis externa in diabetic patients. Infection of the eye, which may lead to rapid destruction of the eye, occurs most commonly after injury or surgical procedures. In infants or debilitated persons, *P aeruginosa* may invade the bloodstream and result in fatal sepsis; this occurs commonly in patients with leukemia or lymphoma who have received antineoplastic drugs or radiation therapy and in patients with severe burns. In most *P aeruginosa*

infections, the symptoms and signs are nonspecific and are related to the organ involved. Occasionally, verdoglobin (a breakdown product of hemoglobin) or fluorescent pigment can be detected in wounds, burns, or urine by ultraviolet fluorescence. Hemorrhagic necrosis of skin occurs often in sepsis due to *P aeruginosa;* the lesions, called **ecthyma gangrenosum,** are surrounded by erythema and often do not contain pus. *P aeruginosa* can be seen on Gram-stained specimens from ecthyma lesions, and cultures are positive. Ecthyma gangrenosum is uncommon in bacteremia due to organisms other than *P aeruginosa*.

Vibrios

Vibrios are among the most common bacteria in surface waters worldwide. They are curved aerobic rods and are motile, possessing a polar flagellum. *V cholerae* serogroups O1 and O139 cause cholera in humans, while other vibrios may cause sepsis or enteritis. Upon first isolation, *V cholerae* is a comma-shaped, curved rod 2–4 μm long . It is actively motile by means of a polar flagellum. On prolonged cultivation, vibrios may become straight rods that resemble the gram-negative enteric bacteria.

V cholerae produces convex, smooth, round colonies that are opaque and granular in transmitted light. *V cholerae* and most other vibrios grow well at 37 °C on many kinds of media, including defined media containing mineral salts and asparagine as sources of carbon and nitrogen. *V cholerae* grows well on **thiosulfate-citrate-bile-sucrose (TCBS)** agar, on which it produces yellow colonies that are readily visible against the dark-green background of the agar. Vibrios are oxidase-positive, which differentiates them from enteric gram-negative bacteria. Characteristically, vibrios grow at a very high pH (8.5–9.5) and are rapidly killed by acid. Cultures containing fermentable carbohydrates therefore quickly become sterile.

In areas where cholera is endemic, direct cultures of stool on selective media such as TCBS, and enrichment cultures in alkaline peptone water are appropriate. However, routine stool cultures on special media such as TCBS generally are not necessary or cost-effective in areas where cholera is rare.

Under natural conditions, *V cholerae* is pathogenic only for humans. A person with normal gastric acidity may have to ingest as many as 10^{10} or more *V cholerae* to become infected when the vehicle is water, because the organisms are susceptible to acid. When the vehicle is food, as few as 10^{2}–10^{4} organisms are necessary because of the buffering capacity of food. Any medication or condition that decreases stomach acidity makes a person more susceptible to infection with *V cholerae*.

Cholera is not an invasive infection. The organisms do not reach the bloodstream but remain within the intestinal tract. Virulent *V cholerae* organisms attach to the microvilli of the brush border of epithelial cells. There they multiply and liberate cholera toxin and perhaps mucinases and endotoxin.

Neisseriae

The neisseriae are gram-negative cocci that usually occur in pairs. *Neisseria gonorrhoeae* (gonococci) and *Neisseria meningitidis* (meningococci) are pathogenic for humans and typically are found associated with or inside polymorphonuclear cells. Some neisseriae are normal inhabitants of the human respiratory tract, rarely if ever cause disease, and occur extracellularly. The typical neisseria is a gram-negative, nonmotile diplococcus, approximately 0.8 µm in diameter . Individual cocci are kidney-shaped; when the organisms occur in pairs, the flat or concave sides are adjacent.

Haemophilus influenzae

Haemophilus influenzae is found on the mucous membranes of the upper respiratory tract in humans. It is an important cause of meningitis in children and occasionally causes respiratory tract infections in children and adults.

Mycobacteria

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. Although they do not stain readily, once stained they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli. *Mycobacterium tuberculosis* causes tuberculosis and is a very important pathogen of humans. *Mycobacterium leprae* causes leprosy. *Mycobacterium avium-intracellulare* (*M avium* complex, or MAC) and other **atypical mycobacteria** frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems.

Mycoplasmas

There are over 150 species in the class of cell wall-free bacteria. At least 15 of these species are thought to be of human origin, while others have been isolated from animals and plants. In humans, four species are of primary importance: *Mycoplasma pneumoniae* causes pneumonia and has been associated with joint and other infections. *Mycoplasma hominis* sometimes causes postpartum fever and has been found with other bacteria in uterine tube infections. *Ureaplasma urealyticum* is a cause of nongonococcal urethritis in men and is associated with lung disease in premature infants of low birth weight. *Mycoplasma genitalium* is closely related to *M pneumoniae* and has been associated with urethral and other infections. Other members of the genus *Mycoplasma* are pathogens of the respiratory and urogenital tracts and joints of animals.

They have the following characteristics: (1) The smallest mycoplasmas are 125 - 250 nm in size. (2) They are highly pleomorphic because they lack a rigid cell wall and instead are bounded by a triple-layered "unit membrane" that contains a sterol (mycoplasmas require the addition of serum or cholesterol to the medium to produce sterols for growth). (3) Mycoplasmas are completely resistant to penicillin because they lack the cell wall structures at which penicillin acts, but they are inhibited by

tetracycline or erythromycin. (4) Mycoplasmas can reproduce in cell-free media; on agar, the center of the whole colony is characteristically embedded beneath the surface. (5) Growth of mycoplasmas is inhibited by specific antibody. (6) Mycoplasmas have an affinity for mammalian cell membranes.

Rickettsia

They are obligate intracellular parasites and, except for Q fever, are transmitted to humans by arthropods. Many rickettsiae are transmitted transovarially in the arthropod, which serves as both vector and reservoir.

Chlamydiae

Chlamydiae that infect humans are divided into three species—*Chlamydia trachomatis*, *Chlamydia pneumoniae*, and *Chlamydia psittaci*—on the basis of antigenic composition, intracellular inclusions, sulfonamide susceptibility, and disease production. A fourth species, *Chlamydia pecorum*, infects a variety of animals but is not known to infect humans. All chlamydiae exhibit similar morphologic features, share a common group antigen, and multiply in the cytoplasm of their host cells by a distinctive developmental cycle. The chlamydiae can be viewed as gram-negative bacteria that lack mechanisms for the production of metabolic energy and cannot synthesize ATP. This defect restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. Thus, chlamydiae are **obligate intracellular parasites.**

Food microbiology

Food microbiology is the study of the microorganisms that inhabit, create, or contaminate food. Of major importance is the study of microorganisms causing food spoilage. "Good" bacteria, however, such as probiotics, are becoming increasingly important in food science. In addition, microorganisms

are essential for the production of foods such as cheese, yogurt, other fermented foods, bread, beer and wine.

Food safety

Food safety is a major focus of food microbiology. Pathogenic bacteria, viruses and toxins produced by microorganisms are all possible contaminants of food. However, microorganisms and their products can also be used to combat these pathogenic microbes. Probiotic bacteria, including those that produce bacteriocins, can kill and inhibit pathogens. Alternatively, purified bacteriocins such as nisin can be added directly to food products. Finally, bacteriophages, viruses that only infect bacteria, can be used to kill bacterial pathogens. Thorough preparation of food, including proper cooking, eliminates most bacteria and viruses. However, toxins produced by contaminants may not be heat-labile, and some are not eliminated by cooking

Fermentation

Fermentation is one way microorganisms can change a food. Yeast, especially *Saccharomyces cerevisiae*, is used to leaven bread, brew beer and make wine. Certain bacteria, including lactic acid bacteria, are used to make yogurt, cheese, hot sauce, pickles, fermented sausages and dishes such as kimchi. A common effect of these fermentations is that the food product is less hospitable to other microorganisms, including pathogens and spoilage-causing microorganisms, thus extending the food's shelf-life. Food fermentations are ancient technologies that harness microorganisms and their enzymes to improve the human diet. Fermented foods keep better, have enhanced flavours, textures and aromas, and may also possess certain health benefits, including superior digestibility. For vegetarians, fermented foods serve as palatable, protein-rich meat substitutes.

Probiotics

Probiotics are living organisms that, when consumed, have beneficial health benefits outside their inherent nutritional effects. There is a growing body of evidence for the role of probiotics in gastrointestinal infections, irritable bowel syndrome and inflammatory bowel disease. *Lactobacillus* species are used for the production of yogurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, chocolate and other fermented foods, as well as animal feeds such as silage.

Microbial biopolymers

A variety of biopolymers, such as polysaccharides, polyesters and polyamides, are naturally produced by microorganisms. Several microbially-produced polymers are used in the food industry.

Xanthan

Plant-pathogenic bacteria of the genus *Xanthomonas* are able to produce the acidic exopolysaccharide xanthan gum. Because of its physical properties, it is widely used as a viscosifer, thickener, emulsifier or stabilizer in the food industry.

Alginate

Alginate is the main representative of a family of polysaccharides that neither show branching nor repeating blocks or unit patterns and this property distinguishes it from to other polymers like xanthan or dextran. Alginates can be used as thickening agents.

Cellulose

Cellulose is a simple polysaccharide, in that it consists only of one type of sugar (glucose), and the units are linearly arranged and linked together by β-1,4 linkages only. The mechanism of biosynthesis is, however, rather complex, partly because in native celluloses, the chains are organized as highly

ordered water-insoluble fibers. Currently, the key genes involved in cellulose biosynthesis and regulation are known in a number of bacteria, but many details of the biochemistry of its biosynthesis are still not clear. In spite of the enormous abundance of cellulose in plants, bacterial celluloses are being investigated for industrial exploitations.

Poly-γ-glutamic acid

Poly-γ-glutamic acid (γ-PGA) produced by various strains of *Bacillus* has potential applications as a thickener in the food industry.

Levan

has great potential as a functional biopolymer in foods, feeds, cosmetics, and the pharmaceutical and chemical industries. Levan can be used as food or a feed additive with prebiotic and hypocholesterolemic effects.

Exopolysaccharides

Microorganisms synthesize a wide spectrum of multifunctional polysaccharides, including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides or exopolysaccharides (EPSs). EPSs generally consist of monosaccharides and some noncarbohydrate substituents (such as acetate, pyruvate, succinate, and phosphate). Owing to the wide diversity in composition, they have found multifarious applications in various food and pharmaceutical industries.

Foodborne pathogens

Foodborne pathogens are the leading causes of illness and death in less developed countries, killing approximately 1.8 million people annually. In developed countries, foodborne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of

dollars in medical care and lost productivity. New foodborne pathogens and foodborne diseases are likely to emerge, driven by factors such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status. There are growing concerns that terrorists could use pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth.

Enteric viruses

Food and waterborne viruses contribute to a substantial number of illnesses throughout the world. Among those most commonly known are hepatitis A virus, rotavirus, astrovirus, enteric adenovirus, hepatitis E virus, and the human caliciviruses consisting of the noroviruses and the Sapporo viruses. This diverse group is transmitted by the fecal-oral route, often by ingestion of contaminated water and food .

Protozoan parasites

Protozoan parasites associated with food and water can cause illness in humans. Although parasites are more commonly found in developing countries, developed countries have also experienced several foodborne outbreaks. Contaminants may be inadvertently introduced to the foods by inadequate handling practices, either on the farm or during processing of foods. Protozoan parasites can be found worldwide, either infecting wild animals or in water and contaminating crops grown for human consumption. The disease can be much more severe and prolonged in immunocompromised individuals.

Mycotoxins

Molds produce mycotoxins, which are secondary metabolites that can cause acute or chronic diseases in humans when ingested from contaminated foods. Potential diseases include cancers and tumors in

different organs (heart, liver, kidney, nerves), gastrointestinal disturbances, alteration of the immune system, and reproductive problems. Species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Claviceps* grow in agricultural commodities or foods and produce the mycotoxins such as aflatoxins.

Yersinia enterocolitica

Yersinia enterocolitica includes pathogens and environmental strains that are ubiquitous in terrestrial and fresh water ecosystems. Evidence from large outbreaks of yersiniosis and from epidemiological studies of sporadic cases has shown that *Y. enterocolitica* is a foodborne pathogen.

Vibrio

Vibrio species are prevalent in estuarine and marine environments, and seven species can cause foodborne infections associated with seafood. *Vibrio cholerae* O1 and O139 serovtypes produce cholera toxin and are agents of cholera.

Staphylococcus aureus

Staphylococcus aureus is a common cause of bacterial foodborne disease worldwide. Symptoms include vomiting and diarrhea that occur shortly after ingestion of *S. aureus* toxin-contaminated food. The symptoms arise from ingestion of preformed enterotoxin, which accounts for the short incubation time. Staphylococcal enterotoxins are superantigens and, as such, have adverse effects on the immune system.

Campylobacter

Campylobacter spp., primarily *C. jejuni* is one of the major causes of bacterial gastroenteritis in the U.S. and worldwide.

Listeria monocytogenes

Listeria monocytogenes is Gram-positive foodborne bacterial pathogen and the causative agent of human listeriosis. *Listeria* infections are acquired primarily through the consumption of contaminated foods, including soft cheese, raw milk, deli salads, and ready-to-eat foods such as luncheon meats and frankfurters.

Salmonella

Salmonella serotypes continue to be a prominent threat to food safety worldwide. Infections are commonly acquired by animal to human transmission though consumption of undercooked food products derived from livestock or domestic fowl.

Shigella

Shigella species are members of the family Enterobacteriaceae and are Gram negative, non-motile rods. Four subgroups exist based on O-antigen structure and biochemical properties: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C) and *S. sonnei* (subgroup D). Symptoms include mild to severe diarrhea with or without blood, fever, tenesmus and abdominal pain. Further complications of the disease may be seizures, toxic megacolon, reactive arthritis and hemolytic uremic syndrome. Transmission of the pathogen is by the fecal-oral route, commonly through food and water.

Escherichia coli

More information is available concerning *Escherichia coli* than any other organism, thus making *E. coli* the most thoroughly studied species in the microbial world. For many years, *E. coli* was considered a commensal of human and animal intestinal tracts with low virulence potential. It is now known that many strains of *E. coli* act as pathogens, inducing serious gastrointestinal diseases and even

death in humans. There are six major categories of *E. coli* strains that cause enteric diseases in humans, including the:

1. enterohemorrhagic *E. coli*, which cause hemorrhagic colitis and hemolytic uremic syndrome,
2. enterotoxigenic *E. coli*, which induce traveler's diarrhea,
3. enteropathogenic *E. coli*, which cause a persistent diarrhea in children living in developing countries,
4. enteroaggregative *E. coli*, which provokes diarrhea in children,
5. enteroinvasive *E. coli* that are biochemically and genetically related to *Shigella* species and can induce diarrhea,
6. diffusely adherent *E. coli*, which cause diarrhea and are distinguished by a characteristic type of adherence to mammalian cells.

Clostridium botulinum* and *Clostridium perfringens

Clostridium botulinum produces extremely potent neurotoxins that result in the severe neuro-paralytic disease, botulism. The enterotoxin produced by *C. perfringens* during sporulation of vegetative cells in the host intestine results in debilitating acute diarrhea and abdominal pain.

Environmental microbiology

Environmental microbiology is the study of the composition and physiology of microbial communities in the environment. The environment in this case means the soil, water, air and sediments covering the planet and can also include the animals and plants that inhabit these areas. Environmental microbiology also includes the study of microorganisms that exist in artificial environments such as bioreactors. An average gram of soil contains approximately one billion (1,000,000,000) microbes representing probably several thousand species. Microorganisms have special impact on the whole biosphere. They are the backbone of ecosystems of the zones where light cannot approach. In such

zones, chemosynthetic bacteria are present which provide energy and carbon to the other organisms there. Some microbes are decomposers which have ability to recycle the nutrients. Microbes have a special role in biogeochemical cycles. Microbes, especially bacteria, are of great importance because their symbiotic relationship (either positive, neutral, or negative) have special effects on the ecosystem.

Microorganisms are used for *in-situ* microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant. Since most sites typically have multiple pollutant types, the most effective approach to microbial biodegradation is to use a mixture of bacterial species and strains, each specific to the biodegradation of one or more types of contaminants. It is vital to monitor the composition of the indigenous and added bacteria in order to evaluate the activity level and to permit modifications of the nutrients and other conditions for optimizing the bioremediation process.

Biodegradation of pollutants

Microbial biodegradation of pollutants plays a pivotal role in the bioremediation of contaminated soil and groundwater sites. Such pollutants include chloroethenes, steroids, organophosphorus compounds.

Oil biodegradation

Petroleum oil is toxic, and pollution of the environment by oil causes major ecological concern. Oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult; much of the oil can, however, be eliminated by the hydrocarbon-degrading activities of microbial communities, in particular the hydrocarbonoclastic bacteria (HCB). These organisms can help remedy the ecological damage caused by oil pollution of marine habitats.

Waste biotreatment

Bio-treatment, the processing of wastes using living organisms, is an environmentally friendly alternative to other options for treating waste material. Bioreactors have been designed to overcome the various limiting factors of bio-treatment processes in highly controlled systems. This versatility in the design of bioreactors allows the treatment of a wide range of wastes under optimized conditions. It is vital to consider various microorganisms and a great number of analyses are often required.

Wastewater treatment

Wastewater treatment processes are geared towards one purpose: cleaning up water. Recent application of molecular techniques is unveiling the microbial composition and architecture of the complex communities involved in the treatment processes. It is now recognized that wastewater processes harbor a vast variety of microorganisms most of which are yet-to-be cultured, hence uncharacterized.

Corynebacteria

Corynebacteria are a diverse group Gram-positive bacteria found in a range of different ecological niches such as soil, vegetables, sewage, skin, and cheese smear. Some, such as *Corynebacterium diphtheriae*, are important pathogens while others, such as *Corynebacterium glutamicum*, are of immense industrial importance. *C. glutamicum* is one of the biotechnologically most important bacterial species with an annual production of more than two million tons of amino acids, mainly L-glutamate and L-lysine.

Legionella

Legionella is common in many environments, with at least 50 species and 70 serogroups identified. *Legionella* is commonly found in aquatic habitats where its ability to survive and to multiply within different protozoa equips the bacterium to be transmissible and pathogenic to humans.

Lactobacillus

Lactobacillus species are found in the environment mainly associated with plant material. They are also found in the gastrointestinal tract of humans, where they are symbiotic and make up a portion of the gut flora.

Aspergillus

Aspergillus spores are common components of aerosols where they drift on air currents, dispersing themselves both short and long distances depending on environmental conditions. When the spores come in contact with a solid or liquid surface, they are deposited and if conditions of moisture are right, they germinate. The ability to disperse globally in air currents and to grow almost anywhere when appropriate food and water are available means that ubiquitous is among the most common adjectives used to describe these moulds.

Microbial nitrogen cycling

Microorganisms that convert gaseous nitrogen (N_2) to a form suitable for use by living organisms are pivotal for life on earth. This process is called nitrogen fixation. Another set of microbial reactions utilise the bioavailable nitrogen creating N_2 and completing the cycle in a process called denitrification. This crucial nutrient cycle has long been the subject of extensive research.

Rhizobia

Symbiotic nitrogen fixation is a mutualistic process in which bacteria reside inside plants and reduce atmospheric nitrogen to ammonia. This ammonia can then be used by the plant for the synthesis of proteins and other nitrogen-containing compounds such as nucleic acids. The Gram-negative soil bacteria that carry out this process are collectively referred to as rhizobia (from the Greek words Riza = Root and Bios = Life).

Microalgae

Algae are a highly diverse group of protists, ranging from simple, unicellular organisms to complex, multicellular entities with a range of differentiated tissues and distinct organs. They are found among diverse aquatic ecosystems and play important roles by supplying carbon and energy as well as providing habitat to other members of the biological communities. Some algae cause significant environmental and health problems.

Anaerobic protozoa

Diplomonads are a group of mitochondrion-lacking, binucleated flagellates found in anaerobic or micro-aerophilic environments. Most research on diplomonads has focused on *Giardia*, which is a major cause of water-borne enteric disease in humans and other animals.

Water microbiology

An adequate supply of safe drinking water is one of the major prerequisites for a healthy life, but waterborne diseases are still a major cause of death in many parts of the world, particularly in young children, the elderly, or those with compromised immune systems. As the epidemiology of waterborne diseases is changing, there is a growing global public health concern about new and reemerging infectious diseases that are occurring through a complex interaction of social, economic, evolutionary, and ecological factors. An important challenge is therefore the rapid, specific and sensitive detection of waterborne pathogens. Presently, microbial tests are based essentially on time-consuming culture methods. However, newer enzymatic, immunological and genetic methods are being developed to replace and/or support classical approaches to microbial detection. Moreover, innovations in nanotechnology and nanosciences are having a significant impact in biodiagnostics, where a number of nanoparticle-based assays and nanodevices have been introduced for biomolecular detection. Molecular techniques based on genomics, proteomics and transcriptomics are rapidly growing as

complete microbial genome sequences are becoming available, and advances are made in sequencing technology, analytical biochemistry, microfluidics and data analysis. While the clinical and food industries are increasingly adapting these techniques, there appear to be major challenges in detecting health-related microbes in source and treated drinking waters. This is due in part to the low density of pathogens in water, necessitating significant processing of large volume samples. From the vast panorama of available molecular techniques, some are finding a place in the water industry: Quantitative PCR, protein detection and immunological approaches, loop-mediated isothermal amplification (LAMP), microarrays.

Soil microbiology

Soil microbiology is the study of organisms in soil, their functions, and how they affect soil properties. It is believed that between two to four billion years ago, the first ancient bacteria and microorganisms came about in Earth's primitive seas. These bacteria could fix nitrogen, in time multiplied and as a result released oxygen into the atmosphere. This release of oxygen led to more advanced microorganisms. Microorganisms in soil are important because they affect the structure and fertility of different soils. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae, and protozoa. Each of these groups has different characteristics that define the organisms and different functions in the soil it lives in.

Bacteria

Bacteria are the smallest organisms in the soil and are the only soil microorganisms that are prokaryotic. All of the other microorganisms are eukaryotic, which means they have a more advanced cell structure with internal organelles and the advanced ability to reproduce sexually. A prokaryote has a very simple cell structure with no internal organelles. Bacteria are the most abundant microorganisms

in the soil, and serve many important purposes, one of those being nitrogen fixation among other biochemical processes.

Biochemical processes

One of the most distinguished features of bacteria as a whole is their biochemical versatility. A species called *Pseudomonas* can metabolize a wide range of chemicals and fertilizers. In contrast, another species known as *Nitrobacters* can only derive its energy by turning nitrite into nitrate, which results in a gain of oxygen and is known also as oxidation. Furthermore, the species *Clostridium* is also an example of bacteria's versatility because it, unlike most species, can actually grow in the absence of oxygen.

Nitrogen fixation

Bacteria are responsible for the process of nitrogen fixation, which is the conversion of atmospheric nitrogen into nitrogen which can be used by plants to uptake. Autotrophic bacteria, or bacteria that derives its energy making its own food by oxidation, like the *Nitrobacters* species, rather than feeding on plants or other organisms. The bacteria that are autotrophic are responsible for nitrogen fixation.

Actinomycetes

Actinomycetes are soil microorganisms. They are a type of bacteria. They are similar to both bacteria and fungi, and have characteristics linking them to both groups. Actinomycetes are often believed to be the missing evolutionary link between bacteria and fungi, but they have many more characteristics in common with bacteria than they do fungi. One of the most notable characteristics of the actinomycetes is their ability to produce antibiotics. Streptomycin, neomycin, erythromycin and tetracycline are only a few examples of the antibiotics derived from actinomycetes. Streptomycin is used to treat tuberculosis and infections caused by certain bacteria and neomycin is used to reduce the risk of

bacterial infection during surgery. Erythromycin is a very important antibiotic that is used to treat certain infections caused by bacteria, such as bronchitis; pertussis (whooping cough); pneumonia; and ear, intestine, lung, urinary tract, and skin infections. This ability to produce these useful antibiotics is the basis of our entire pharmaceutical industry and has saved human lives.

Fungi

Next to bacteria, fungi are abundant in soil population compared to other microorganisms. Fungi are important in the soil as food sources for other, larger organisms, pathogens, beneficial symbiotic relationships with plants or other organisms and help to reduce crop residues and biochemically process nutrients to improve the soil they inhabit. Fungi can be split into different species based on primarily on the size, shape and color of their spores, which are used to reproduce.

Algae

Algae can make its own nutrients through a process known as photosynthesis. Photosynthesis is when light energy is converted to chemical energy that can be stored as nutrients. For algae to grow, it must be exposed to areas of light because photosynthesis requires light, so algae is typically distributed evenly wherever sunlight and moderate moisture is available. Algae, however, do not have to be on the soil surface or directly exposed to sun rays, but it can live below the soil surface as long as the algae has uniform temperature and moisture conditions. Bacteria are not the only organism that can fix nitrogen, because algae are capable of performing nitrogen fixation as well.

Protozoa

Protozoa are eukaryotic organisms which are some of the first microorganisms to develop a means of sexual reproduction, which is a huge evolutionary step from duplication of spores, like many of the other soil microorganisms depend on. Protozoa can be split up into three categories: flagellates,

amoebae, and ciliates. Flagellates are the smallest members of the protozoa group, and can be divided further based on whether they can participate in photosynthesis. Amoebae are larger than flagellates and move in a different way. Ciliates are the largest of the protozoa group

It is important to understand the many different groups and species of microorganisms in different soils because they affect so much of the soil. Microorganisms contribute to nutrient availability in soil, manage soil stability by means of different biochemical processes such as nitrogen fixation, and they contribute to the growth and success of the plants and overall ecosystem of a soil environment.

Industrial microbiology

Industrial microbiology or microbial biotechnology encompasses the use of microorganisms in the manufacture of food or industrial products. The use of microorganisms for the production of food, either human or animal, is often considered a branch of food microbiology. The microorganisms used in industrial processes may be natural isolates, laboratory selected mutants or genetically engineered organisms.

Antibiotics

Industrial microbiology is perhaps best known for its development of antibiotics and pharmaceutical agents. Penicillin, streptomycin, and a host of other antimicrobial agents originated from industrial microbiology in the 1950s and 1960s.

Food microbiology

Yogurt, cheese, chocolate, and silage (animal food) are all produced by industrial microbiology processes. Beneficial bacteria such as probiotics are becoming increasingly important in the food industry. Lactic acid bacteria and *Bifidobacteria* are amongst the most important groups of microorganisms used in the food industry. These bacteria are thought to have health-promoting

abilities and many are used as probiotics for the prevention, alleviation and treatment of intestinal disorders in humans and animals.

Biopolymers

A huge variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are produced by microorganisms. These products range from viscous solutions to plastics. The genetic manipulation of microorganisms has permitted the biotechnological production of biopolymers with tailored material properties suitable for high-value medical application such as tissue engineering and drug delivery. Industrial microbiology can be used for the biosynthesis of xanthan, alginate, cellulose, cyanophycin, poly(gamma-glutamic acid), levan, hyaluronic acid, organic acids, oligosaccharides and polysaccharides, and polyhydroxyalkanoates.

Bioremediation

Microbial biodegradation of pollutants can be used to clean up contaminated environments. These bioremediation and biotransformation methods harness naturally occurring microbes to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals.

Bioremediation of mercury

Mercury is a heavy metal with extreme toxicity, the ability to biomagnify, and long range atmospheric transport of its gaseous form. Past and present industrial uses of mercury have resulted in the pollution of soils, groundwater, rivers and marine ecosystems worldwide, the clean-up of which, using standard technology, is either not feasible or is prohibitively costly. A low cost and environmentally friendly alternative is bioremediation.

Waste bio-treatment

Microorganisms are used to treat the vast quantities of wastes generated by modern societies. Biotreatment, the processing of wastes using living organisms, is an environmentally friendly, relatively simple and cost-effective alternative to physico-chemical clean-up options. Confined environments, such as bioreactors can be employed in biotreatment processes.

Wastewater treatment

Biological wastewater treatment is undoubtedly one of the most important biotechnological processes, which have been used for over a century to treat municipal and industrial wastewaters. Culture-independent molecular techniques have been used to study the diversity and physiology of ecologically important microorganisms in wastewater treatment processes. Microbes play a vital role in the cycling of nitrogen in wastewater treatment processes (including anaerobic ammonia oxidation processes) and methane fermentation processes.

Health-care and medicine

Microorganisms are used to produce human or animal biologicals such as insulin, growth hormone, and antibodies. Diagnostic assays that use monoclonal antibody, DNA probe technology or real-time PCR are used as rapid tests for pathogenic organisms in the clinical laborarory. Microorganisms may also help in the treatment of diseases such as cancer. Research shows that *Clostridium* can selectively target cancer cells. Various strains of non-pathogenic *Clostridium* have been shown to infiltrate and replicate within solid tumous. *Clostridium* therefore have the potential to deliver therapeutic proteins to tumors. *Lactobacillus* spp. and other lactic acid bacteria possess numerous potential therapeutic properties including anti-inflammatory and anti-cancer activities. Vaccines are used to combat infectious disease, however the last decade has witnessed a revolution in the approach to vaccine design and development. Sophisticated technologies such as genomics, proteomics, functional

genomics, and synthetic chemistry can be used for the rational identification of antigens, the synthesis of complex glycans, and the generation of engineered carrier proteins.

Archaea

Examination of microbes living in unusual environments (e.g. high temperatures, salt, low pH or temperature, high radiation) lead to discovery of microbes with new abilities that can be harnessed for industrial purposes.

Corynebacteria

Corynebacteria are a diverse group Gram-positive bacteria found in a range of different ecological niches such as soil, vegetables, sewage, skin, and cheese smear. *Corynebacterium glutamicum* is of immense industrial importance and is one of the biotechnologically most important bacterial species with an annual production of more than two million tons of amino acids, mainly L-glutamate and L-lysine. The genome sequence of *C. glutamicum* has been published.

Xanthomonas

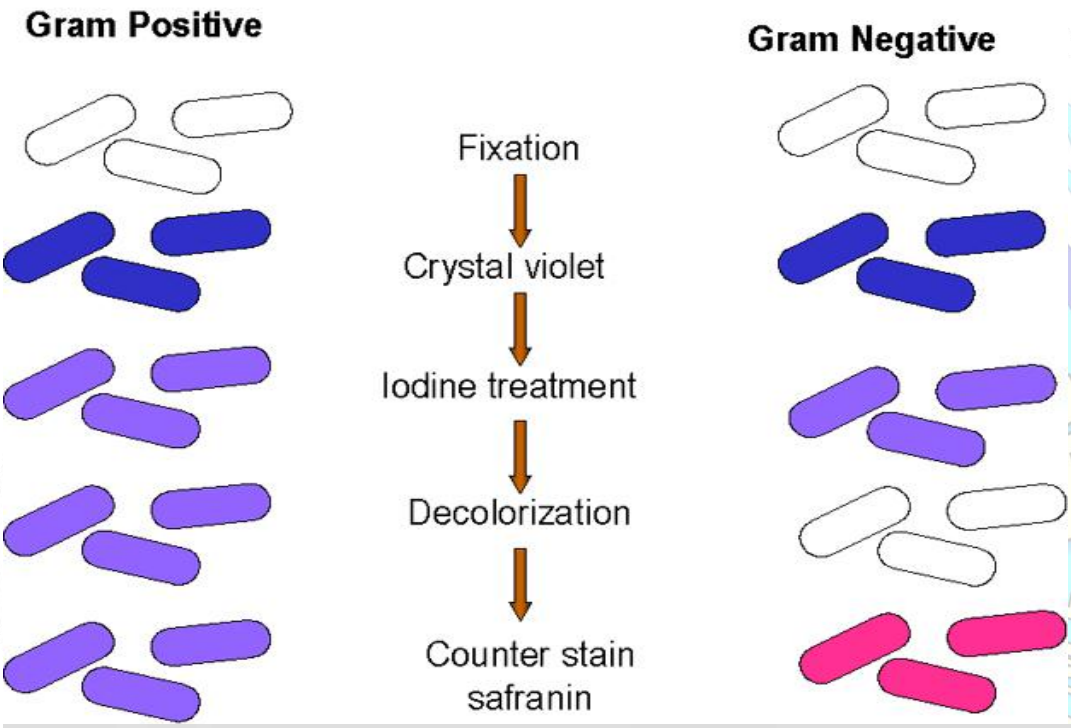
Bacteria of the genus *Xanthomonas* are able to produce the acidic exopolysaccharide xanthan gum. Because of its physical properties, it is widely used as a viscosifer, thickener, emulsifier or stabilizer in both food and non-food industries.

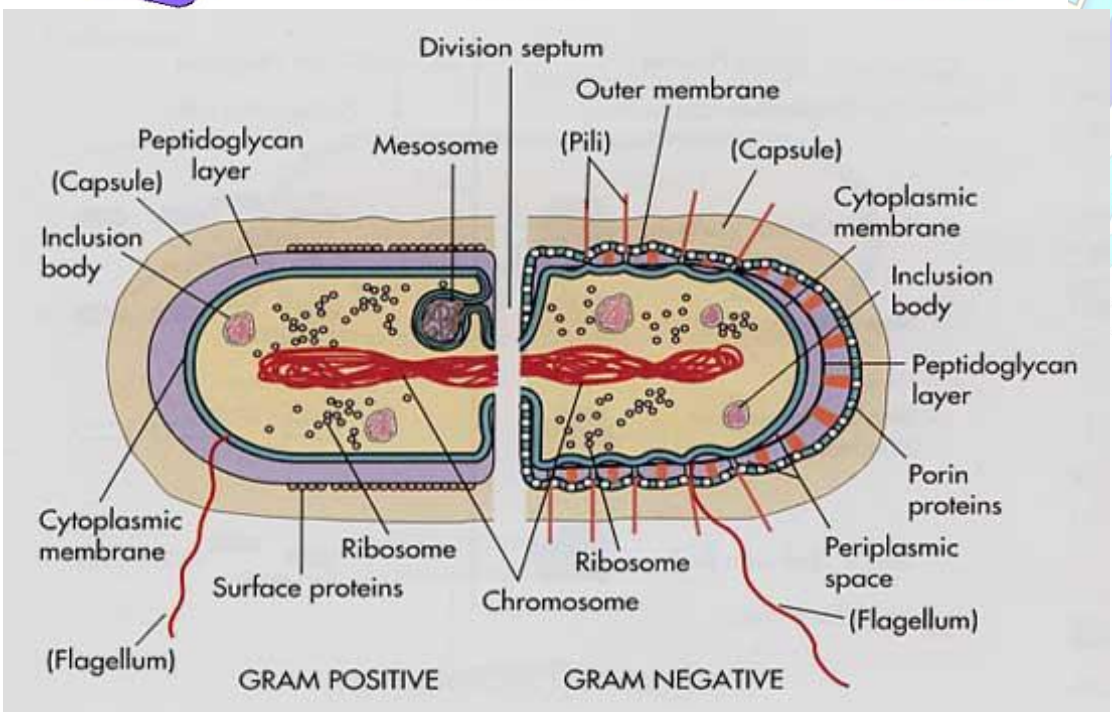
Aspergillus

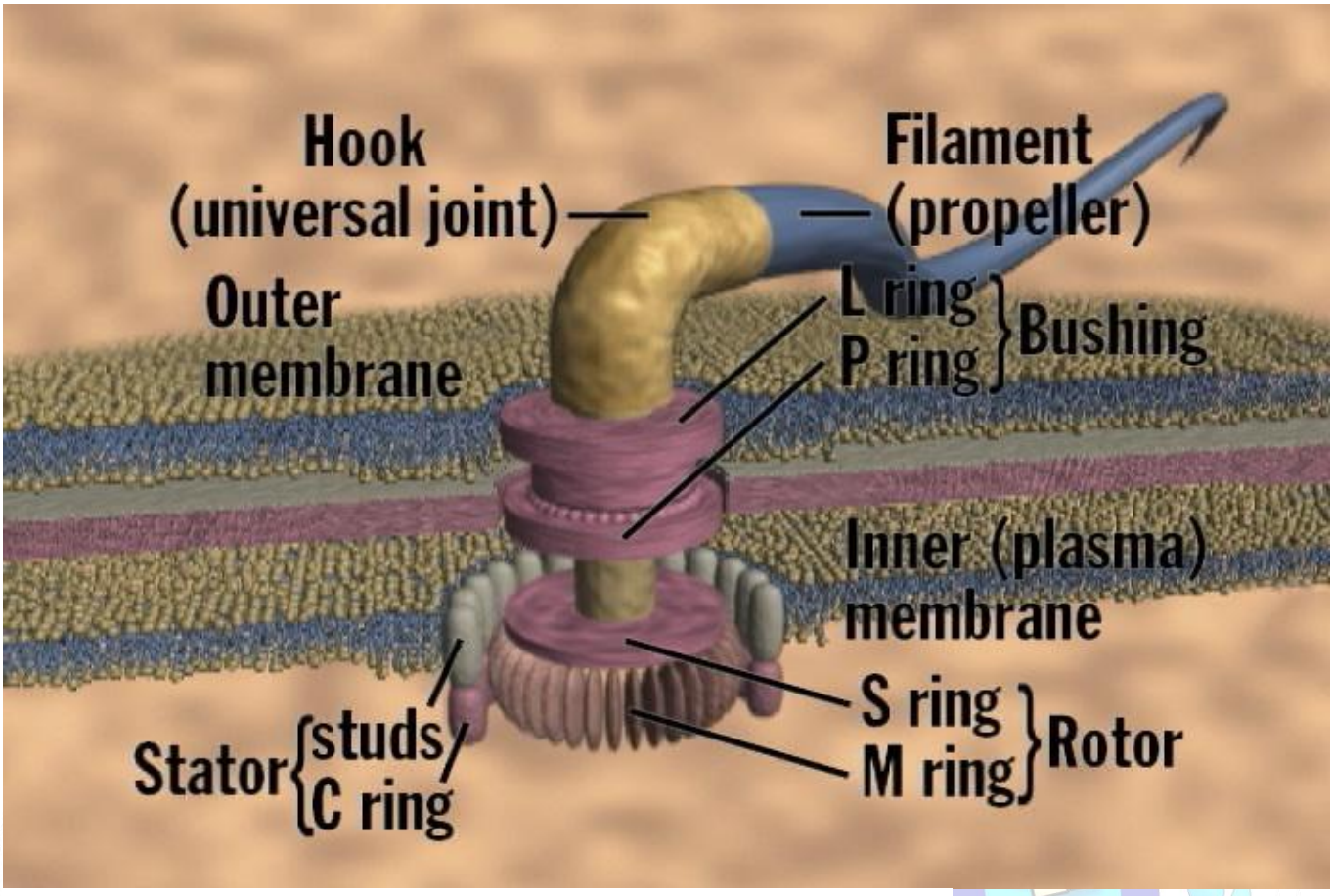
Species within the genus *Aspergillus* have a large chemical repertoire. Commodity products produced in *Aspergillus* cell 'factories' include citric, gluconic, itaconic, and kojic acid. The use of *Aspergillus niger* in citric acid production dates back to 1917. Citric acid is one of the most widely used food ingredients. It also has found use in the pharmaceutical and cosmetic industries .

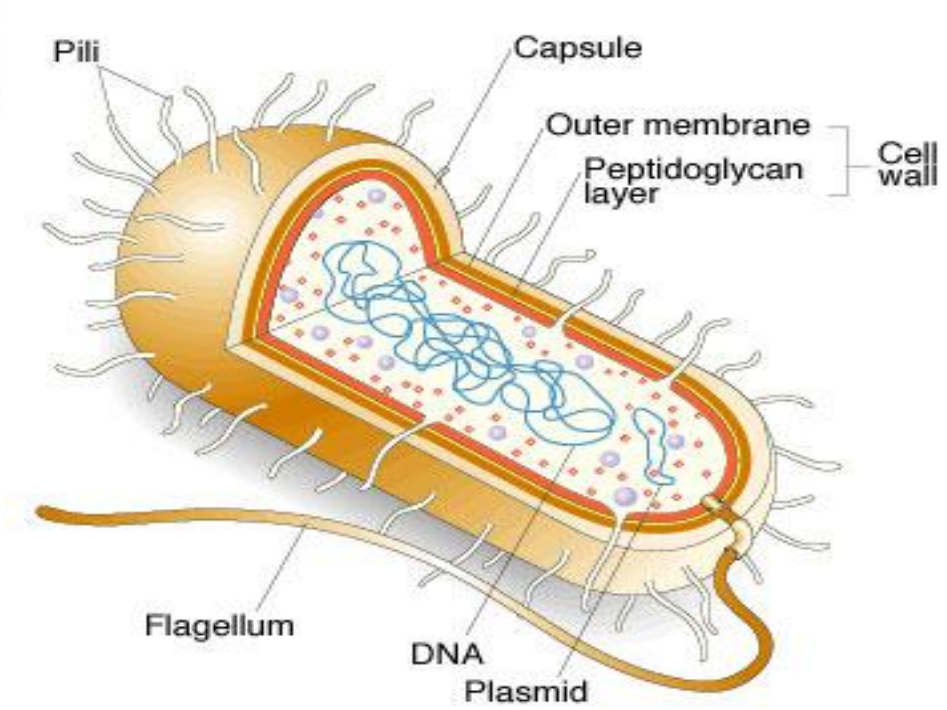
Viruses

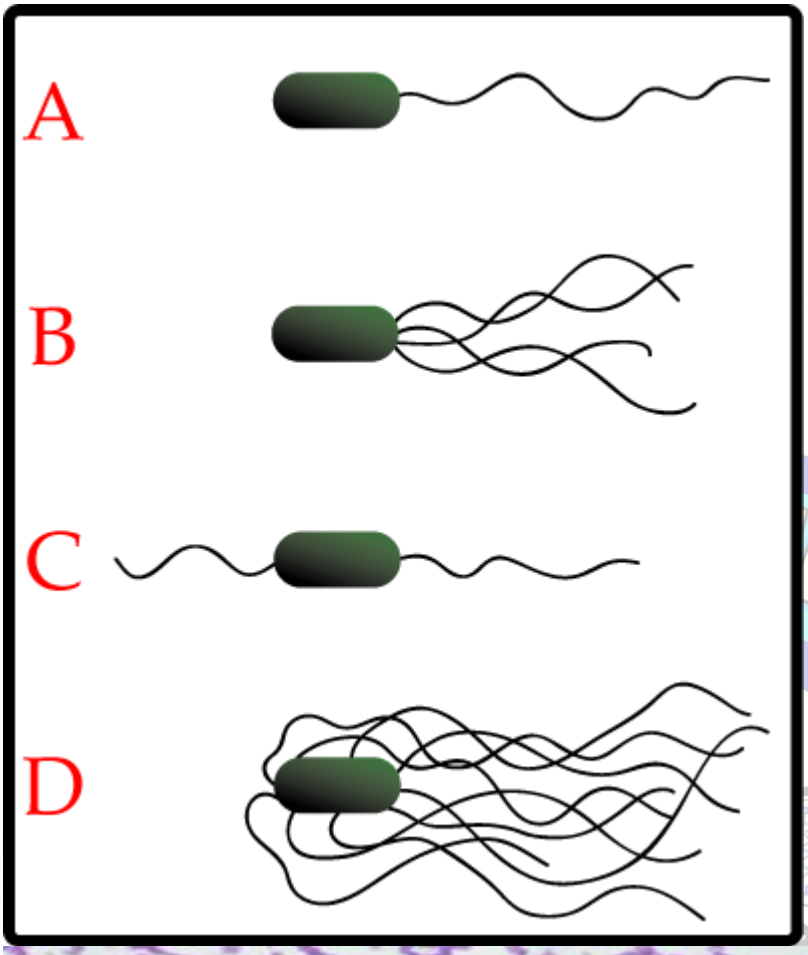
Viruses that are pathogenic to insect pests can be exploited as biological control agents. Some viruses such as baculoviruses have been exploited for use as gene expression and delivery vectors in both insect and mammalian cells.

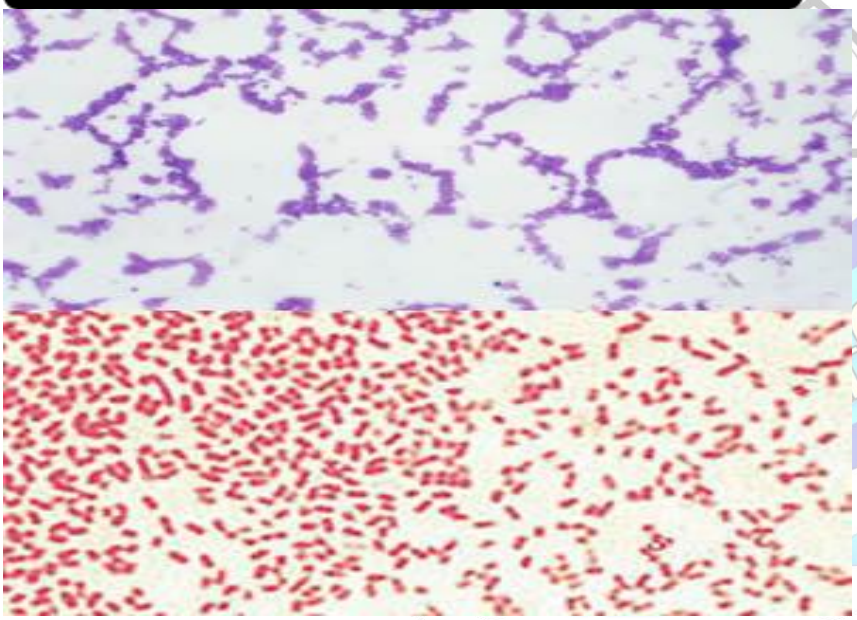


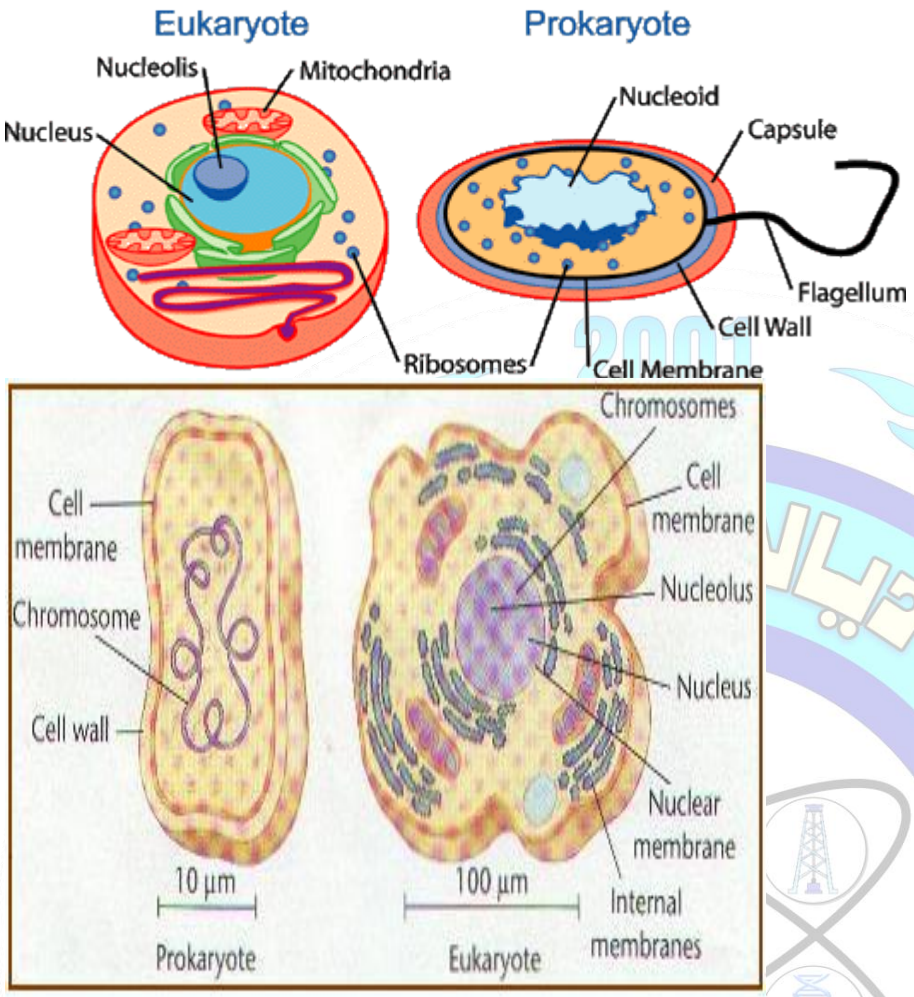


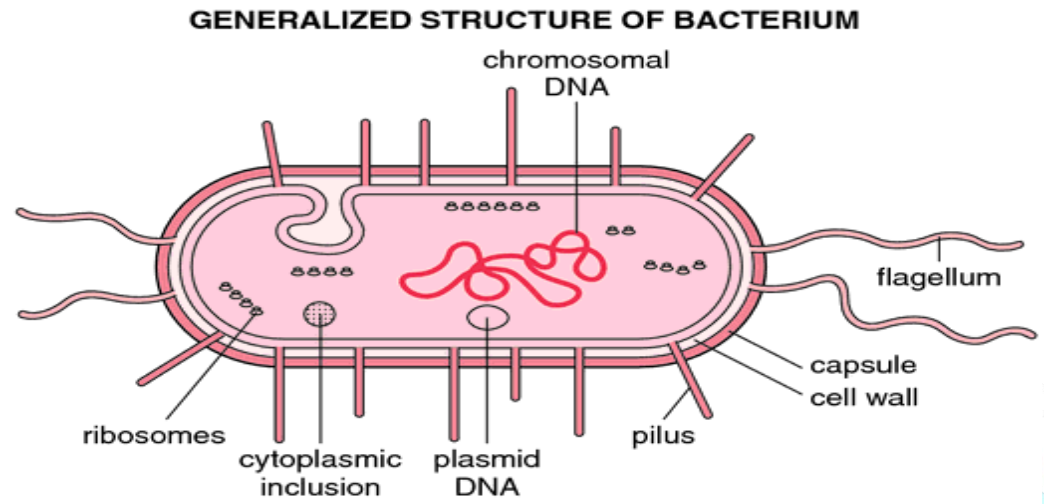

Hook
(universal joint)
Filament
(propeller)
Outer
membrane
L ring
P ring
Bushing
Inner (plasma)
membrane
S ring
M ring
Rotor
Stator
studs
C ring

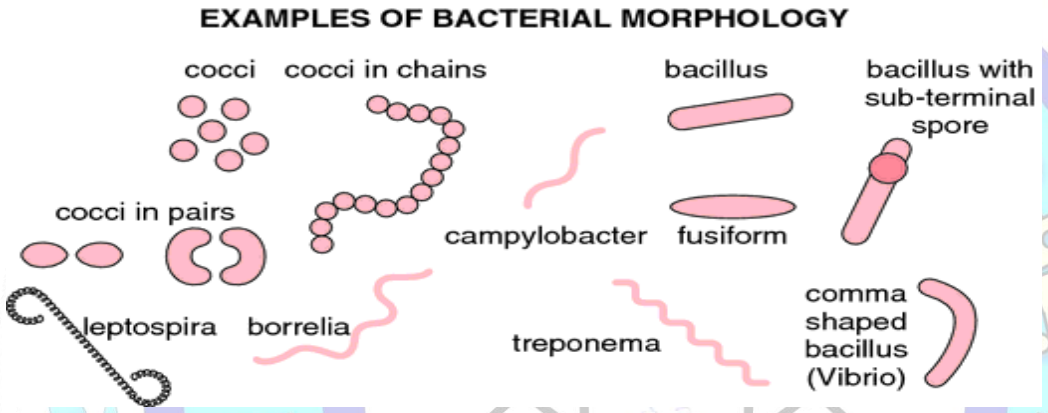

Pili
Capsule
Outer membrane
Peptidoglycan layer
Cell wall
Flagellum
DNA
Plasmid

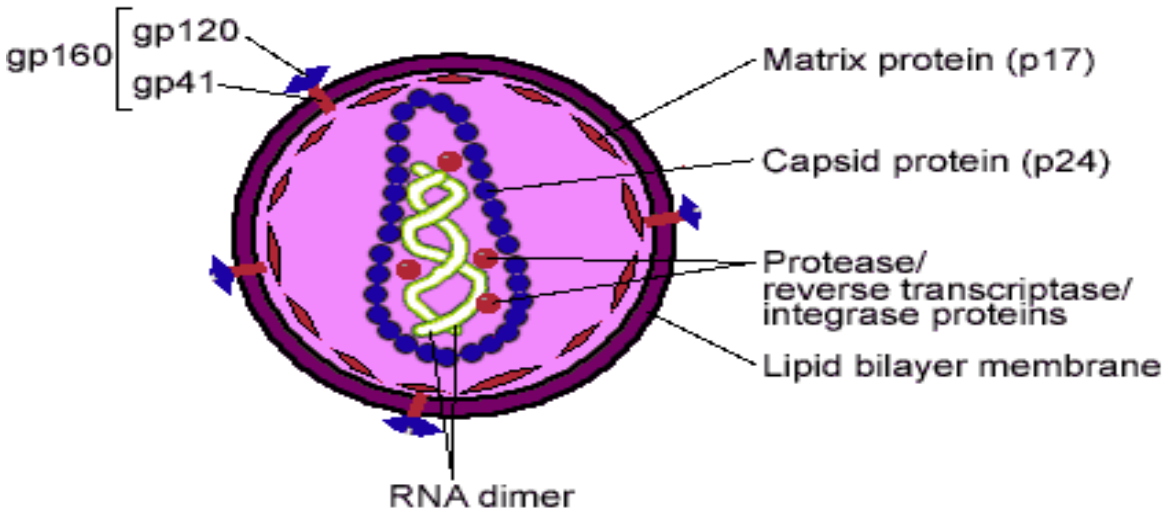

A
B
C
D



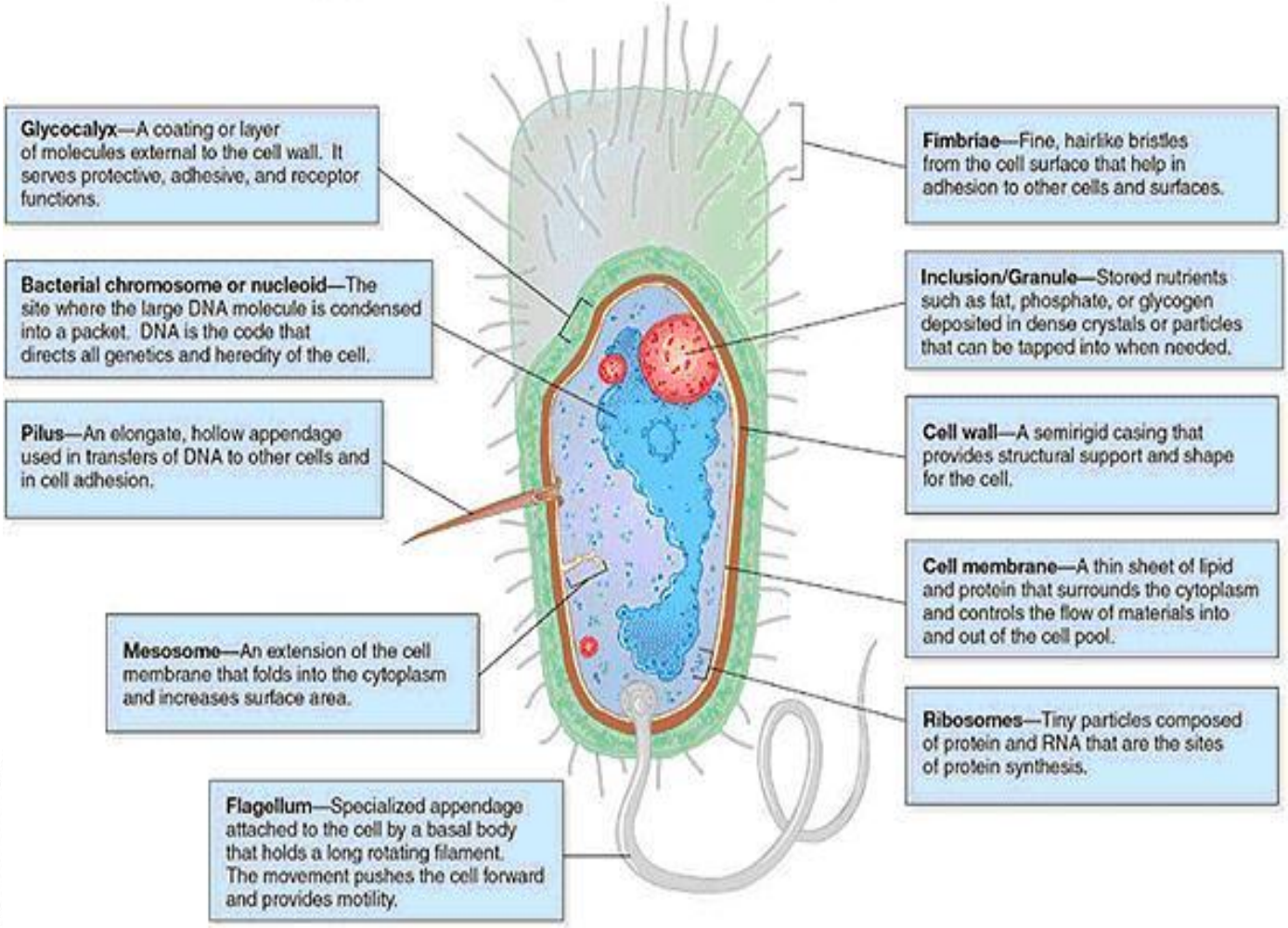

Eukaryote
Prokaryote
Nucleolis
Mitochondria
Nucleus
Nucleoid
Capsule
Flagellum
Cell Wall
Ribosomes
Cell Membrane
Chromosomes
Cell membrane
Nucleolus
Nucleus
Nuclear membrane
Internal membranes
Cell membrane
Chromosome
Cell wall
10 µm
100 µm
Prokaryote
Eukaryote

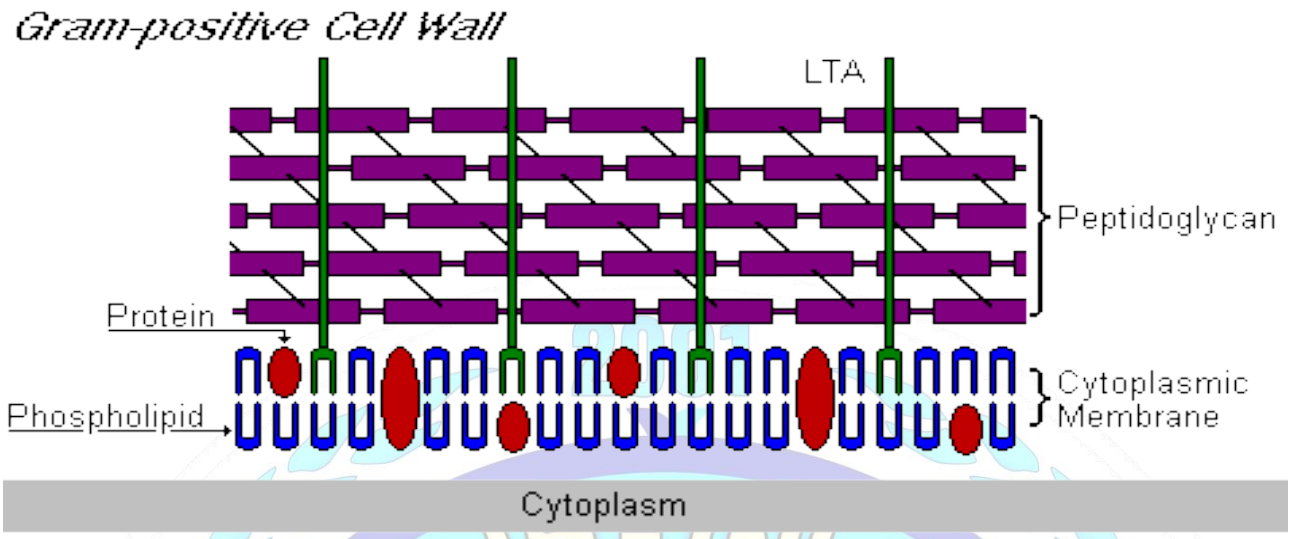


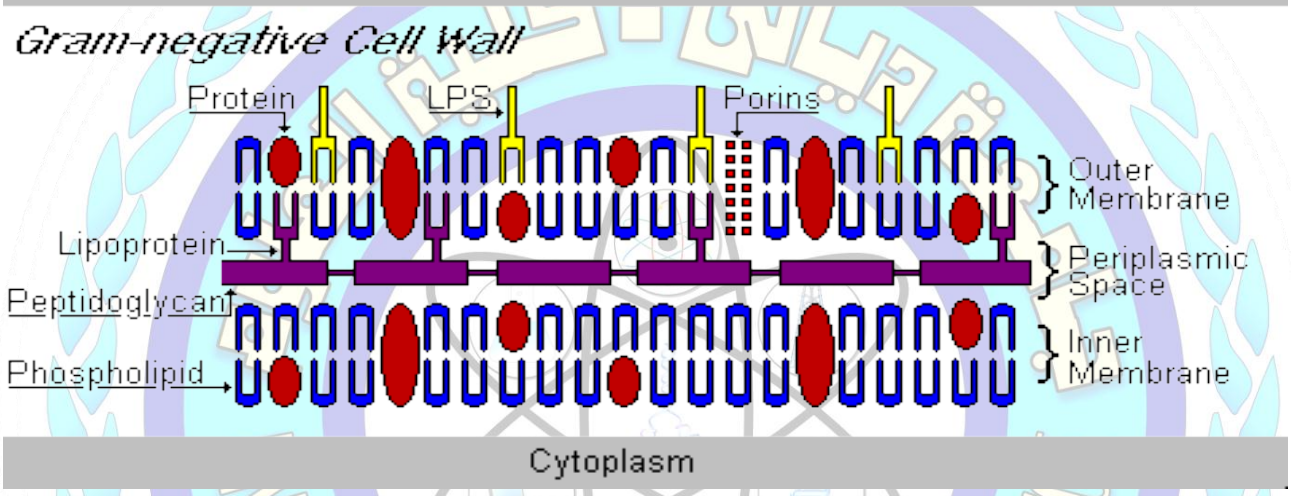


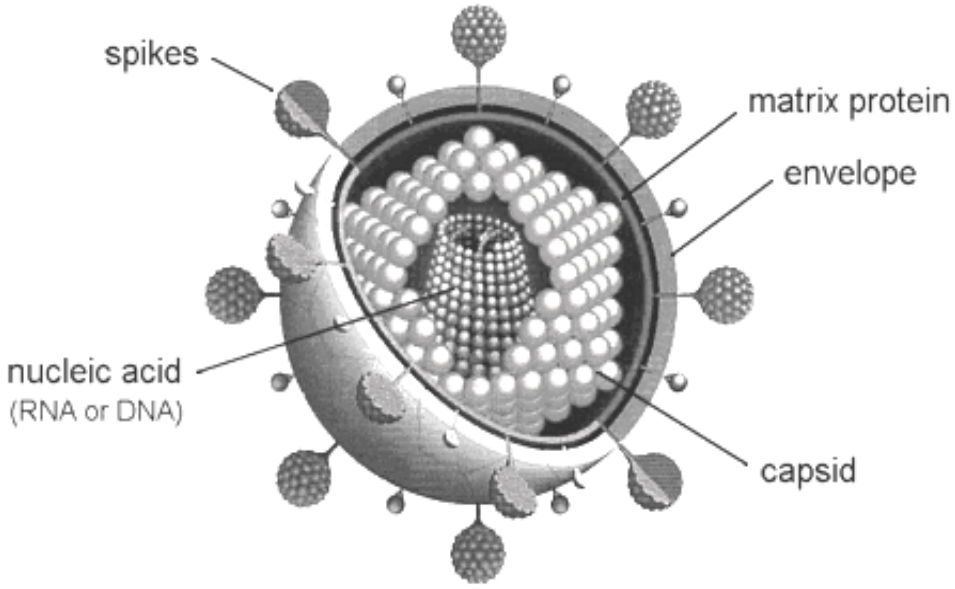


virus particle (ex: HIV)

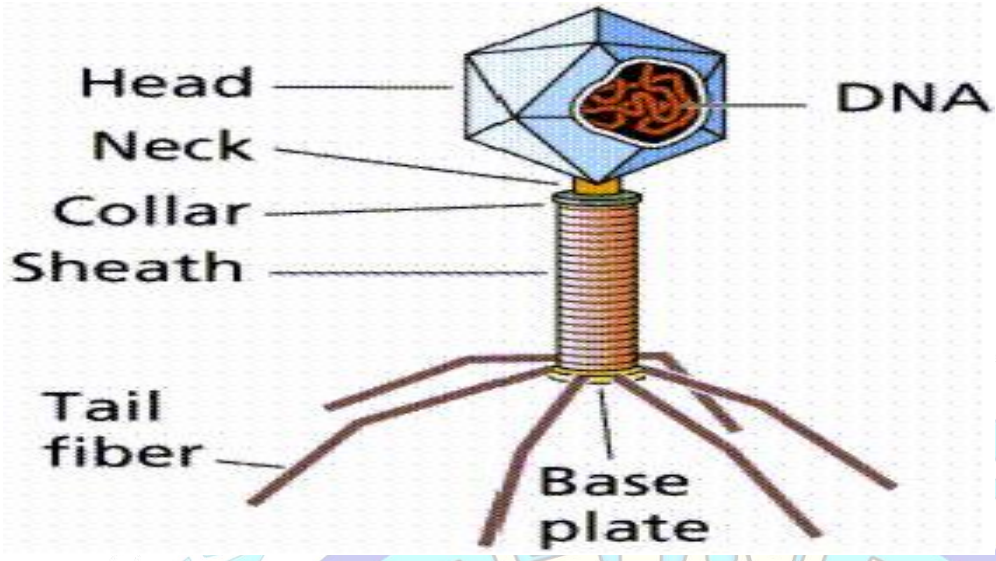

Glycocalyx—A coating or layer of molecules external to the cell wall. It serves protective, adhesive, and receptor functions.
Bacterial chromosome or nucleoid—The site where the large DNA molecule is condensed into a packet. DNA is the code that directs all genetics and heredity of the cell.
Pilus—An elongate, hollow appendage used in transfers of DNA to other cells and in cell adhesion.
Mesosome—An extension of the cell membrane that folds into the cytoplasm and increases surface area.
Flagellum—Specialized appendage attached to the cell by a basal body that holds a long rotating filament. The movement pushes the cell forward and provides motility.
Fimbriae—Fine, hairlike bristles from the cell surface that help in adhesion to other cells and surfaces.
Inclusion/Granule—Stored nutrients such as fat, phosphate, or glycogen deposited in dense crystals or particles that can be tapped into when needed.
Cell wall—A semirigid casing that provides structural support and shape for the cell.
Cell membrane—A thin sheet of lipid and protein that surrounds the cytoplasm and controls the flow of materials into and out of the cell pool.
Ribosomes—Tiny particles composed of protein and RNA that are the sites of protein synthesis.



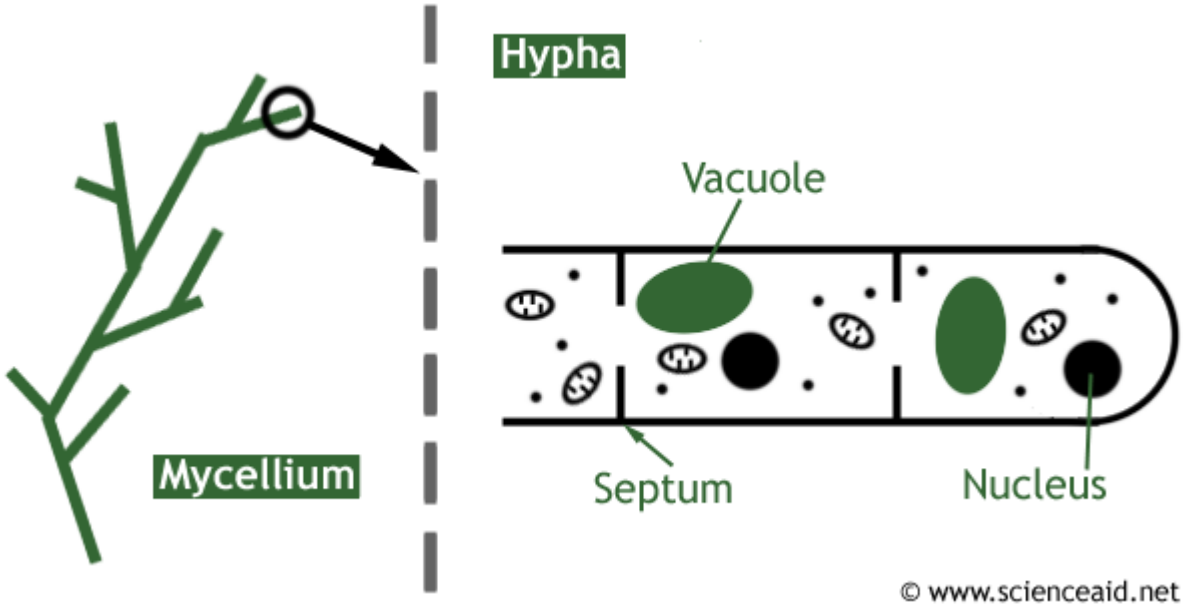


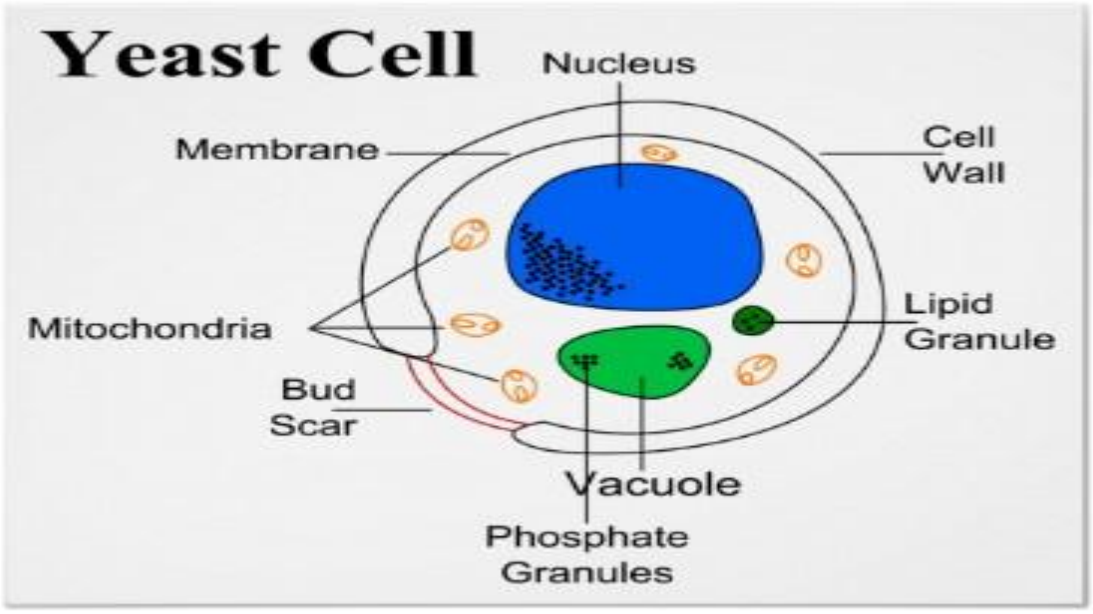


virus particle



Bacteriophage




Yeast Cell
Nucleus
Membrane
Cell Wall
Mitochondria
Lipid Granule
Bud Scar
Vacuole
Phosphate Granules