Lec.1 Introduction to Microbiology

- Microbiology, the study of microscopic organisms, derived its name from three Greek words: *mikros* ("small"), *bios* ("life"), and *logos* ("science"). Taken together they mean the study of microorganisms (MOs) which are very small and cannot be seen by unaided eye. They are generally 1 millimeter (mm) or less in diameter.
- MOs include organisms such as protozoa, algae, fungi, bacteria and virus (which are not technically classified as living organisms but do contain genetic material).
- MOs are present in vast numbers everywhere on the bodies of animals and humans, on plant surfaces, in the air, food, water, dust, soil, and even inside the intestinal canal of all insects, birds, animals and human beings.
- The MOs that live inside and on humans (normal microbiota) are estimated to outnumber human cells by a factor of 10.
- Some of these MOs are harmful (disease causing MOs), others benefit by association with biological activity of the host.
- Microbiology research encompasses all aspects of these MOs such as their behavior, evolution, ecology, biochemistry, and physiology, along with the pathology of diseases that they cause.

Our goal in this lecture is to introduce you to this amazing group of organisms and to outline the history of their evolution and discovery.

History of Microbiology

Pre-microbiology

The possibility that microorganisms existed was discussed for many centuries before their actual discovery in the 17th century.

- In 1546, Girolamo Fracastoro proposed that epidemic diseases were caused by transferable seed-like entities that could transmit infection by direct or indirect contact or even without contact over long distances.

Microorganisms were not described correctly until the 17th century. Why?

The reason for this was that all these early studies lacked the microscope.

The Microscope and Discovery of Microorganisms

- **Antonie van Leeuwenhoek (1632–1723)** was one of the first people to observe microorganisms, using a microscope of his own design, and made one of the most important contributions to biology.

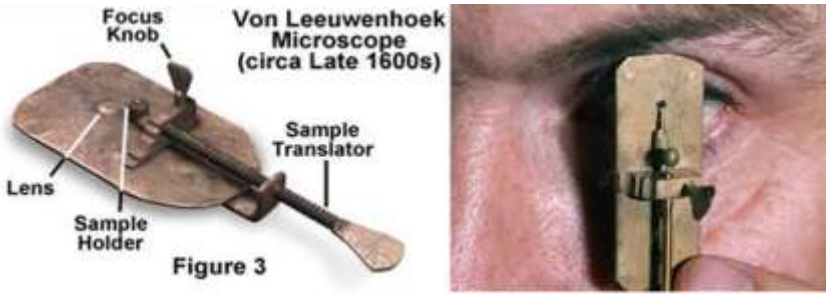
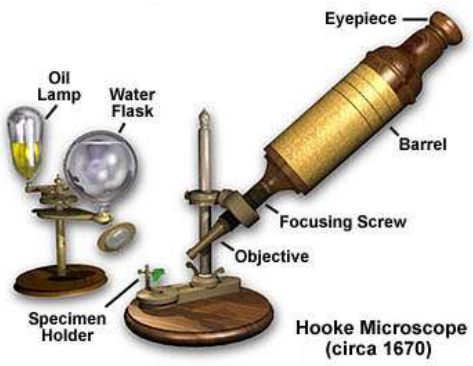
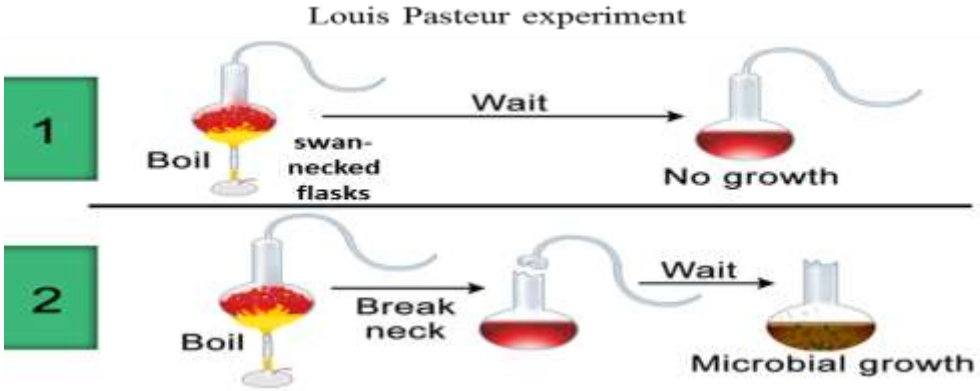


Figure 3

- **Robert Hooke** was the first to use a microscope to observe living things. Hooke's 1665 book, *Micrographia*, contained descriptions of plant cells. Before Van Leeuwenhoek's discovery of microorganisms in 1675, it had been a mystery why grapes could be turned into wine, milk into cheese, or why food would spoil. Van Leeuwenhoek did not make the connection between these processes and microorganisms, but using a microscope, he did establish that there were forms of life that were not visible to the naked eye. Van Leeuwenhoek's discovery, along with subsequent observations by Spallanzani and Pasteur, ended the long-held belief that life spontaneously appeared from non-living substances during the process of spoilage.



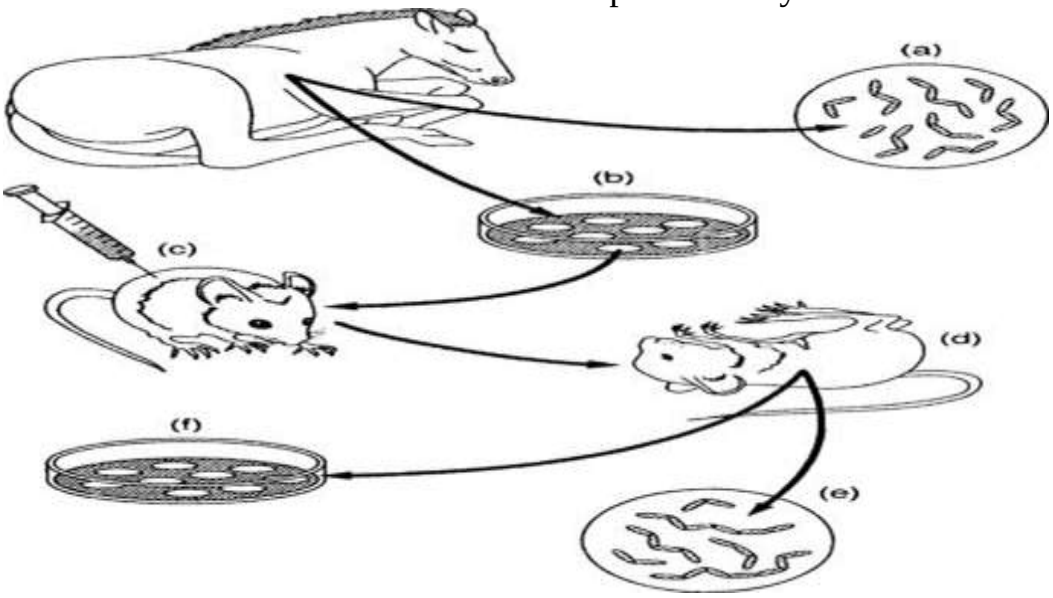
- **Lazzaro Spallanzani (1729–1799)** found that boiling broth would sterilize it and kill any microorganisms in it. He also found that new microorganisms could settle only in a broth if the broth was exposed to the air.
- **Louis Pasteur (1822–1895) (father of biotechnology)** expanded upon Spallanzani's findings by exposing boiled broths to the air in vessels that contained a filter to prevent all particles from passing through to the growth medium. He also did this in vessels with no filter at all, with air being admitted via a curved tube (swan-necked flasks) that prevented dust particles from coming in contact with the broth. By boiling the broth beforehand, Pasteur ensured that no microorganisms survived within the broths at the beginning of his experiment. Nothing grew in the broths in the course of Pasteur's experiment. This meant that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth. Thus, Pasteur dealt the death blow to the theory of spontaneous generation and supported germ theory instead. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame.



- **In the 1860's, an English surgeon, Joseph Lister** showed the role of MO in the wound contamination, and developed Lister system which came to be known as Antiseptic Surgery, which includes the heat sterilization of instruments and the application of phenol to wound by means of dressings.
- **Ferdinand Julius Cohn (1828 –1898)** was a German biologist. His classification of bacteria into four groups based on shape (sphericals, short rods, threads, and spirals) is still in use today.
- **In 1876, Robert Koch (1843–1910)** established that microbes can cause disease. He found that the blood of cattle who were infected with anthrax always had large numbers of *Bacillus anthracis*. Koch found that he could transmit anthrax from one animal to another by taking a small sample of blood from the infected animal and injecting it into a healthy one, and this caused the healthy animal to become sick. He also found that he could grow the bacteria in a nutrient broth, then inject it into a healthy animal, and cause illness. Based on these experiments, he devised criteria for establishing a causal link between a microbe and a disease and these are now known as Koch's postulates. Although these postulates cannot be applied in all cases, they do retain historical importance to the development of scientific thought and are still being used today.

  Koch postulates:

  1. The suspected causative agent must be found in every case of disease.
  2. This MO must be isolated from the infected individual and grown in a culture with no other types of MO.
  3. When inoculation into normal healthy susceptible animal a pure culture of the agent must be producing the specific disease.
  4. The same MO must be isolated from the experimentally infected host.



The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

- **Paul Ehrlich (1909)** by him chemotherapy was introduced and the modern era of control treatment began with the use of chemicals that would kill or interfere with the growth of the disease agent without damaging the infected individual.

- **In 1929, Alexander Fleming** isolated a mold produced substance that inhibited bacteria but was nontoxic to lab animal. He named this antibacterial material Penicillin, which is one type of antibiotics. Up to data, many new approaches and techniques are developing that aid in the isolation, treatment, controlling, and prevention of infectious disease.
- Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the electron microscope was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

Key Points

- Van Leeuwenhoek is largely credited with the discovery of microbes, while Hooke is credited as the first scientist to describe live processes under a microscope.
- Spallanzani and Pasteur performed several experiments to demonstrate that microbial life does not arise spontaneously.
- Cohn laid the groundwork for discovering and cataloging microbes, while Koch conclusively showed that microbes can cause diseases.
- Clearly, these early microbiologists not only disproved spontaneous generation but also contributed to the rebirth of microbiology. They developed liquid media for culturing microbes. They also developed methods for sterilizing media and maintaining their sterility. These techniques were next applied to understanding the role of microorganisms in disease.
- **In the late 1800s and for the first decade of the 1900s**, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age of Microbiology** during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.
- **19th Century**, widespread use of the compound microscope and the development of staining techniques in order to better visualize microorganisms. In addition, people began to realize that microorganisms could cause disease, and did experiments on immunity.
- **20th Century,** was a time of great advancement for all forms of science, including microbiology. The first vaccines and antibiotics were developed, and the first chemotherapeutic agents were used to treat bacterial diseases such as syphilis. Deoxyribonucleic acid (DNA) was discovered to be the genetic material of the cell, which opened up the field of genetics research and allowed more recently for sequencing the genomes of microorganisms.

Timeline

1600 1700 1800 1900

1677 Observed "little animals" (Antony Leeuwenhoek)

1796 First scientific Small pox vaccination (Edward Jenner)
1850 Advocated washing hands to stop the spread of disease (Ignaz Semmelweis)
1861 Disproved spontaneous generation (Louis Pasteur)
1862 Supported Germ Theory of Disease (Louis Pasteur)
1867 Practiced antiseptic surgery (Joseph Lister)
1876 First proof of Germ Theory of Disease with *B. anthracis* discovery (Robert Koch)
1881 Growth of Bacteria on solid media (Robert Koch)
1882 Outlined Kochs postulates (Robert Koch)
1882 Developed acid-fast Stain (Paul Ehrlich)
1884 Developed Gram Stain (Christian Gram)
1885 First Rabies vaccination (Louis Pasteur)
1887 Invented Petri Dish (R.J. Petri)
1892 Discovered viruses (Dmitri Iosifovich Ivanovski)
1899 Recognized viral dependence on cells for reproduction (Martinus Beijerinck)
1900 Proved mosquitoes carried the yellow fever agent (Walter Reed)
1910 Discovered cure for syphilis (Paul Ehrlich)
1928 Discovered Penicillin (Alexander Fleming)
1977 Developed a method to sequence DNA (W. Gilbert & F. Sanger)
1983 Polymerase Chain Reaction invented (Kary Mullis)
1995 First microbial genomic sequence published (*H. influenzae*) (TIGR)

Microbiology can be broadly classified as:

1. **Pure Microbiology;** is exploratory and conducted in order to better understand a scientific phenomenon.
2. **Applied Microbiology;** is based on information gleaned from pure research and used to answer specific questions or solve problems. Applied Microbiology has many branches such as; medical microbiology, industrial microbiology, pharmaceutical microbiology, microbial biotechnology, food microbiology, agricultural microbiology, veterinary microbiology, environmental microbiology, water microbiology and aeromicrobiology.

Some branches of Pure Microbiology

	Branch	Definition
1	Bacteriology	the study of bacteria
2	Mycology	the study of fungi
3	Protozoology	the study of parasites
4	Virology	the study of viruses
5	Phycology/algology	the study of algae
6	Parasitology	the study of parasites
7	Nematology	the study of nematodes
8	Immunology	the study of the immune system

9	Microbial cytology	the study of microscopic and submicroscopic details of microorganisms
10	Microbial physiology	the study of how the microbial cell functions biochemically. Includes the study of microbial growth, metabolism and cell structure.
11	Microbial ecology	the relationship between MOs and their environment.
12	Microbial genetics	the study of how genes are organized and regulated in microbes in relation to their cellular functions.
13	Cellular microbiology	a discipline bridging microbiology and cell biology.
14	Molecular microbiology	the study of the molecular principles of the physiological processes in MOs.
15	Astro microbiology	the study of MOs in outer space
16	Phylogeny	the study of the genetic relationships between different organisms
17	Nano microbiology	the study of those organisms on nano level.

Lec.2 Microbial Taxonomy

- **Taxonomy** (Gk. taxon =arrangement; eg, the classification of organisms in an ordered system that indicates a natural relationship).
- **A taxon** is a collection of related organisms grouped together for purposes of classification. Thus, genus, family, etc. are taxons.
- **Microbial taxonomy** is a means by which microorganisms can be grouped together. Organisms having similarities with respect to the criteria used are in the same group, and are separated from the other groups of microorganisms that have different characteristics.
- **Linnaean taxonomy** is the system most familiar to biologists. It uses the formal taxonomic ranks of kingdom, phylum, class, order, family, genus, and species. The lower ranks are approved by a consensus of experts in the scientific community. Of these ranks, the family, genus, and species are the most useful.
- Microorganisms have wide taxonomic distribution and include organisms such as protozoa, algae, fungi, bacteria and virus.

Components of taxonomy

- **Classification** is the categorization of organisms with like characteristics into taxonomic groups.
- **Nomenclature** refers to the naming of an organism by international rules according to its characteristics, using binomial system of nomenclature.
- **Identification** is application of classification and nomenclature to assign proper name to unknown organism and place it in its proper position within classification system.

In any discussion on biological classification, it is impossible to avoid mentioning **Linnaeus**, the Swedish botanist who attempted to bring order to the naming of living things by giving each type a Latin name.
Linnaeus was responsible for introducing the *binomial* system of nomenclature, by which each organism was assigned a *genus* and a *species* (Table 1).

Note the following conventions, which apply to the naming of all living things (the naming of viruses is something of a special case):

- the generic (genus) name is always given a *capital* letter
- the specific (species) name is given a *small* letter
- the generic and specific name are *italicised*, or, if this isn't possible, underlined

Many species are named after people, either the discoverer or a famous person in the field of microbiology, for example Salmonella is after D.E. Salmon, who discovered it (albeit as "*Bacillus typhi*").

- For the generic epithet, all names derived from people must be in the female nominative case, either by changing the ending to -a or to the diminutive -ella, depending on the name.

- For the specific epithet, the names can be converted into either adjectival form (adding -nus (m.), -na (f.), -num (n.) according to the gender of the genus name).

Table 1: Some examples on binomial system of nomenclature

Type	Genus name	Species name	Full name of organism
Bacteria	*Escherichia*	*coli*	*Escherichia coli*
Archaea	*Thermococcus*	*litoralis*	*Thermococcus litoralis*
Mold	*Penicillium*	*chrysogenum*	*Penicillium chrysogenum*
Yeast	*Saccharomyces*	*cerevisiae*	*Saccharomyces cerevisiae*
Protozoa	*Entamoeba*	*histolytica*	*Entamoeba histolytica*
Algae	*Arthrospira*	*platensis*	*Arthrospira platensis*
Helminthes	*Ascaris*	*lumbricoides*	*Ascaris lumbricoides*

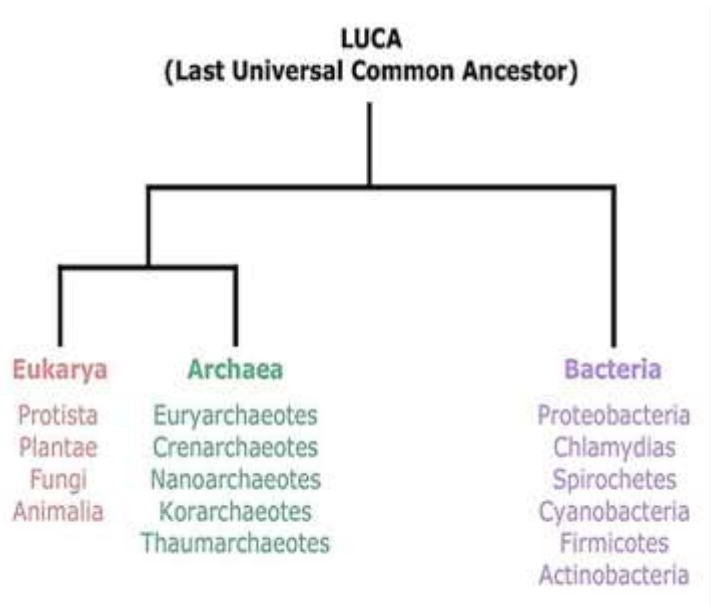
An important breakthrough in microbial taxonomy arose from studies of their cellular architecture, when it was discovered that cells exhibited one of two possible "floor plans", which are either prokaryotes (Greek pro, before, and karyon, nut or kernel; organisms with a primordial nucleus) or eukaryotes (Greek eu, true, and karyon, nut or kernel)

Organisms were divided into five kingdoms: Monera, Protista, Fungi, Animalia, and Plantae, but the five-kingdom system (proposed by Robert Whittaker in 1969) is no longer accepted by microbiologists. Why?

This is because not all "prokaryotes" are the same and therefore should not be grouped together in a single kingdom. Furthermore, it is currently argued that the term prokaryote is not meaningful and should be abandoned.

Three domains system

Molecular phylogenies divide all living organisms into three domains – Bacteria (“true bacteria”), Archaea (means ‘ancient’), and Eukarya (eukaryotes: protists, fungi, plants, animals). These domains represent a level of classification that goes even higher than the kingdoms (figures 1and 2).



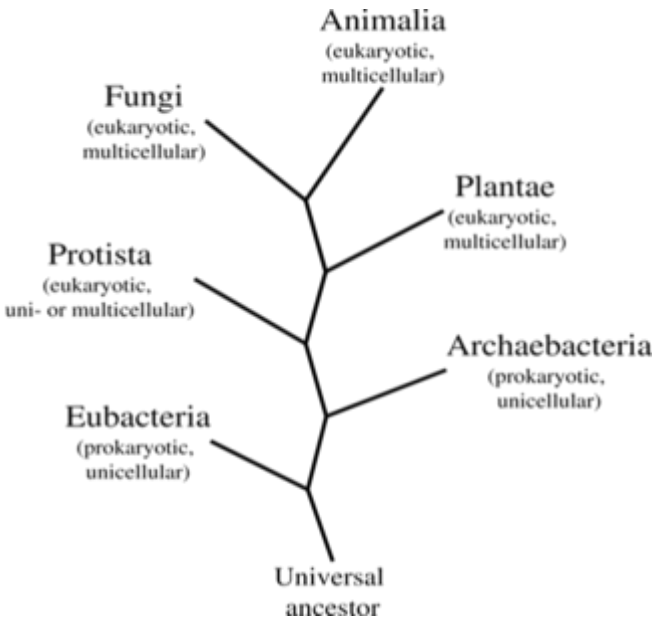


Figure 1: Left; three domains system, Right; tree of life shows the three domains system

Domain	Bacteria
Phylum	Proteobacteria
Class	Zymobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*coli*

Figure 2: A modern hierarchical classification for *E. coli*

Bacteria (sometimes referred to as true bacteria or eubacteria)

The techniques such as microscopic examination of specimens, culturing (growing) microbes in the laboratory and isolating pure cultures from mixed-culture populations, originally used for studying bacteria, have been modified for the study of all microorganisms—hence the transition from bacteriology to microbiology.

Members of domain Bacteria are:

- Usually single-celled (unicellular) organisms and they are described as prokaryotic because they lack a nucleus.
- Few of bacteria are pathogens, most play beneficial roles; they break down dead plant and animal material and, in doing so, cycle elements in the biosphere. Furthermore, they are used extensively in industry to make bread, cheese, antibiotics, vitamins, enzymes, and other products.

Archaea (sometimes called archaeobacteria or archaebacteria)

Through a microscope the archaea look much like bacteria, but there are important differences in their chemical composition, biochemical activities, and environments.

Members of domain Archaea are distinguished from bacteria by many features:

- Most notably their distinctive rRNA sequences
- Lack of peptidoglycan in their cell walls
- Have unique membrane lipids
- Many archaea are found in extreme environments, and they are divided into; Methanogens, Halophiles, Thermophiles and Psychrophilic (Figure 3).
- Although some archaea are members of a community of microbes involved in gum disease in humans, their role in causing disease has not been clearly established.
- Archaeans use different energy sources like hydrogen gas, carbon dioxide, sulphur and some of them use sunlight.

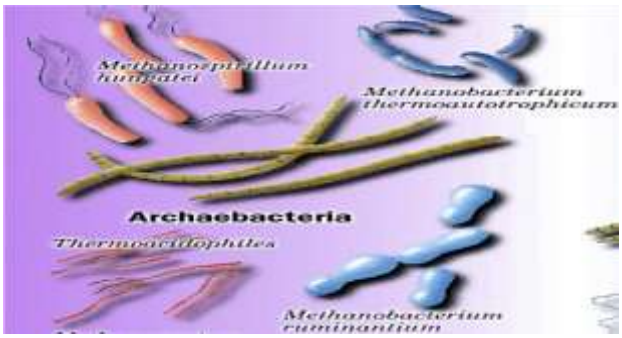


Figure 3: Some examples on Archaebacteria

Eukarya (all eukaryotic organisms)

Domain Eukarya includes microorganisms classified as protists or fungi. Animals and plants are also placed in this domain. Protists are generally unicellular but larger than most bacteria and archaea. They have traditionally been divided into protozoa and algae.

Fungi

- They are eukaryotic cells (with a true nucleus).
- Fungi (Figure 4) are either unicellular (yeast) or multicellular (molds) and their cell wall is composed of chitin.
- They obtain nutrients by absorbing organic material from their environment (decomposers), through symbiotic relationships with plants (symbionts), or harmful relationships with a host (parasites).
- They form characteristic filamentous tubes called hyphae (microscopic in size), the collection of hyphae is called mycelium (visible to the naked eye).
- Fungi reproduce by releasing spores.
- Some may be microscopic in size, while others form much larger structures, such as mushrooms and bracket fungi that grow in soil or on damp logs.
- Molds have considerable value; they are used to produce antibiotics- penicillin, cephalosporin etc, fermented products like soy sauce, Camembert chees and other products. But they are also implicated in various human, animal and plant diseases including athlete's foot and the moldy spoilage of grains and peanuts. The unicellular yeasts are widely used in Baking industry and for the production of all alcoholic beverages like wine, beer etc. On the other hand, some yeasts cause food spoilage and diseases such as vaginitis and thrush (an oral infection).

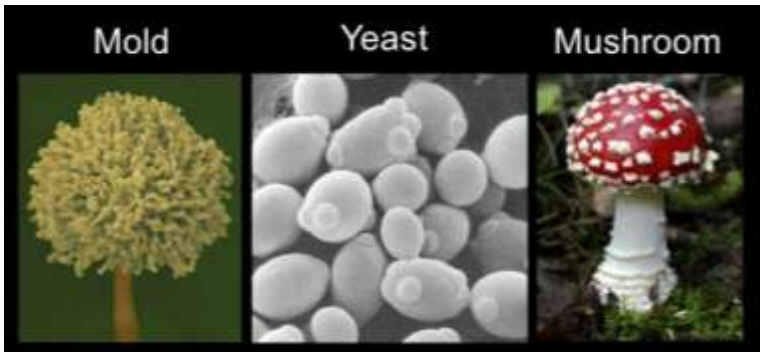


Figure 4: Types of fungi

Protozoa

- They make up the largest group of organisms in the world in terms of numbers, biomass, and diversity and present in soil, water and marshy places.
- Protozoa are unicellular eukaryotes; their size varies from 5-200 µm (Figure 5).
- Some protozoa are oval or spherical, others elongated. Still others have different shapes at different stages of the life cycle
- Motile having cilia, flagella and pseudopodia.
- Obtain nourishment by absorption or ingestion through specialized structures.
- Their cell walls are made up of cellulose.
- Protozoa have been traditionally divided based on their mode of locomotion into;

Flagellates, Ciliates, Amoeboid and Protozoans.

- They also have different means of nutrition, which groups them as autotrophs or heterotrophs. saprophytic or parasitic.
- They are animal-like in that:
  1. They ingest particulate food,
  2. Lack a rigid cell wall,
  3. Are able to move at some stage of their life cycle
  4. Do not contain chlorophyll.
- Their role in nature is varied, but the best known protozoa are the few that cause disease in human beings and animals, such as malaria in humans. Some protozoa are beneficial, such as those found in stomach of cattle, sheep and termites that help digest food.

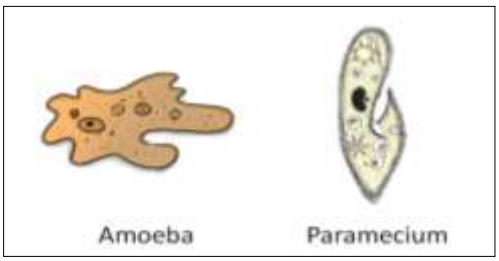


Figure 5: Some examples on protozoa

Algae (cyanobacteria or blue-green algae)

- They are relatively simple unicellular or multicellular eukaryotes (Figure 6).
- They are autotrophic and live in water, damp soil, and rocks and produce oxygen and carbohydrates used by other organisms.
- It is believed that cyanobacteria are the origins of green land plants.
- Their size varies from 1 µm up to 120 meters in length and exhibit a variety of shapes
- They are considered plant-like because they:
  1. contain the green pigment chlorophyll, carry out photosynthesis,
  2. have rigid cell walls.
- They are either motile or non-motile.
- They cause problems by clogging water pipes, releasing toxic chemicals into water bodies, or growing in swimming pools. But extracts of some species have commercial uses: as emulsifiers for foods such as ice-creams; as a source of agar used as solidifying agent in microbial media and as anti-inflammatory drugs for ulcer treatment.

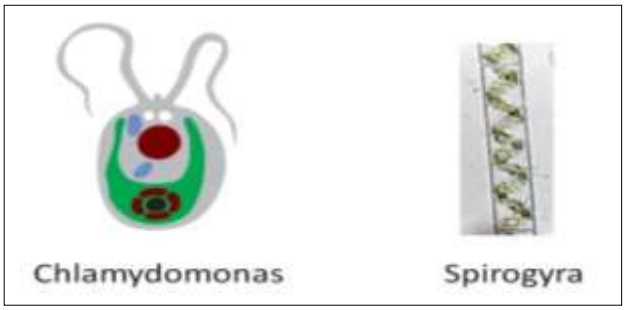


Figure 6: Some examples on algae

Multicellular Animal Parasites

They are eukaryotic organisms consisting of the flatworms and roundworms, collectively known as the helminths. Although they are not microorganisms by definition, since they are large enough to be easily seen with the naked eye, they live a part of their life cycle in microscopic form (Figure 7). Since the parasitic helminths are of clinical importance, they are often discussed along with the other groups of microbes.

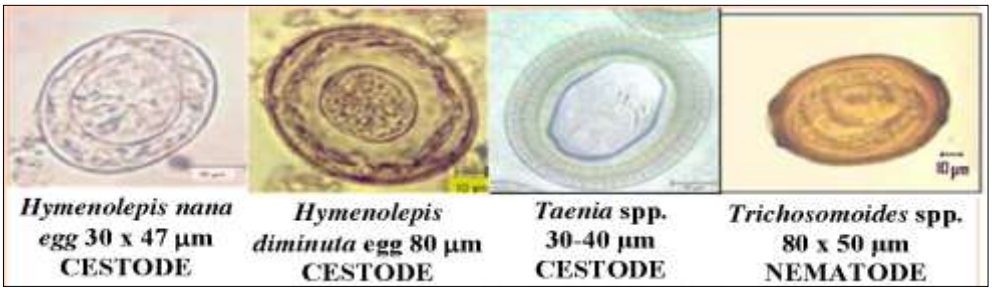


Figure 7: Some examples on helminth eggs

Viruses

- Although viruses are classified as MOs, The place of viruses in the phylogenetic tree of life is uncertain. Viruses differ from all other organisms in three major respects:
  1. They contain only one kind of nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)
  2. Only the nucleic acid is necessary for their reproduction;
  3. They are unable to reproduce outside of a host's living cell.
- Consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat (Figure 8).
- Ultra-microscopic, they can be seen only under an electron microscope.
- Non-cellular obligate parasites of plants, animals and bacteria as well as other protists.
- Their size varies from 0.015µm -0.2 µm and shapes from spherical, rod, flexuous to cozohedral.
- Viruses cause large number of diseases in humans (such as AIDS, common cold, hepatitis etc), plants (tobacco mosaic disease, papaya ring spot disease etc) and foot-and-mouth disease of animals. In addition, some retroviruses have also been implicated in the growth of some malignant tumors.

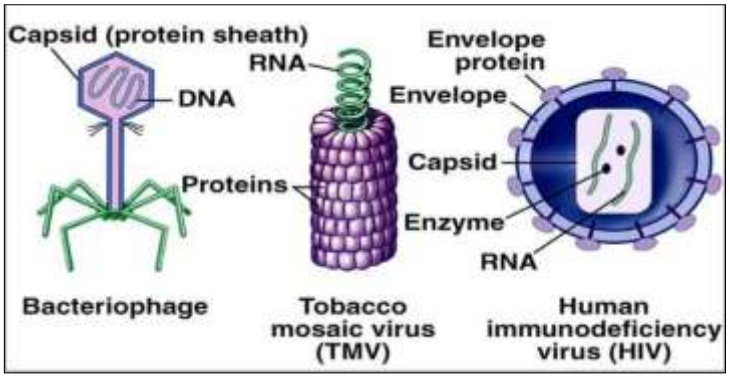


Figure 8: Viral structure

Lec.3 Bacterial Cell Structure

- The basic unit of all living things is the cell.
- Among microorganisms, all bacteria and protozoans are unicellular; fungi may be unicellular or multicellular, while algae may exist in unicellular, multicellular and colonial.
- All organisms are either **prokaryotic** 'before nucleus' or **eukaryotic** 'true nucleus' cells, both of which exist in the microbial world.

Shape and arrangement of bacteria

Bacteria are much smaller than eukaryotic cells; most fall into a size range of about 1–5μm, although some may be larger than this. Some of the smallest bacteria, such as the mycoplasma measure less than 1μm.

Because of their extremely small size, it was only with the advent of the electron microscope that we were able to learn about the detailed structure of bacterial cells. Using the light microscope however, it is possible to recognize differences in the shape and arrangement of bacteria; most have one of three basic shapes (Figure 1):

- rod shaped (*bacillus*)
- spherical (*coccus*)
- curved: these range from comma-shaped (*vibrio*) to corkscrew-shaped (*spirochaete*)

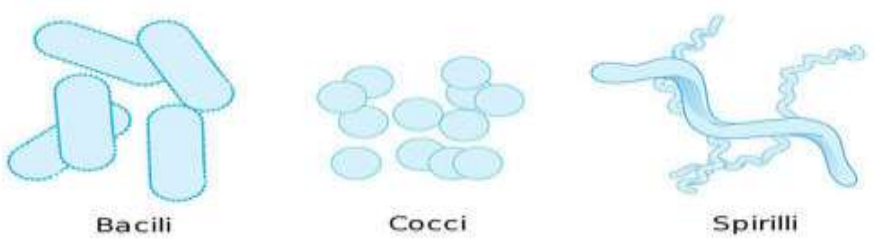


Figure1: Three basic shapes of bacteria

- In recent years, square, triangular and star-shaped bacteria have all been discovered.

As well as these characteristic cell shapes, bacteria may also be found grouped together in particular formations (Table 1). When they divide, they may remain attached to one another, and the shape the groups of cells assume reflects the way the cell divides. Cocci, for example, are frequently found as chains of cells, a reflection of repeated division in one plane. Other cocci may form regular sheets or packets of cells, as a result of division in two or three planes. Yet others, such as the staphylococci, divide in several planes, producing the irregular and characteristic 'bunch of grapes' appearance. Rod-shaped bacteria only divide in a single plane and may therefore be found in chains, while spiral forms also divide in one plane, but tend not to stick together. Blue–greens form filaments; these are regarded as truly multicellular rather than as a loose association of individuals.

Table 1: Common Prokaryotic Cell Arrangements		
Name	**Description**	**Illustration**
Coccus (pl. cocci)	Single coccus	
Diplococcus (pl. diplococci)	Pair of two cocci	
Tetrad (pl. tetrads)	Grouping of four cells arranged in a square	
Streptococcus (pl. streptococci)	Chain of cocci	
Staphylococcus (pl. staphylococci)	Cluster of cocci	
Bacillus (pl. bacilli)	Single rod	
Streptobacillus (pl. streptobacilli)	Chain of rods	

Prokaryotic cell structure

When compared with the profusion of elaborate organelles encountered inside a typical eukaryotic cell, the interior of a typical bacterium looks rather empty. The only internal structural features are:

- A bacterial chromosome or nucleoid, there may be additional DNA in the form of a plasmid
- Thousands of granular ribosomes
- A variety of granular inclusions associated with nutrient storage

All of these are contained in a thick aqueous soup of carbohydrates, proteins, lipids and inorganic salts known as the **cytoplasm**, which is surrounded by a **plasma membrane**. This in turn is wrapped in a **cell wall**, whose rigidity gives the bacterial cell its characteristic shape. Depending on the type of bacterium, there may be a further surrounding layer such as a capsule or slime layer and/or structures external to the cell associated with motility (flagella) or attachment (pili/fimbriae) (Figure 2).

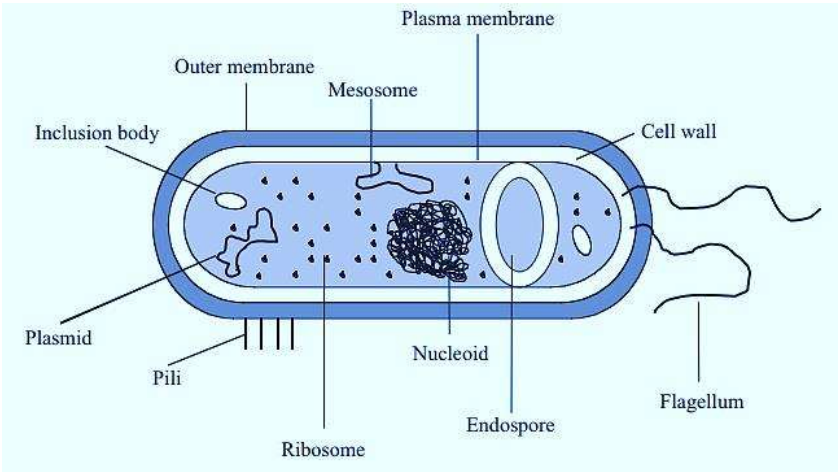


Figure 2: Structure of a generalized prokaryotic cell

The nucleoid

- The genetic material of prokaryotes lacks a surrounding nuclear membrane.
- The nucleoid or bacterial chromosome comprises a closed circle of double stranded DNA, many times the length of the cell and highly folded and compacted.
- The DNA may be associated with certain bacterial proteins.
- Some bacteria contain additional DNA in the form of small, self-replicating extrachromosomal elements called plasmids. These do not carry any genes essential for growth and reproduction, and thus the cell may survive without them.
- Not all bacteria conform to the model of a single circular chromosome; some have been shown to possess two with genes shared between them, while examples of linear chromosomes are also known.

Ribosomes

- They are composed of a complex of protein and RNA, and are the site of protein synthesis in the cell. They are measured in Svedberg units (S)
- Although they carry out a similar function, the ribosomes of prokaryotic cells are smaller and lighter than their eukaryotic counterparts.
- All ribosomes comprise two unequal subunits; each subunit contains its own RNA and a number of proteins (Figure 3).

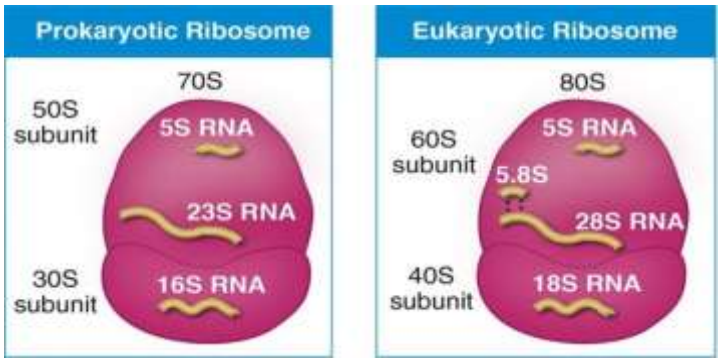


Figure 3: Prokaryotic and eukaryotic ribosomes

Inclusion bodies

- They are granular structures found within the cytoplasm of certain bacteria. These act as food reserves, and may contain organic compounds such as starch, glycogen or lipid (Figure 4). In addition, sulphur and polyphosphate can be stored as inclusion bodies, the latter being known as volutin or metachromatic granules. Some inclusion bodies are actually membranous vesicles or intrusions into the cytoplasm which contain photosynthetic pigments or enzymes.

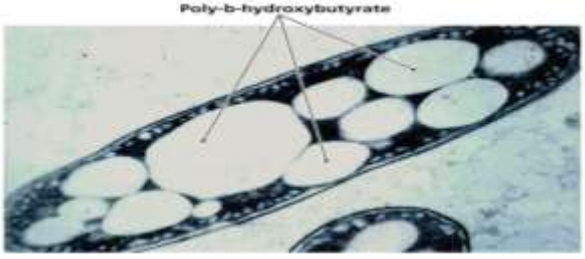


Figure 4: PHB granules (bacterial inclusions)

Endospores

Certain bacteria such as *Bacillus* and *Clostridium* produce endospores. They are dormant forms of the cell that are highly resistant to extremes of temperature, pH and other environmental factors, and germinate into new bacterial cells when conditions become more favorable. The spore's resistance is due to the thick coat that surrounds it (Figure 5).

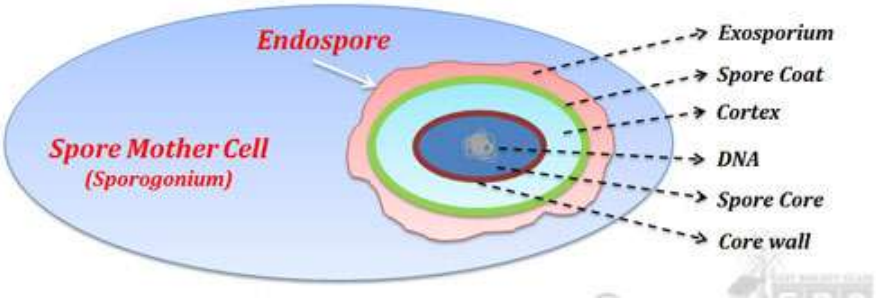


Figure 5: Bacterial endospore

The plasma membrane

- The cytoplasm and its contents are surrounded by a plasma membrane, which can be thought of as a bilayer of phospholipid arranged like a sandwich, together with associated proteins (Figure 6).
- Phospholipids comprise a compact, hydrophilic (water-loving) head and a long hydrophobic (water-hating) tail region.
- Also included in the membrane are a variety of proteins; these may pass right through the bilayer or be associated with the inner (cytoplasmic) or outer surface only. These proteins may play structural or functional roles in the life of the cell. Many enzymes associated with the metabolism of nutrients and the production of energy, are associated with the plasma membrane in prokaryotes.
- The majority of bacterial membranes does not contain sterols but contain molecules called **hopanoids** which thought to assist in maintaining membrane stability.

The function of the plasma membrane is to:

- keep the contents in,
- allowing the selective passage of certain substances in and out of the cell (it is a semipermeable membrane)

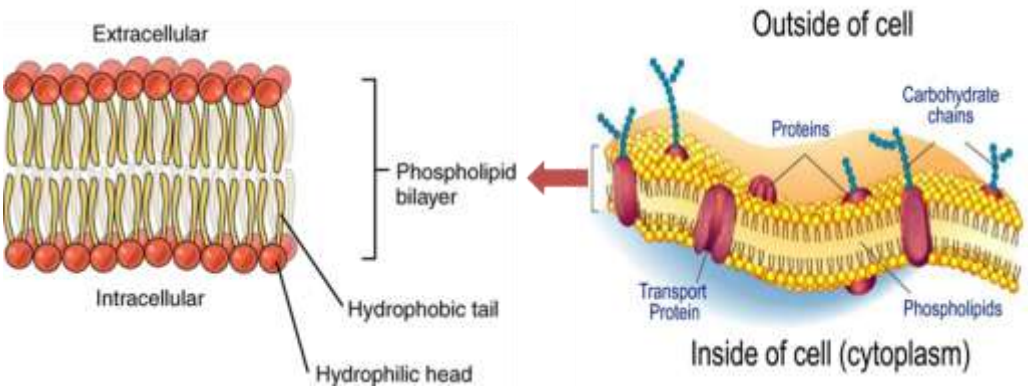


Figure 6: Structure of bacterial cell membrane

The bacterial cell wall

- Bacteria have a thick, rigid cell wall, which maintains the integrity of the cell, and determines its characteristic shape.
- The major component of the cell wall, which is responsible for its rigidity, is a substance unique to bacteria, called **peptidoglycan (murein)**. This is a high molecular weight polymer whose basic subunit is made up of three parts: *N*-acetylglucosamine, *N*-acetylmuramic acid and a short peptide chain (Figure 7). The latter comprises the amino acids l-alanine, d-alanine, d-glutamic acid and either l-lysine or diaminopimelic acid (DAP). DAP is a rare amino acid, only found in the cell walls of prokaryotes.

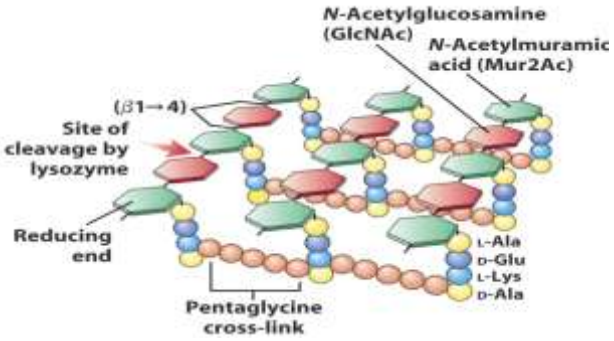


Figure 7: Peptidoglycan structure

Although all bacteria (with a few exceptions) have a cell wall containing peptidoglycan, there are two distinct structural types. These are known as **Gram-positive** and **Gram-negative**. The names derive from the Danish scientist Christian Gram, who, in the 1880s developed a rapid staining technique that could differentiate bacteria as belonging to one of two basic types.

- **Gram-positive cell walls** are relatively simple in structure, comprising several layers of peptidoglycan (as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall) connected to each other by cross-linkages to form strong, rigid scaffolding. In addition, they contain acidic polysaccharides called **teichoic acids**; these contain phosphate groups that impart an overall negative charge to the cell surface (Figure 8).

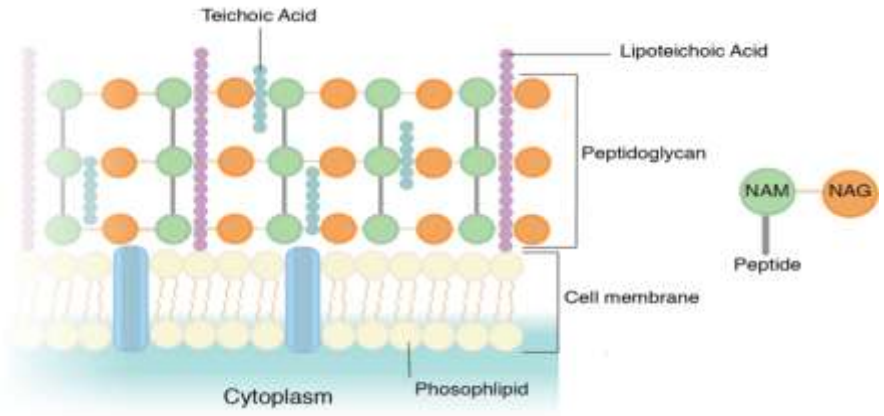


Figure 8: Gram positive cell wall

- **Gram-negative cell wall** (Figure 9), is multilayers and consists of a thin, inner wall composed of:

1. Peptidoglycan; is just 2-3 layers and generally 2-3 nm thick. Chemically, only 10 to 20% of the Gram-negative cell wall is peptidoglycan.
2. An outer membrane; is a lipid bilayer about 7 nm thick. It is composed of phospholipids, lipoproteins, lipopolysaccharides (LPS), and proteins.

- Phospholipids are located mainly in the inner layer of the outer membrane,
- The lipoproteins connect the outer membrane to the peptidoglycan.
- The lipopolysaccharides, located in the outer layer of the outer membrane, consist of a lipid portion called lipid A embedded in the membrane and a polysaccharide portion extending outward from the bacterial surface. The LPS portion of the outer membrane is also known as endotoxin.
- In addition, pore-forming proteins called **porins** span the outer membrane. The porins function as channels for the entry and exit of solutes through the outer membrane of the Gram-negative cell wall. The outer membrane of the Gram-negative cell wall is studded with surface proteins that differ with the strain and species of the bacterium.

  - The periplasm is the gelatinous material between the outer membrane, the peptidoglycan, and the cytoplasmic membrane. This periplasmic space is about 15nm wide and contains a variety of hydrolytic enzymes for nutrient breakdown, periplasmic binding proteins for transport via the ATP-binding cassette (ABC) system, and chemoreceptors for chemotaxis.

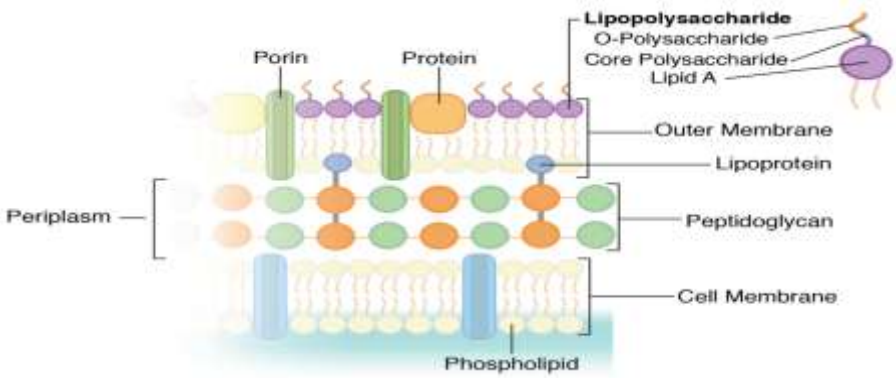


Figure 9: Gram negative cell wall

Beyond the cell wall

A number of structural features are to be found on the outer surface of the cell wall; these are mainly involved either with locomotion of the cell or its attachment to a suitable surface. Perhaps the most obvious extracellular structures are:

1. **Flagella (sing: flagellum),** thin hair-like structures often much longer than the cell itself, and used for locomotion in many bacteria. There may be a single flagellum, one at each end, or many, depending on the bacteria concerned (Figure 10).

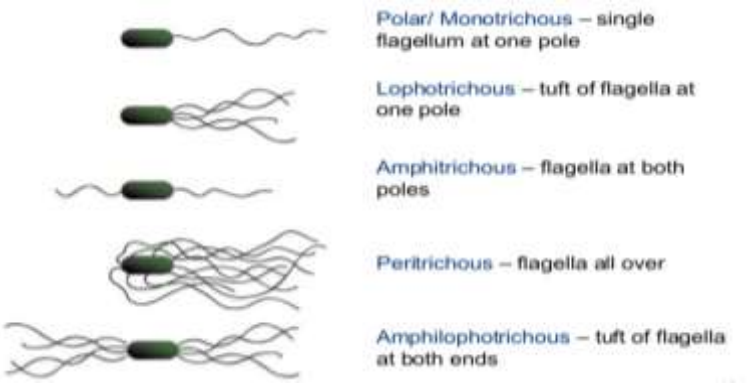


Figure 10: Types of flagella arrangement

Each flagellum is a hollow but rigid cylindrical filament made of the protein flagellin, attached via a hook to a basal body, which secures it to the cell wall and plasma membrane (Figure 11). The basal body comprises a series of rings, and is more complex in Gram-negative than Gram-positive bacteria. Rotation of the flagellum is an energy-dependent process driven by the basal body, and the direction of rotation determines the nature of the resulting cellular movement.

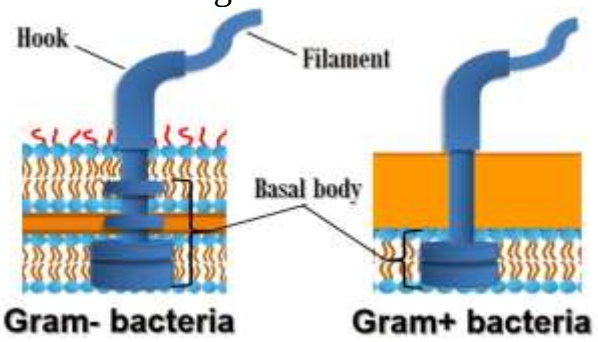


Figure 11: Structure of bacterial flagellum

2. **Pili (sing: pilus)** are structures that superficially resemble short flagella (Figure 12). They differ from flagella, in that they:

- do not penetrate to the plasma membrane,
- are not associated with motility.

Their function, rather, is to anchor the bacterium to an appropriate surface. Pathogenic (disease-causing) bacteria have proteins called adhesins on their pili, which adhere to specific receptors on host tissues. Attachment pili are sometimes called fimbriae, to distinguish them from another distinct type of pilus, the sex pilus, which as its name suggests, is involved in the transfer of genetic information by conjugation.

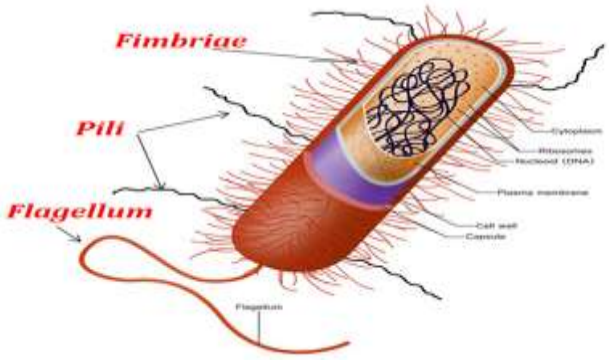


Figure 12: Cell-surface appendages of a bacterial cell

3. Outside the cell wall, most bacteria have a polysaccharide layer called a **glycocalyx**, which could be as slime layer or as capsule (Figure 13).

- Slime layer is a diffuse and loosely bound. It helps protect against desiccation, and is instrumental in the attachment of certain bacteria to a substratum (the bacteria that stick to your teeth are a good example of this).

- Capsule is a better defined and generally thicker. It offers protection to certain pathogenic bacteria against the phagocytic cells of the immune system.

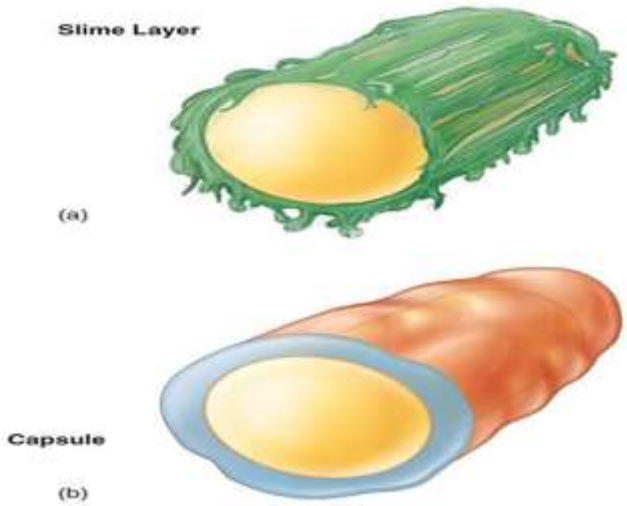


Figure 13: Bacterial polysaccharide layer (glycocalyx);a, slime layer ; b, capsule

Lec:4

The growth

means an increase in size, number, weight, and mass. It is a group of reactions and events led to an increase the macromolecules number and then cell division and reproduction.

Cell cycle

A group of steadily successive events are interrupted with periods which depending on environmental conditions. The required time from the beginning to the end of division known as generation time and the resulting growth called growth rate.

Growth rate and generation time

Generation time (doubling time): The time for a single cell to undergo fission.

It takes short time in prokaryote (ex: 20-25 min in *E coli*). While in eukaryotes it takes two hours to several days.

Generation time varies with:

1- Species of M.O.

2- Nutrients.

3- Environmental conditions: PH, and temperature.

4- Growth phase.

Prokaryotic cell cycle:

Most of studies on prokaryotic cell cycle were done on *E. coli* because of it is easy to handle. Prokaryotic cell cycle includes:

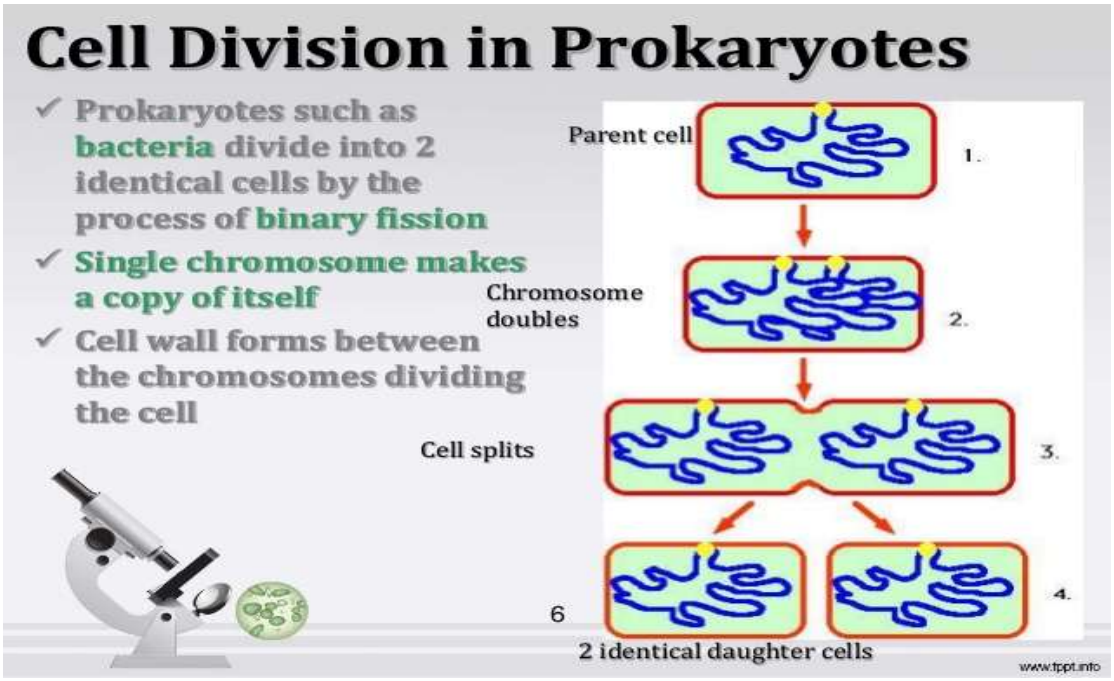
1- **First stage:**
This period is still under speculating. Mostly, under the optimal condition it disappears due to the shortage of generation time, also the environmental conditions greatly affect the cell.

2- **Second stage:**

A- A stage of DNA synthesis abbreviated as C instead of S, it means chromosome replication.
B- It required most of cycle time.
C- It controls the continuity of the cycle, since when the DNA synthesis is interrupted the cell will not divide.
D- It is affected, a little, by the environmental conditions.

2- **Third and fourth stages:**

A- After the DNA synthesis stage, there is a gap before the cell is dividing into tow daughter cells.
B- It represents both third and fourth stages, G2 and M.
C- It referred as to D.
D- It is affected, a little bit, by the environmental conditions.



Growth curve of bacteria:

When bacteria are inoculated into a new culture media, it shows a characteristic growth curve which has four phases:

1- Lag phase:

During this phase, bacteria exhibit growth in size but no increase in cell number and the bacteria are preparing for synthesis of DNA, various enzymes, and other components, which are for cell division. The lag phase varies in length with the conditions of the M.O and the nature of the media, this mean that the phase may be long if the inoculum is from an old culture or if the culture is refrigerated.

2- Logarithmic(exponential) phase:

During this period the cells divide steadily at a constant rate. The log of the number of cells is plotted against time results in a straight line. Under appropriate conditions, the growth rate is maximal during this phase, and the population is most nearly uniform in terms of chemical compositions of cells, metabolic activity and other physiological characteristics.

3- Stationary phase:

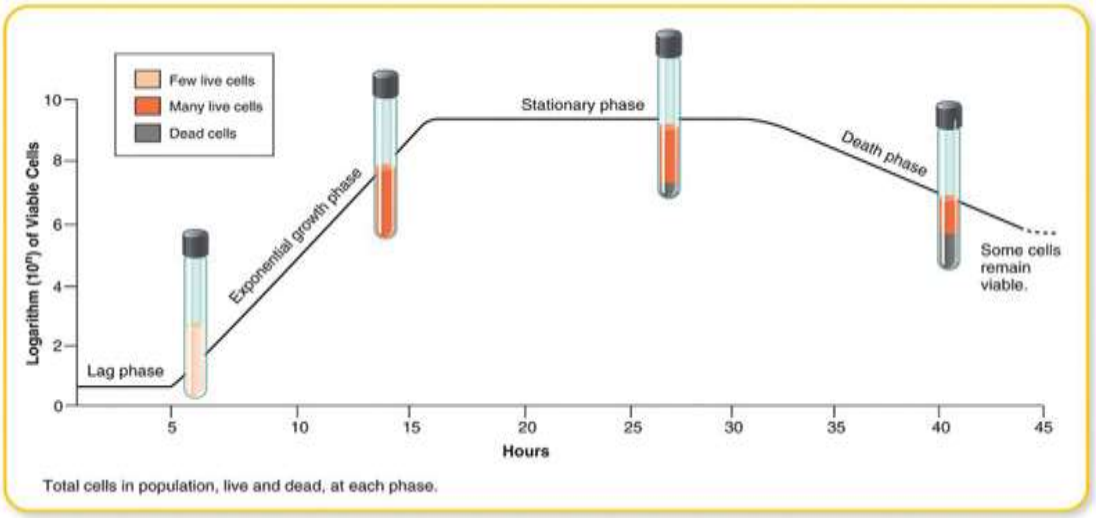
During this phase the growth rate is equal to the death rate. Food begins to run out, poisonous waste products accumulate, PH changes, hydrogen acceptors are used up, and energy transfers are diminished. The rate of fission begins to decline, and the organisms die in increasing numbers.

4- **Death (decline) phase**:

Eventually the number of viable bacterial cells begins to decline, signaling the onset of the death phase. The kinetics of bacterial death, like those of growth, are exponential.

Recently, some authors are dividing the growth curve into six phases by the letters A to F as follows (a) Lag phase- Growth rate is zero. (b) Acceleration phase- Increasing growth rate. C Exponential phase — Constant growth rate. (c) Retardation phase- Growth rate is

decreasing. (e) Maximum stationary phase- Growth rate is zero. F Decline phase- Growth rate is negative (death).



- When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase).

1.Methods for measurement of cell mass:

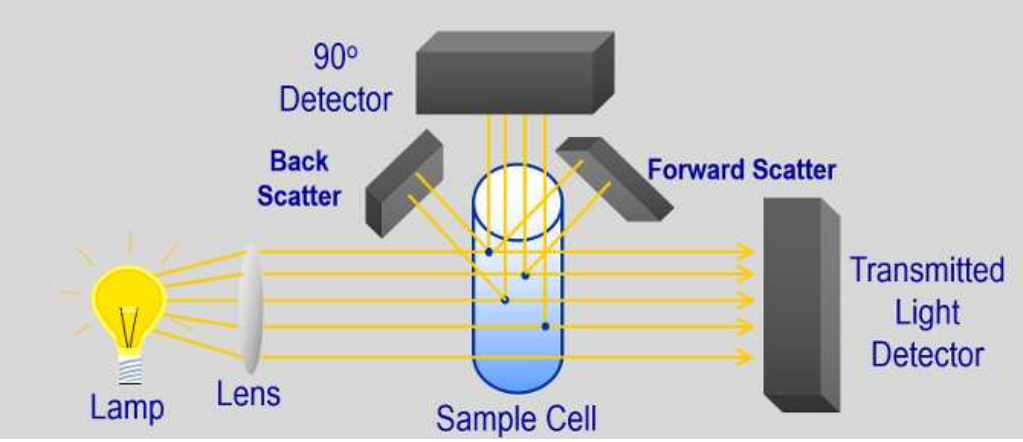
Methods for measurement of the cell mass involve both **direct** and **indirect** techniques:

A- Direct physical measurement of dry weight, wet weight, or volume of cells after centrifugation. This is the most direct approach for quantitative measurement of a mass of cell. The sample is centrifugation or filtered and the residue or the pellet is washed a number of times to remove all extraneous matter, the residue is then dried and weight. It can be used only with very dense cell suspension. It is commonly used for measuring growth of moulds in certain phases of industrial work.

B- Direct chemical measurement of some chemical component of the cells such as total N, total protein, or total DNA content.

C- Indirect measurement of chemical activity such as rate of O2 production or consumption, CO2 production or consumption.

D- Turbidity measurements employ a variety of instruments to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve.



1. Source of Light of a single wave-length (monochromatic)

2. Filter

3. Tube with cell free medium

4. Tube with suspension of microorganisms

5. Photocell or Detector

2. Methods for measurement of cell numbers:

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

A- Direct microscopic count also called Breed method or Haemocytometer, is possible using special slides known as counting chamber. Dead cells cannot be distinguished from living ones. Only dense suspension can be counted (cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.

B- Electronic counting chamber; count numbers and measure size distribution of cells. Such electronic devices are more often used to count eukaryotic cells such as blood cells.

C- Indirect viable cell count also called plate count. Involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in nontoxic diluents (water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a colony forming unit (CFU). And the number of CFU is related to the viable number of bacteria in the sample. Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted. Disadvantages are 1- only living cells develop colonies that are counted, 2- clumps or chains of cells develop into a single colony, 3- colonies develop only from those organisms for which the cultural condition are suitable for growth.

- **Determination of cell activity:**

  Measurement of a specific chemical changes produced on a constituent of the medium, ex: acid production from sugar in the nutrient medium. The amount of acid produced is proportional to the magnitude of the cell suspension. Also, specific enzyme may be assayed to measurement cell growth.

Nutritional Types of Microorganisms

Lec:5

The Ways of food entrance to M.O.s:

Entrance of food substances into all plant cells, and most animal cells, is by passage through the cell membrane and cell wall if present of nutrients in an aqueous solution by the processes of diffusion and osmosis. **This type of nutrition is to be osmotrophic.**

Phagotrophic cells

Animal cells are typically without cell walls, have the ability to ingest solid particles of food by drawing them into the cell through the cell membrane by the process called **phagocytosis.** Phagocytic cells are said to have a phagotrophic type of nutrition.

Pinocytosis:

Many kinds of animas cell, though lacking cell walls, are not phagocyte. However, they can engulf fluids, and possibly pass minute particles inward through the cell membrane by a process called **pinocytosis.**

A similar process called endocytosis, large, complex molecules such as proteins, nucleic acids, some phages, and possibly colloids like sulfur, taken into the mammalian cell via minute invaginations of the cytoplasm membrane.

Requirements of nitrogen, phosphorus, and sulfur

- **Carbon**:

The sole inorganic source of carbon is Co_2(M.O called **autotrophs**) while glucose and amino acids are the sole source of organic carbon, which is needed for anabolism and release energy and M.O called **heterotroph.**

- **Nitrogen**:

Is needed for the synthesis of amino acids, purine, pyrimidine, nucleic acids, enzymes, and vitamins.

- **Phosphorus**:

Is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and other cell components.

- **Sulfur**:

Is needed for the synthesis of substances like amino acids (cysteine, and methionine), thiamine, biotin, and some carbohydrate

Nutritional types of microorganisms:

There are two sources of energy available to organisms:

1-Light energy

2-The energy derived from oxidizing organic or inorganic molecules.

- Some of nutrients transport method need energy such as in anaerobic bacteria, while the other doesn't need it like in aerobic bacteria.
- Let us focus on carbon first. All organisms are carbon-based with macromolecules – proteins, carbohydrates, lipids, nucleic acid – having a fundamental core of carbon. On one hand, organisms can use reduced, preformed organic substances as a carbon source. These are the heterotrophs or "other eaters." Alternatively, they can rely on carbon dioxide (CO2) as a carbon source, reducing or "fixing" it this inorganic form of carbon into an organic molecule. These are the autotrophs or "self-feeders."
- For energy, there are two possibilities as well: light energy or chemical energy. Light energy comes from the sun, while chemical energy can come from either organic or inorganic chemicals. Those organisms that use light energy are called phototrophs ("light eaters"), while those that use chemical energy are called **chemotrophs** ("chemical eaters"). Chemical energy can come from inorganic sources or organic sources. An organism that uses inorganic sources is known as a **lithotroph** ("rock eater"), while an organism that uses organic sources is called an **organotroph** ("organic eater").

These terms can all be combined, to derive a single term that gives you an idea of what an organism is using to meet its basic needs for energy, electrons, and carbon.

Phototrophs:

Microorganismsuse light as an energy source, such as *Chlorobium*

Chemotrophs:

Microorganisms obtain energy from the oxidation of chemical compounds.

Lithtrophs:

Microorganisms use reduced inorganic substances as an electron source.

Organotrophs:

Microorganisms extract electrons or hydrogen from organic compounds.

Mixotrophs:

Bacteria that depending on inorganic energy sources and organic carbon sources.

Nutritional type	Energy source	Carbon source	Examples
Photoautotroph	light	Co_2	*Cyanobacteria*,

photoheterotoph **photoorganotroph**	light	Organic compounds	*Rhodospirillum*
Chemoautotrophs	Inorganic compounds,e.g.H_2, NH_3,NO_2,H_2S.	CO_2	A few bacteria and many archaea
Chemoheterotrophs	inorganic compounds	Organic compounds	Pathogenic bacteria, some archaea.

The common nutrients requirements:

Analysis of microbial cell composition shows that 95% or more of cell dry weight is made up few major elements (carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium and iron). These are referred to as macronutrients or macro elements because M.O require them in large amounts. The first six are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four exist in the cell as cations and play a variety of roles.

Potassium (K^+):

It is required for activity by a number of enzymes including some of those involved in proteins synthesis.

Calcium (Ca^{++}):

It has many functions and the most important one is the contribution to heat resistance of bacterial end spore formation.

Magnesium (Mg^+):

It serves as a cofactor for many enzymes also makes a complex with ATP, stabilizes ribosome and cell membrane.

Iron (Fe^{++} or Fe^{+++}):

It issued in synthesis of cytochromes, as a cofactor for enzymes and electron carrying proteins.

Growth factors:

An autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors.** Growth factors are organized into three categories:

1-Purines and pyrimidines:

Required for synthesis of nucleic acids (DNA and RNA).

2-Amino acids:

Required for the synthesis of proteins.

3-Vitamins:

Needed as coenzymes and functional groups of certain enzymes.

- The growth factors are not metabolized directly as sources of carbon or energy; rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotroph**. Thus, a strain of *E. coli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph.
- Some vitamins that are frequently required by certain bacteria as some of growth factors are listed in the following:

1- **Folic acid:** synthesis of thymine, purine bases, serine, methionine .

2- **Biotin:** Biosynthetic reactions that require CO2 fixation

3- **Pyridoxine (B6):** decarboxylation and recreation of amino acids.

4- **Vitamin K:** electron transport processes.

Mineral salts:

Microorganisms need mineral salts in a small quantity of inorganic ions (cations and anions) such as:

1-Macronutrients elements:

They are needed for activation of enzymes, enzymes cofactor, and controlling the osmotic pressure inside the cell. They including Mg^{+2}, Ca^{+2}, Na^{+}, and Cl^{-}.

2-Micronutrients elements:

They are required in low concentration.

- There is a group of M.O, which needed Na^{+} and Cl^{+} in high concentration Called **Halophiles that can be classified into:**

1-Slightly halophiles:

Those who needed a small quantity of NaCl (2-5%), including most of marine bacteria.

2-Moderately halophiles:

NaCl is needed in a range about (5-10%) such as ***Pseudomonas***, ***Lactobacillus.***

3-Extremely halophiles:

NaCl is needed in a range about (20-30%) such as ***Halobacterium*** and ***Micrococcus.*** NaCl is necessary to stabilize the binding of cell wall proteins to each other

The effect of oxygen:

Oxygen is a universal component of cells and is always provided in large amounts by H_2O.Procaryotes display a wide range of responses to molecular O_2. (Table 2)

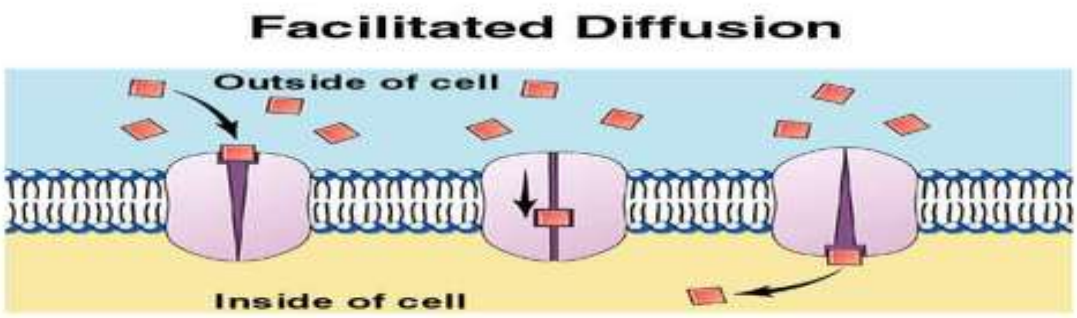
Group	Aerobic	Anaerobic	O_2 effect
Obligate aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate anaerobe	No growth	Growth	
Facultative anaerobe **(Facultative aerobe)**	Growth	Growth	Not required for growth but utilized when available
Aerotolerant anaerobe	Growth	Growth	Not required and not utilized

Uptake of Nutrients

In order to support its' activities, a cell must bring in nutrients from the external environment across the cell membrane. In bacteria and archaea, several different transport mechanisms exist., the most important of them:

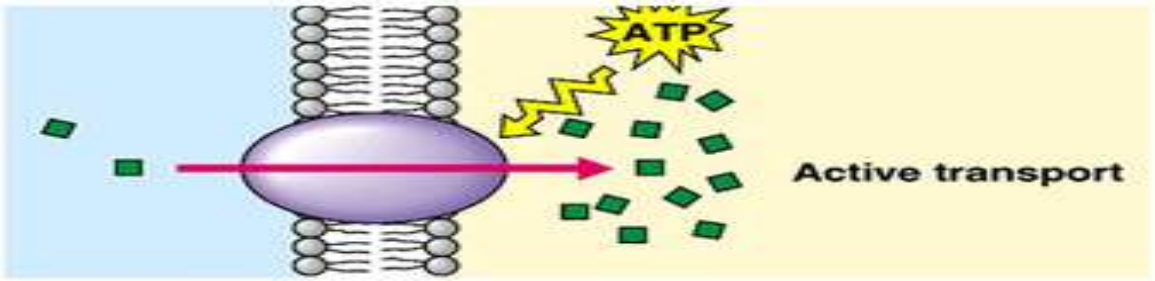
1-Facilitated diffusion:

A few substances can cross the cytoplasm membrane by passive diffusion. In this process molecules move from a region of higher concentration to one of lower concentration.



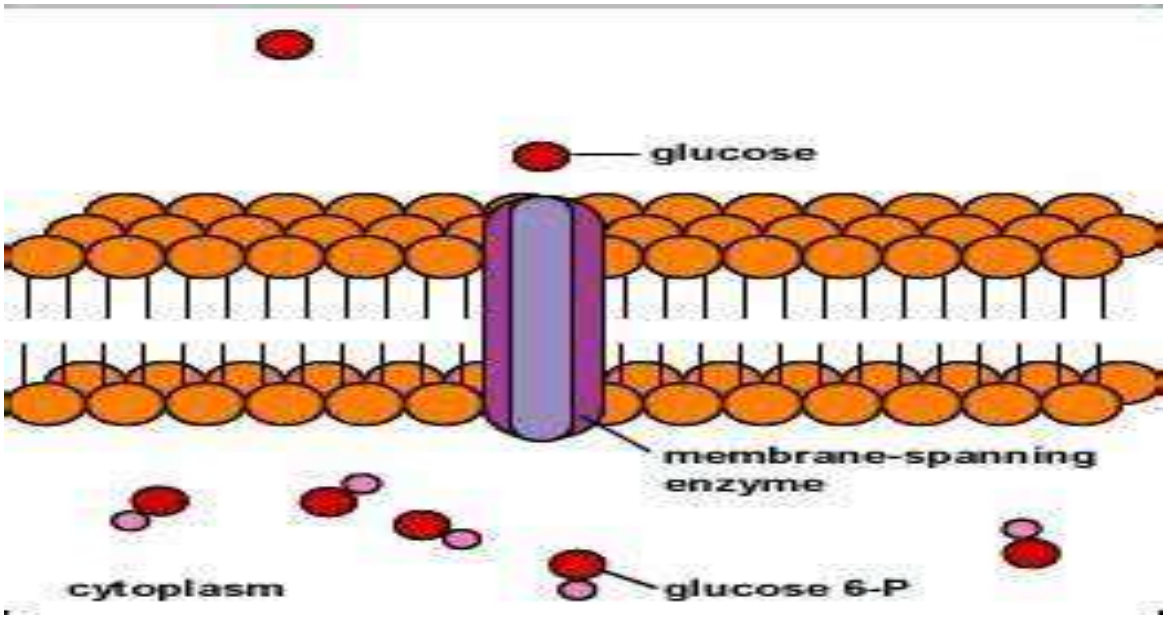
2-Active transport:

It is transport of solute to higher concentration or against the concentration gradient with the use of metabolic energy in input.



3-Group translocation:

A substrate is becoming phosphorylated during the transport process.



Control of microbial growth Lec.:6

The rate of microbial growth and death influenced by a number of environmental parameters. Some agents destroy all forms of microbes, whereas other agents only inhibit their growth. A nutrient may be essential for growth in low concatenation; it may be **Toxic** at higher concentration.

Control of microbial growth by antimicrobial agents:

A number of inhibitory chemicals are employed for the control of microbial growth.

The principal reasons for microbial control are:

1- To prevent transmission of disease and infection.
2- To prevent decomposition and spoilage.
3- To prevent contamination of materials used in pure culture work in laboratories.

- **Microorganisms can be inhibited or destroyed by physical or by chemical agents.**

Antimicrobial agents are classified according to their application and action into:

1- **Biocide:** A general term describing a chemical agent, usually broad spectrum that inactivates M.O.
2- **Bacteriostatic:** a chemical agent is able to inhibit bacterial multiplication.
3- **Bacteriocidal**: a chemical agent is able to kill bacteria.
4- **Sterilization:** a physical or chemical process that completely destroys or removes all microbial life, including spores.
5- **Disinfectants**: can be either germicides or microstatic agents that kill or prevent the growth of pathogenic M.O but not necessarily the spores and are applied only to inanimate.
6- **Antiseptic**: a substance that destroys or inhibit the growth of M.O in or on living tissue.
7- **Antimicrobial agent**: Chemical that kills M.O or prevent the growth of M.O.

Microbicidal and Microbiostatic agents act by:

1-Destruction by:

i- Heat (boilers, ovens).

ii- Chemical agent(disinfectants).

iii- Radiation (X-ray, UV light).

iv- Mechanical agents (crushing, scattering by ultrasonic vibrations).

2-Removal (especially bacteria) by:

i- Filtering

ii- High-speed centrifugation

3-inhibition by:

i- Low temperature (refrigeration, dry ice)

ii- Desiccation.

iii- Freeze-drying.

iv- High osmotic pressure.

v- Chemical and drugs such as:

a- Dyes: eosin, methylene blue and crystal violet.

b- Chemotherapeutic drugs such as: antibiotics.

Types of cell damage:

1- Denaturation and coagulation of protein and enzymes
2- Damage of cell wall or prevent the formation of cell wall.
3- Damage the permeability of cytoplasmic membrane.
4- Prevent the synthesis of protein and nucleic acids
5- Interfere with the activity of enzymes.

A- physical parameters:

1- **Temperature (heat):** it influences the rates of chemical reactions and the proteins, thereby affecting the rates of enzymatic activities. The higher the temperature above the maximal growth temperature the higher the death rate for that M.O Higher temperature can be used to kill M.O and it used to sterilize materials. Sterilization by heat can be divided into:

A. Moist heat: moisture at elevated temperature causes the coagulation of macromolecules. Moist heat at temperature of 40-80C, applied for 30 min is sufficient to kill vegetative bacteria, virus and fungi but not bacterial spores. they exposed to steam at 121C and 15-pound inch pressure for 15 min. in autoclave, which will kill all M.O including endospores.

B. Dry heat: It is a process that dehydrates the cell, causing solutes precipitation and oxidation of macromolecules father than their coagulation. Dry heat is used in the range of 160-180C for 1-2 hr. it is generally used for sterilization of certain glassware.

Pasteurization: destroys vegetative pathogens but does not affect many on the M.O that spoil milk.

2-Filteration: it is a common technique for separating of different size, but it is also useful for sterilization.

3-Radiation: it is used for controlling growth can be characterized as either non-ionizing such as ultraviolet (UV) light (which is absorbed by DNA at wavelength 240-280. it has low penetrability and is used primarily for irradiation of air and flats, also it can be used in hospitals)

4- Osmotic pressure.

5-PH: acidic (2-6), alkaline (8-above), neutral (6.5-7.5)

6-Drying: It ceases of metabolism in cells because of unavailable humidity.

7-Lyophiliation (freeze-drying) is a more practical way of preserving M.O for storage. The principle of lyophilization is that the culture is dried in a glass vial while in the frozen state by removing the water through a process called sublimation. That is water is removed from the frozen as a vapour by using a high vacuum system.

8-Surface tension: it affects the permeability of cytoplasmic membrane, which led to leak the components of the cell outside.

B- Chemical agents:

Compounds have the ability to kill or prevent growth or metabolism of M.O.

Typical properties must found in these chemical agents are:

1- Antimicrobial activity.
2- Solubility.
3- Stability.
4- Non-toxic to human and animal and should be extremely toxic to M.O at room temperature.
5- Homogenicity.
6- Acting only with M.O and not with other organic compound Capacity to penetrate into M.O cell.
7- Non-corrosive and non-staining to human.
8- Have a good smell.
10-Detergent capacities.
11-Availability.

The major antimicrobial chemical agent is:

1- **Phenol (carbolic acid) and phenol derivatives:** it can be used as a disinfectant for organic matter or at high concentration; it acts as a cytoplasmic poison by disrupting the cell wall. Phenol inactivates important enzyme system in the cell and denatures cell proteins.

2-Alcohols: are capable of killing vegetative forms of bacteria and fungi but are inactive against spores. They are more effective at concentration between60-90%. Alcohols also act as **proteins denaturants** and **lipid solvents** and exert their activity on microbial membranes as well as the lipid envelope of viruses. They are also **dehydrating agents**.

3-Halogens: The halogens chlorine, bromine, fluorine, and iodine are very strong oxidizing agents. They inactivate proteins by oxidizing **sulfurdryl groups**.

4-Dyes: such as malachite green, birilliant green, and crystal violet.

5-Ammonia: it has an affect mainly on gram-positive bacteria.

6-Quaternary ammonium compounds: the bactericidal effect is high against gram positive and also quite active against gram negative bacteria. It action is inactivate certain enzyme of M.O through their capacity to combine with and denature proteins and cause some damage to cytoplasmic membrane.

7-Acids and alkalines: the killing action of mineral acids such as HCl ,and H2SO4 is a function of the degree of dissociation of the final hydrogen ion concentration. Alkalies action is dependent on dissociation and the resulting concentration of hydroxyl ions.

8-Gaseous: it highly flammable even in low concentration therefore it mixed with CO2 because of its power to penetrate, it is used to sterilize large packages, bundles of cloths, and even certain plastics. The mode of action is alkylation reaction with organic compounds such as enzymes and other proteins.

9-Antibiotics: major chemotherapeutic agents based on mechanism of action:

1- **Inhibitors of cell membrane function**: they change permeability of the microbial cell, such as nystatin.
2- **Inhibitors of cell wall synthesis**: such as penicillins and cephalosporins.
3- **Inhibitors of nucleic acids synthesis**: such as rifampin.
4- **Inhibitors of protein synthesis**: there is many drugs used today that are classified as inhibitors of protein synthesis and in most instances they exhibit greater affinity for bacterial ribosomes. The major inhibitors or protein synthesis are tetracycline.

Lec. 7 Immunology

Microorganisms that cause pathology in humans and animals enter the body at different sites and produce disease by a variety of mechanisms. Many different infectious agents can cause pathology, and those that do are referred to as **pathogenic microorganisms** or **pathogens**. Invasions by microorganisms are initially countered, in all vertebrates, by innate defense mechanisms that preexist in all individuals and act within minutes of infection. Only when the innate host defenses are bypassed, adaptive immune response required.

Our bodies are constantly exposed to microorganisms present in the environment, including infectious agents that have been shed from infected individuals. Contact with these microorganisms may occur through external or internal epithelial surfaces: the **respiratory tract mucosa** provides a route of entry for airborne microorganisms, the **gastrointestinal mucosa** for microorganisms in food and water; insect bites and wounds allow micro-organisms to penetrate the skin; and direct contact between individuals offers opportunities for infection of the skin and reproductive mucosa. The **normal flora** can also produce antimicrobial substances, such as the **colicins** (anti-bacterial proteins made by Escherichia coli) that prevent colonization by other bacteria.

Routes of infection for pathogens

Route of entry	Mode of transmission	Pathogen	Disease
Mucosal surfaces			
Airway	Inhaled droplets	Influenza virus *Neisseria meningitidis*	Influenza Meningococcal meningitis
Gastrointestinal tract	Contaminated water or food	*Salmonella typhi* Rotavirus	Typhoid fever Diarrhea
Reproductive tract	Physical contact	*Treponema pallidum*	Syphilis

Infectious disease occurs when a microorganism succeeds in evading or overwhelming innate host defenses to establish a local site of infection and replication that allows its further transmission. In some cases, the initial infection remains local and does not cause significant pathology. In other cases, the infectious agent causes significant pathology as it spreads through the lymphatics or the bloodstream, or as a result of secreting toxins.

Our surface epithelia are more than physical barriers to infection; they also produce chemical substances that are microbicidal or inhibit microbial growth. For example, the antibacterial enzyme **lysozyme** is secreted in tears and saliva. The **acid pH** of the stomach and the **digestive enzymes** of the upper gastrointestinal tract create a substantial chemical barrier to infection.

Intrinsic epithelial barriers to infection	
Mechanical	Epithelial cells joined by tight junctions Longitudinal flow of air or fluid across epithelium Movement of mucus by cilia
Chemical	Fatty acids (skin) Enzymes: lysozyme (saliva, sweat, tears), pepsin (gut) Low pH (stomach) Antibacterial peptides: defensins (skin, gut), cryptidins (intestine)
Microbiological	Normal flora compete for nutrients and attachment to epithelium and can produce antibacterial substances

Surface epithelia provide mechanical, chemical, and microbiological barriers to infection

The immune system can be divided into three basic lines of defense against pathogenic infection:

- The first line of defense against infection are the **surface barriers** that prevent the entry of pathogens into the body
- The second line of defense are the **non-specific phagocytes** and other internal mechanisms that comprise **innate** immunity
- The third line of defense are the **specific lymphocytes** that produce antibodies as part of the adaptive immune response

The Immune System: Three Lines of Defense

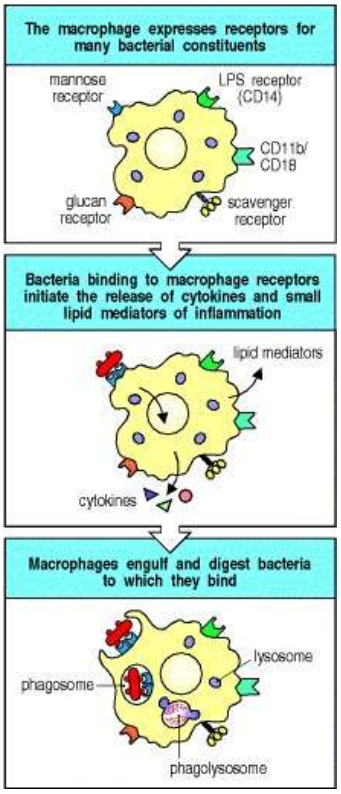
NON-SPECIFIC DEFENCES (INNATE IMMUNITY)		SPECIFIC DEFENCES (ADAPTIVE IMMUNITY)
First line of defense	Second line of defense	Third line of defense
• Skin • Mucous membranes • Secretions of skin and mucous membranes	• Phagocytic leukocytes • Antimicrobial proteins • Inflammatory response • Fever	• Lymphocytes • Antibodies • Memory cells

First Line of Defense

- The primary defence against infectious disease are the surface barriers that prevent pathogens from entering the body
- These surface barriers include intact **skin** (protect external boundaries) and **mucous membranes**.
- Both the skin and mucous membranes release chemical secretions which restrict the growth of microbes on their surfaces
- If pathogens cannot enter the host body, they cannot disrupt normal physiological functions and cause disease

Second Line of Defense

- The second line of defence against infection are the non-specific cellular and molecular responses of the innate immune system
- These defences do not differentiate between different types of pathogen and respond the same way upon every infection
- Phagocytic leukocytes migrate to infection sites and engulf foreign bodies (**dendritic cells** then present antigens to **lymphocytes**)
  - Inflammatory responses increase capillary permeability at infected sites, recruiting leukocytes but leading to localised swelling
  - Antimicrobial proteins (such as cytokines and complement proteins) regulate immune activity within the body
  - Fever increases body temperatures to activate heat-shock proteins and suppress microbial growth and propagation



Phagocytes bear several different receptors that recognize microbial components and induce phagocytosis.

Third Line of Defense

- The final line of defence against infection are the lymphocytes that produce **antibodies** to specific antigenic fragments
- Each **B cell** produces a specific antibody, and the body has millions of different B cells capable of detecting distinct antigens
- **Helper T cells** regulate B cell activation, ensuring that antibodies are only mass-produced at the appropriate times
- Both B and T cells will differentiate to form **memory cells** after activation, conferring long-term immunity to a particular pathogen

Lec.8 Microbial genetics

Nucleic acids types

The genetic information of prokaryotic and eukaryotic microorganisms encoded within the DNA (deoxyribonucleic acid) molecule and sometimes (as in viruses) in the RNA (ribonucleic acid) molecule. These molecules are known as macromolecules and they are responsible for the transition of hereditary information from one generation to the other. Protein macromolecule is the result of the genetic code into its structural or functional form.

The structure of nucleic acids and their replication

The genetic information of a cell forms a GENOME. The genome of a microorganism is divided into segments consisting of DNA nucleotides sequences known as a GENE. These genes may have structural or functional, metabolic functions .

DNA structure

The DNA is a double helix where each strand is composed of a sequence of nucleotides; phosphodiester bonds link these nucleotides to each other. Each nucleotide is formed of **a deoxyribose sugar, a nitrogen base** and **a phosphate group** (Figure 1 a).

Four nitrogen bases are found in DNA: adenine (A), guanine (G), cytosine (C), and thymine (T). A and G are purines, while C, T and U are pyrimidines (Figure 1 c).

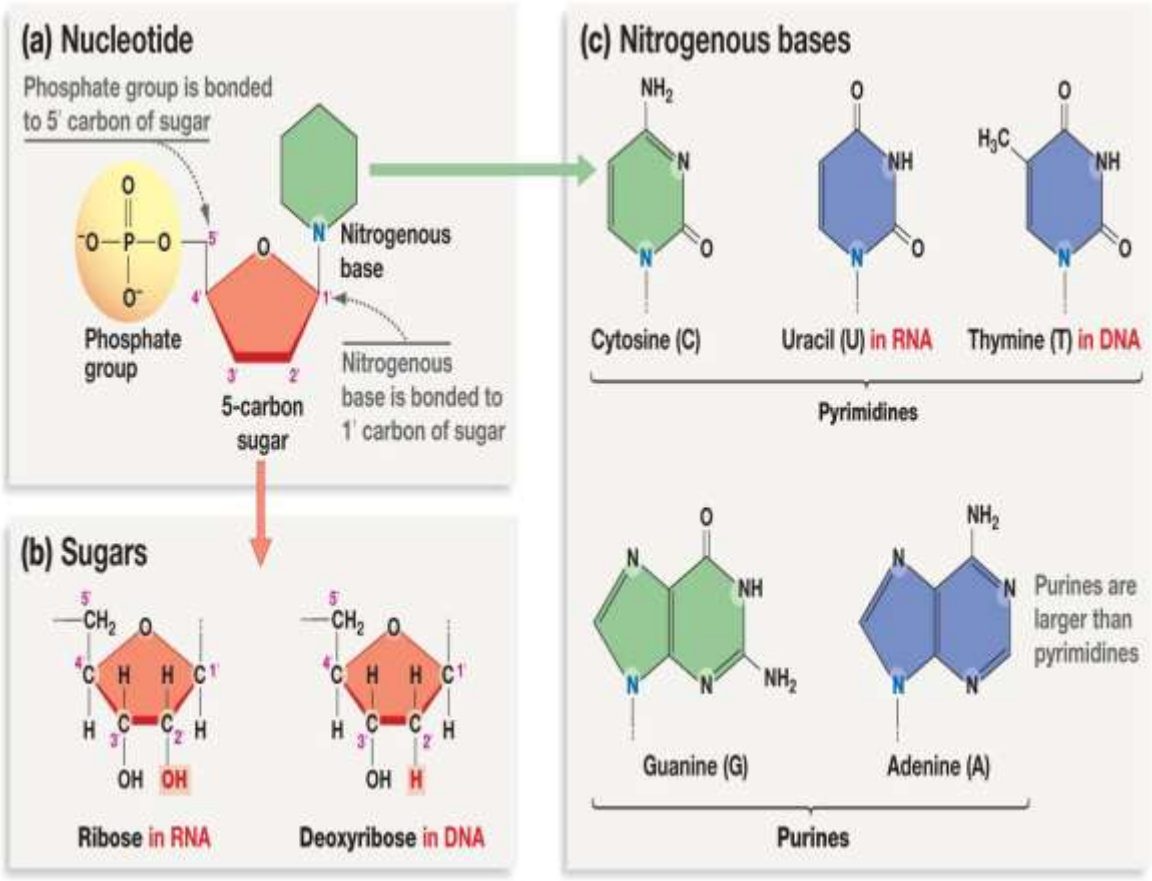


Figure 1: DNA structure

The primary structure of DNA

It is resembled by the sequence of nucleotides in a single strand. In this structure when the nitrogen base is bound to the sugar it is known as a **nucleoside**, when a phosphate group is linked to the nucleoside it is known as **nucleotide** (Figure 2).

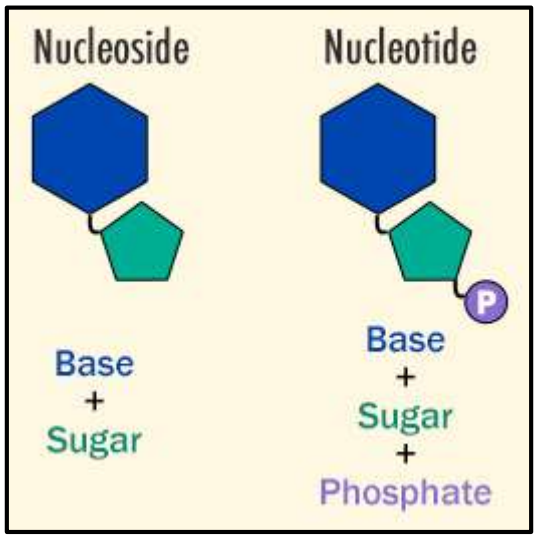


Figure 2: Nucleoside and Nucleotide structure

The secondary structure of DNA

The two strands of the double - helix are complementary and antiparallel. They are complementary because **A** in one strand always connected by a **double** hydrogen bonds with **T** of the complementary strand (forming what is called a base pair); **C** always connected (base pair) by **triple** hydrogen bonds with **G** of the complementary strand (Figure 3).

They are antiparallel because the 5' ⟶ 3' strand starts with a 5' -$PO_4^{=}$ group and ends with a 3'–OH free group while the complementary strand has inverse polarity starting with 3' -OH ending with 5'-$PO_4^{=}$ (3' - 5')

This DNA double-helix model was proposed by Watson and Crick in 1953.

In a DNA molecule, each turn in a double helix has 10 bases, and the diameter of a single turn is two nm (1 Kilobase = 1000 base pairs and has a molecular weight of 3.3 x 10^5 per strand).

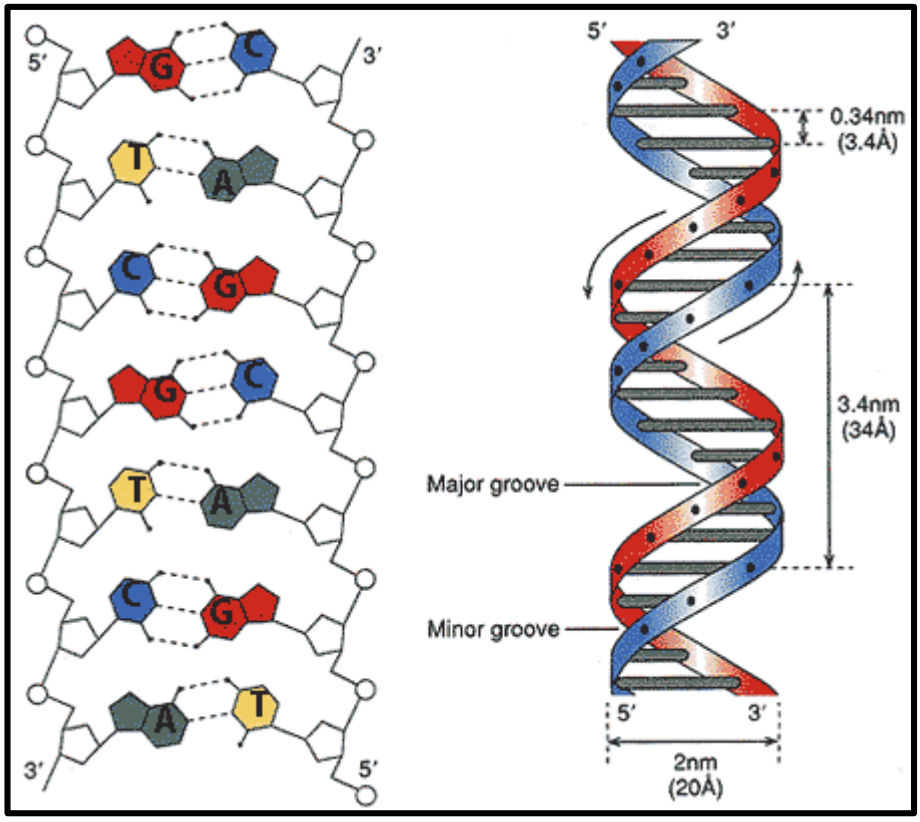


Figure 3: Secondary structure of DNA

A DNA molecule always carries a negative charge due to the PO_4 groups. These charges are neutralized by alkaline proteins known as histones in eukaryotes, histone-like proteins in prokaryotes.

Structure of RNA molecule

An RNA molecule is usually single stranded; it has a sequence of ribonucleotides each is formed of a ribose sugar, a nitrogen base (A, G, C, and **Uracil (U) instead of thymine** (Figure 4)), and a phosphate group. A ribonicleoside is formed of a **ribose** (Figure 1 b) sugar and a nitrogen base.

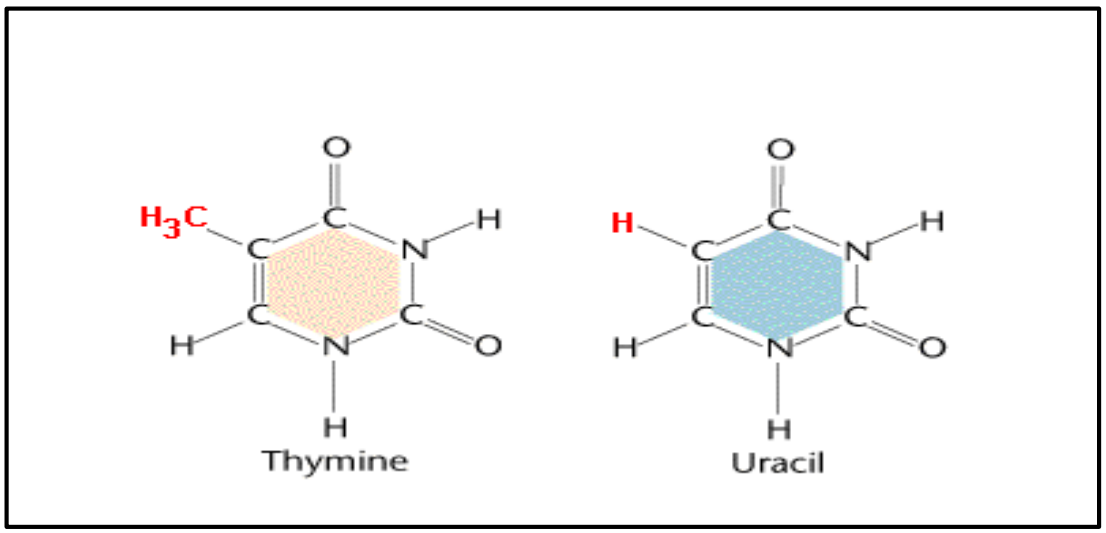


Figure 4: Uracil structure

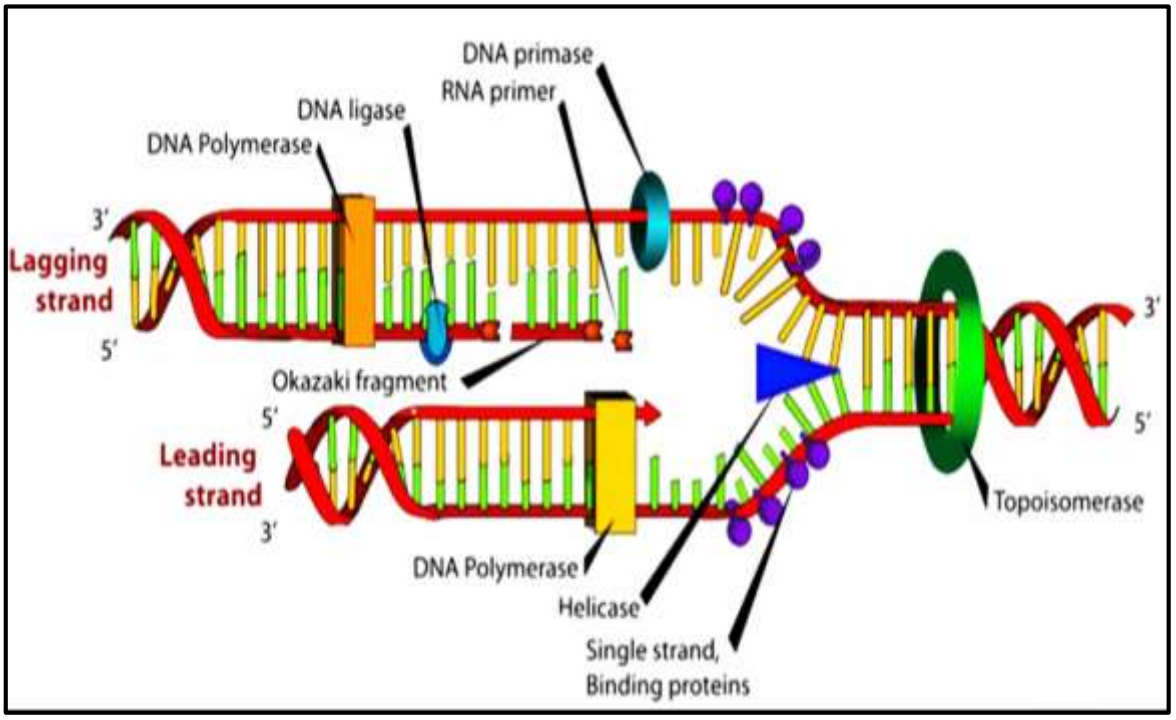


Figure 5: Semi-conservative DNA replication

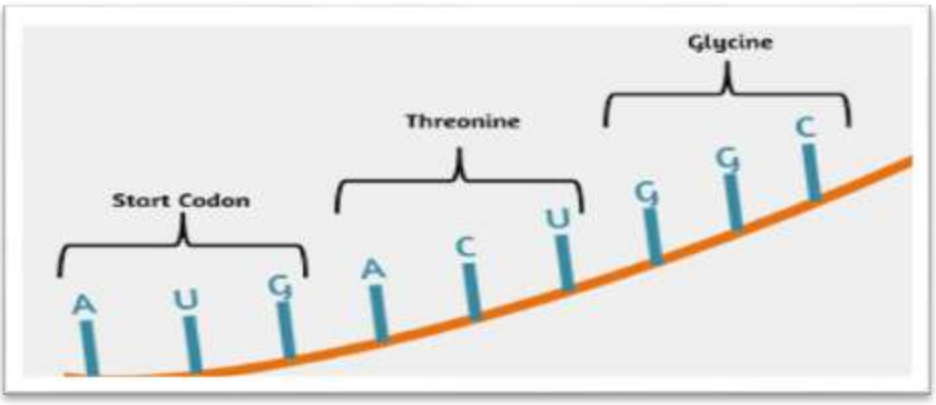
Types of RNA and steps in proteins synthesis

There are three types of RNA (mRNA, tRNA, and rRNA). Their roles will be described within the process of protein synthesis.

1-Messenger RNA (mRNA):

It is formed in the nucleus of eukaryotes and nuclear region of prokaryotes. It carries the information transcribed from the DNA to the ribosomes (in the cytoplasm) where protein is synthesized. It is transcribed from a single strand of DNA and is complementary to that strand.

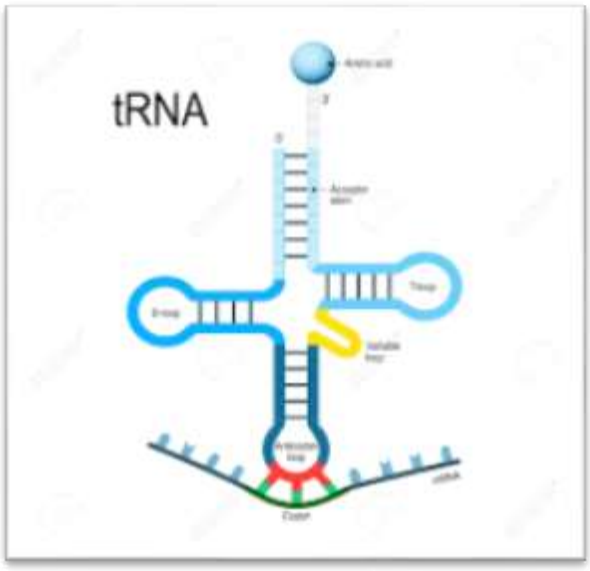
mRNA is a single strand with a sequence of ribonucleotides to be translated by the ribosomes to the required protein.



Transcription is the first step in protein synthesis. The second step in protein synthesis is translation; it requires the presence of the other two types of RNA; transfer RNA tRNA and ribosomal RNA (rRNA).

2-Transfer RNA (tRNA)

It is also known as soluble RNA has a distinguished clover leaf structure and two recognition sites; one binds to an activated amino acid, the second is known as the anticodon that recognizes the codon on the mRNA.

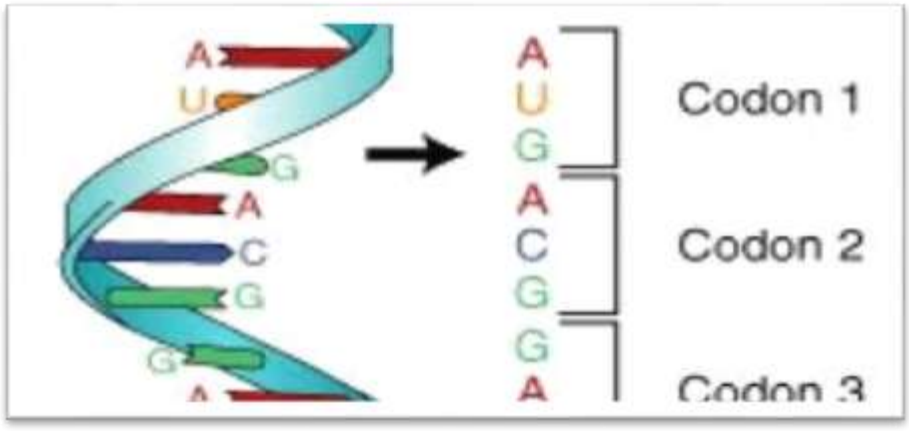


3- Ribosomal RNA (rRNA)

rRNA is a type of non-coding RNA that is a primary and permanent component of ribosomes. As non-coding RNA, rRNA itself is not translated into a protein, but it does provide a mechanism for decoding mRNA into amino acids and interacting with the tRNAs during translation by providing peptidyltransferase activity.

The genetic code

Every **codon** is made of three nucleotides coding for one amino acid, and since there are four nitrogen bases the probabilities of the number of genetic codes are $4^3 = 64$. There are 20 amino acids therefore there could be more than one code for most amino acids. For each amino acid there is one or more tRNA that carries thespecific activated amino acid to the ribosome.



Plasmids

They are DNA molecules other than the chromosomal DNA and are found in prokaryotic microorganisms and some eukaryotes such as yeasts. A plasmid is a double stranded DNA segment found in the cytoplasm unrelated to the chromosome and can replicate independently.

Transfer of genetic materials bacteria

There are three different mechanisms for genetic exchange in bacteria:

1-Transformation

A free DNA molecule is transferred to a recipient cell of bacteria. The double stranded DNA could enter as it is like in Gram negative *Haemophilus* cells or it could enter single stranded as mostly happens in Gram positive cells. After entering the cell the single strand DNA recombines with the DNA of the recipient cell and the new cell is known as the **transformed cell**. A special treatment is needed to facilitate the entrance of the DNA to the recipient cell; this could be accomplished by treatment of recipient cells with $CaCl_2$, then it is known as a competentcell (ready to receive the new DNA).

2-Transduction

It occurs when the DNA of a donor is transferred to the recipient bacteria by a bacteriophage. Transfer of the genetic material by this method is more frequent than by transformation.

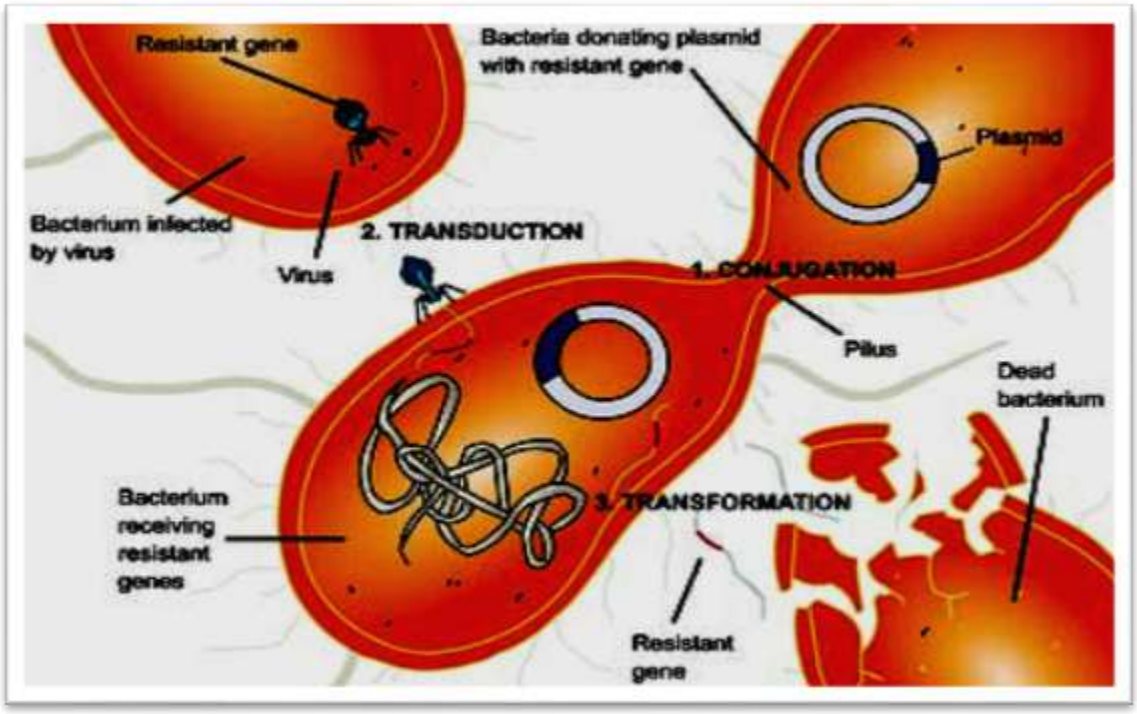
3-Conjugation

Another method for genetic transfer, this method requires the presence of the F (fertility or sex) factor present on the F-plasmid, which is responsible for the formation of sex pili or Conjugation Bridge from donor (r) cells to the recipient (F) cells. Some donor (r) cells are known as high frequency recombinants (HFr) they are (1000) times more competent than the recipientcells in transferring the genetic material.

Mutation in bacteria

The term mutation applies to all heritable changes in nucleotide sequence arising within an organism, they may be: 1- Spontaneous (naturally occurring) 2- Induced by

some mutagenic agents, this could be chemical or physical. When the mutant is not altered phenotypically but only genotypically it is known as **Silent mutation**. A mutation could occur by deletion, insertion of a nucleotide, transition, (purine into pyrimidine or pyrimidine into purine) or transversion (purine into purine or pyrimidine into pyrimidine). **A point mutation** occurs when one nucleotide is inserted or deleted.



Lec.9 Pathogenic Microorganism

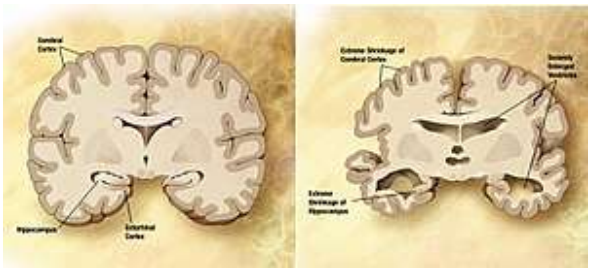
There are different types of microorganisms. These microorganisms may be harmless, harmful or beneficial to their hosts. Microorganisms may cause kinds of communicable diseases by competing metabolic resources, destroying cells or tissues, or secreting toxins.

Pathogenic microorganism may be viruses, bacteria, mycobacteria, fungi, protozoa and infects people or animals in direct or indirect ways. Virus often results in serious diseases. Recent years, human have been challenged by several diseases caused by virus, such as SARS, Ebola virus, hepatitis B, HIV etc. Pathogenic microorganism cause disease worldwide regardless of location, age or socio-economic status. Human infectious diseases come in many forms and span all domains of life.

Types of pathogens

Prions

Prions are misfolded proteins that can transfer their misfolded state to other normally folded proteins of the same type. They do not contain any DNA or RNA and cannot replicate other than to convert already existing normal proteins to the misfolded state. These abnormally folded proteins are found characteristically in some diseases such as bovine spongiform encephalopathy (mad cow disease) and Creutzfeldt–Jakob disease, Alzheimer's disease.



Comparison of a normal aged brain (left) and the brain of a person with Alzheimer's (right).

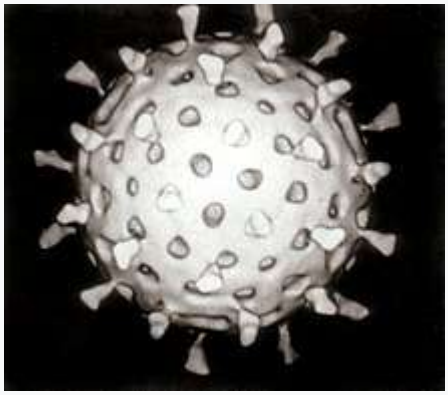
Viruses

Viruses are small particles, typically between 20 and 300 nanometers in length. containing RNA or DNA. Viruses require a host cell to replicate. Some of the diseases that are caused by viral pathogens include smallpox, influenza, mumps, measles, chickenpox, ebola, HIV, and rubella.

Pathogenic viruses are diseases mainly of the families of: Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, and Togaviridae. HIV is a notable member of the retroviridae family which affected 37.9 million people across the world in 2018.

Viruses and diseases:

Type	Transmission	Diseases
Hepatitis A virus	• fecal-oral	• acute hepatitis
Hepatitis B virus	• bodily fluids Vertical and sexual	• acute hepatitis • chronic hepatitis • hepatic cirrhosis • hepatocellular carcinoma
Hepatitis C virus	• blood • sexual contact	• acute hepatitis • chronic hepatitis • hepatic cirrhosis • hepatocellular carcinoma
HIV	• sexual contact • blood • breast milk • vertical transmission	• AIDS
Influenza virus	• droplet contact	• influenza
Measles virus	• droplet contact	• measles
Poliovirus	• fecal-oral	• poliomyelitis[
Rabies virus	• animal bite • droplet contact	• rabies (fatal encephalitis}



Rotavirus

Bacteria

The vast majority of bacteria, which can range between 0.15 to 700 μM in length, are harmless or beneficial to humans. However, a relatively small list of pathogenic bacteria can cause infectious diseases. Pathogenic bacteria have several ways that they can cause disease. They can either directly affect the cells of their host, produce endotoxins that damage the cells of their host, or cause a strong enough immune response that the host cells are damaged.

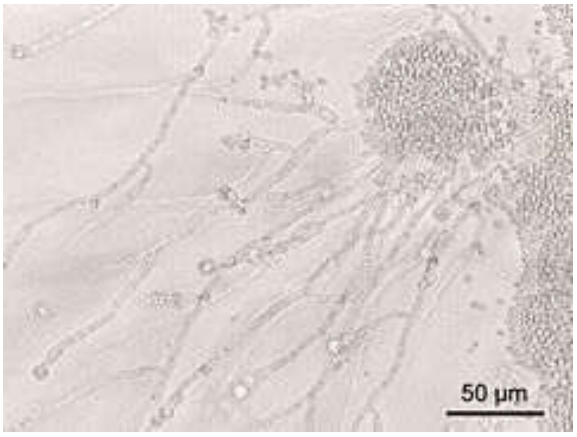
One of the bacterial diseases with the highest disease burden is tuberculosis, caused by the bacterium Mycobacterium tuberculosis, which killed 1.5 million people in 2013, mostly in sub-Saharan Africa. Pathogenic bacteria contribute to other globally significant diseases, such as pneumonia, which can be caused by bacteria such as Streptococcus and Pseudomonas, and foodborne illnesses, which can be caused by bacteria such as Shigella, Campylobacter, and Salmonella. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy.

Examples of bacterial pathogens and diseases

Bacterium	Disease
Staphylococcus aureus	toxic shock syndrome
E. coli	hemorrhagic colitis; hemolytic uremic syndrome
Helicobacter pylori	gastric and duodenal ulcers
Vibrio cholerae	epidemic cholera
Salmonella	salmonellosis

Fungi

Fungi are eukaryotic organisms that can function as pathogens. There are approximately 300 known fungi that are pathogenic to humans including Candida albicans, which is the most common cause of thrush, and Cryptococcus neoformans, which can cause a severe form of meningitis. The typical fungal spore size is <4.7 μm micrometers in length, but some spores may be larger.



***Candida albicans* at 200× magnification**

Algae

Algae are single-celled plants that are generally non-pathogenic although pathogenic varieties do exist. Protothecosis is a disease found in dogs, cats, cattle, and humans caused by a type of green alga known as prototheca that lacks chlorophyll.

Algae

Human parasites

Some eukaryotic organisms, including a number of protozoa and helminths, are human parasites.

Types:

There are three main types of parasites.

Protozoa: Examples include the single-celled organism known as Plasmodium. A protozoa can only multiply, or divide, within the host.

Helminths: These are worm parasites. Schistosomiasis is caused by a helminth. Other examples include roundworm, pinworm, trichina spiralis, tapeworm, and fluke.

Ectoparasites: These live on, rather than in their hosts. They include lice and fleas, such as giardiasis and amebic dysentery, can cause abdominal pain.

Lec. 10 Food microbiology

Food microbiology is the study of the microorganisms that inhibit, create, or contaminate food, including the study of microorganisms causing food spoilage, pathogens that may cause disease especially if food is improperly cooked or stored, those used to produce fermented foods such as cheese, yogurt, bread, beer, and wine, and those with other useful roles such as producing probiotics.

Food spoilage:

Food spoilage Caused by,

1. Bacteria

2. Yeasts and Molds

3. Viruses

The food processor reduces potential problems from microorganisms in several ways:

- Removing or destroying them by trimming, washing, heating, pickling, by adding chemicals, or by encouraging competition by acid- or alcohol-forming organisms.
- Minimizing contamination from equipment, people, the environment, and from unprocessed food.
- Minimizing microbial growth on equipment, by cleaning and sanitizing, and in the product itself by adjusting storage temperature, pH, Water activity, Oxygen and other environmental factors.

Although each factor affecting growth is considered separately in the following discussion, these factors occur simultaneously in nature. When more than one condition is somewhat averse to microbial growth, their inhibitory effects are cumulative.

ex. of Microrganisms cause foodborne disease:

Salmonella

Clostridium botulinum

Clostridium perfringens

Staphylococcus aureus

Vibrio parahaemolyticus

The "Indicator" Organisms

The "indicator" organisms are so called because their presence in large numbers in food signifies one of three contamination possibilities: disease bacteria or filth; spoilage or low quality; or preparation under insanitary conditions.

- Failure of sorting, trimming, washing, and destroying operations to remove or destroy bacteria from raw ingredients adequately.
- Inadequate heat processing.
- Insanitary equipment, particularly near the end of the process.
- The food has reached or is approaching the end of its refrigerated shelf-life.
- The food has been stored at or above room temperature for too long.
- The food is at least partly decomposed.

Coliform Bacteria

The coliform bacteria are non-spore forming rods that occur in large numbers in human and animal feces. They are normally present on raw animal products, such as meats, milk, and eggs, and also occur naturally in soil, water, and surfaces of plants. They are heat sensitive and die rapidly during blanching or pasteurizing. Large numbers of coliforms after a heat process indicate an unacceptable degree of post-heating contamination or indicate time-temperature abuse of the food sufficient to permit growth. High coliform levels warrant investigations to determine the source of contamination or temperature mishandling.

The presence of Escherichia colia, member of the coliform group, in food usually indicates direct or indirect human or animal fecal contamination. Although this may be true in a broad sense, one must not assume a quantitative relationship between the numbers of E. coli and the degree of contamination with feces. E. coli grows well outside the animal body and thrives in unclean food handling equipment.

Food Poisoning

Human illnesses caused by foodborne microorganisms are popularly referred to as food poisoning. The common use of a single classification is due primarily to similarities of symptoms of various food-related diseases (see Table 5). Apart from illness due to food allergy or food sensitivity, foodborne illness may be divided into two major classes, food infection and food intoxication. Food infection results when foods contaminated with pathogenic, invasive, food poisoning bacteria are eaten. These bacteria then proliferate in the human body and eventually cause illness. Food intoxication follows the ingestion of preformed toxic substances which accumulate during the growth of certain bacterial types in foods.

The period of time between the consumption of contaminated foods and the appearance of illness is called the incubation period. The incubation period can range anywhere from less than one hour to more than three days, depending on the causative organisms or the toxic product.

Table 5. Characteristics of the important bacterial food intoxications and foodborne infections. (NAS-NRC, 1975)*

Disease	Etiologic Agent	Incubation Period	Symptons
Botulism	*Clostridium botulinum* A.B.E.F toxin	Usually 1 to 2 days; range 12 hours to more than 1 week	Difficulty in swalling, double vision, difficulty in speech. Occasionally nausea, vomiting, and diarrhea in early stages. Constipation and subnormal temperature. Respiration becomes difficult, often followed by death from paralysis of muscles of respiration.
Salmonellosis	Specific infection by *Salmonella* spp.	Average about 18 hours; range 7 to 72 hours	Abdominal pains, diarrhea, chills, fever, frequent vomiting, prostration. Duration of illness: 1 day to 1 week.
Shigellosis (bacillary dysentery)	Shigella sonnei, s. flexneri, s. dysenteriae, s. boydii	Usually 24 to 48 hours; range 7 to 48 hours	Abdominal cramps, fever, chills, diarrhea, watery stool (frequently containing blood, mucus, or pus), spasm, headache, nausea, dehydration, prostration. Duration: a few days.

Food safety

is a major focus of food microbiology? Numerous agents of disease, pathogens, are readily transmitted via food, including bacteria, and viruses. Microbial toxins are also 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.[5] Thorough preparation of food, including proper cooking, eliminates most bacteria and viruses. However, *toxins produced* by contaminants may not be liable to change to non-toxic forms by heating or cooking the contaminated food due to other safety conditions.

Food testing

To ensure safety of food products, microbiological tests such as testing for pathogens and spoilage organisms are required. This way the risk of contamination under normal use conditions can be examined and food poisoning outbreaks can be prevented. Testing of food products and ingredients is important along the whole supply chain as possible flaws of products can occur at every stage of production.[9] Apart from detecting spoilage, microbiological tests can also determine germ content, identify yeasts and molds, and salmonella. For salmonella, scientists are also developing rapid and portable technologies capable of identifying unique variants of Salmonella .

Polymerase Chain Reaction (PCR) is a quick and inexpensive method to generate numbers of copies of a DNA fragment at a specific band ("PCR (Polymerase Chain Reaction)," 2008). For that reason, scientists are using PCR to detect different kinds of viruses or bacteria, such as HIV and anthrax based on their unique DNA patterns. Various kits are commercially available to help in food pathogen nucleic acids extraction,[11] PCR detection, and differentiation.[12] The detection of bacterial strands in food products is very important to everyone in the world, for it helps prevent the occurrence of food borne illness. Therefore, PCR is recognized as a DNA detector in order to amplify and trace the presence of pathogenic strands in different processed food.

Fermentation

Fermentation is one of the methods to preserve food and alter its quality. 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. Some cheese varieties also require molds to ripen and develop their characteristic flavors.

Microbial biopolymers

Several microbially produced biopolymers are used in the food industry

Alginate

Alginates can be used as thickening agents.[7] Although listed here under the category 'Microbial polysaccharides', commercial alginates are currently only produced by extraction from brown seaweeds such as *Laminaria hyperborea* or *L. japonica*.

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.

Lec: 11 Soil Microbiology

Soils and potting media provide plants and other organisms with nutrients and habitats. Because bacterial and fungal microorganisms in soils and potting media are constantly vying for food, water, and space, soils are regarded as dynamic living environments. Microbes in these substrates obtain nutrients by competing with each other for dead organic matter, feeding on other living organisms (including each other), and/or interacting cooperatively with other organisms.

How soil microorganisms directly or indirectly affect plant growth and health determines if they are considered beneficial, harmful, or insignificant to plants. Microbes that harm plants are plant pathogens since the harm that they cause is considered disease. On the other hand, beneficial microorganisms can either enhance plant growth, suppress plant diseases, or both.

Plant Growth Promotion

Plant growth is improved by beneficial microbes through at least two modes of action . Increasing nutrient availability is one of them. Numerous soil microorganisms help plants obtain otherwise unavailable nutrients by converting these nutrients into plant-available form in exchange for energy from their hosts. Another modes of action is the stimulation of plant growth without actually increasing nutrient availability to plants. Certain beneficial bacteria and fungi stimulate plant growth through the production of metabolites or by their physical interactions with host plants. Several bacterial strains and fungal isolates that promote plant growth without improving nutrient uptake have been identified, though the specific mechanisms that cause these stimulatory effects are unclear.

Plant Disease Suppression

Beneficial microbes help to control plant diseases by the following mechanisms:

• Predation and Hyperparasitism = feeding on pathogens

• Antagonism, competitive exclusion and microbiostasis = competing for nutrients or space by producing metabolites that kill pathogens or inhibit their growth and movement;

• Rhizospere competency = blocking pathogen access to plant roots;

• Induced systemic resistance and systemic acquired resistance = stimulating or priming the plant’s own natural defense system.

As a general rule, disease-suppressive microorganisms work best at preventing rather than curing diseases.

Hyperparasitism: Holes in Rhizoctonia solani hyphal strand produced by digestive enzymes of Trichoderma harzianum strain T-22

Commercially Available Beneficial Microorganisms

Examples of commercially available beneficial microbes include bacterial strains belonging to the genera Bacillus, Streptomyces, and Pseudomonas, and fungal isolates belonging to the genera Trichoderma and Glomus.

Lec. 12 Aquatic Microbiology

Aquatic microorganisms include members of the plant kingdom, protozoa, bacteria, and fungi. These organisms differ radically, and share only their small size; most are not visible without a microscope, though colonies of some can be seen with the naked eye.

Microorganisms are present in large quantities everywhere and can survive extreme physical and chemical conditions. Many microorganisms play foundational roles in aquatic ecosystems, capturing the sun's energy through photosynthesis and, through their role in decomposition, releasing nutrients stored in organic tissue.

Bacteria

Some of the smallest and most ancient organisms on earth, bacteria are present in virtually every environment and are abundant in all aquatic systems. In rivers and streams, many of the bacteria wash in from the surrounding land, and their abundance can increase dramatically after a rainfall. The abundance of bacteria is typically in the millions per millilitre (mL), and in the hundreds of millions per millilitre in especially productive or polluted waters.

If conditions are right, bacteria reproduce extremely rapidly by simple division to produce very large numbers in a short period of time. Bacteria can be found suspended in the water, associated with decaying material (such as dead wood or leaves), or coating the surface of rocks, stones and sand grains as part of the biofilm (the slippery coating on hard surfaces in rivers). They can make up a large fraction of the living material in aquatic systems.

Bacteria display the greatest range in metabolic ability of any group of organisms. There are both autotrophic and heterotrophic bacteria. Heterotrophic bacteria are a crucial link in the decomposition of organic matter and the cycling of nutrients in aquatic system.

Autotrophic bacteria are primary producers in aquatic systems as are true algae. For this reason, autotrophic bacteria (predominantly cyanobacteria) are often categorized as 'algae', though the organisms are by no means closely related. Cyanobacteria used to be mistakenly called 'blue-green algae'. Ecologically, much of what applies to algae is relevant to autotrophic bacteria.

Fungi

Fungi occur as single cells, and in filaments called hyphae. Most aquatic fungi are microscopic; those known as hyphomycetes are the most abundant and important. Fungi are heterotrophic, and, like heterotrophic bacteria, obtain their nutrition by secreting exoenzymes into their immediate environment, which break compounds down into simpler substances the fungi can absorb. Fungi are critical to the decomposition of plant matter in aquatic systems, because they are among the few organisms that can break down certain plant structural compounds such as cellulose and lignin.

Protozoa

Protozoa are microscopic, single-celled organisms that sometimes group together into colonies. There are both autotrophic and heterotrophic types of protozoa. Unlike bacteria and fungi, which absorb dissolved organic compounds from their environment, heterotrophic protozoa (such as the amoebas and *Paramecium*) consume other organisms such as algae, bacteria, or other protists. Together with other microorganisms, protozoa make up the biofilm coating sediments and hard surfaces on riverbeds, though some protozoa are free-swimming. Certain protozoa are parasites and cause diseases such as giardia (beaver fever).

Algae and Phytoplankton

Several groups of largely autotrophic protists are referred to as algae. Like the term 'microorganisms' it is an informal term, used for convenience to describe microorganisms that carry out photosynthesis; the cyanobacteria are often included as algae. Algae vary in size from microscopic to large colonies that can be considered macrophytes. Several types of algae—including phytoplankton—play an important role in supplying the energy at the base of many aquatic food webs.

Phytoplankton are small, microscopic plants that live suspended in the open water. Phytoplankton are generally more abundant in lakes than rivers, and are absent from fast-flowing streams, or where the rate at which the plants are washed downstream is greater than the rate at which they reproduce. Damming a river leads to still-water conditions more suitable for phytoplankton, and nuisance algal blooms may develop in reservoirs. Inputs of nutrients, including nitrogen and phosphorus, can also lead to algal blooms.

Phytoplankton can exist as single cells or in chains or colonies. Phytoplankton are direct food sources for many zooplankton and some fish, and are the base of the food web in deep waters. Phytoplankton vary in their requirements for nutrients, light, and other conditions. Waterbodies support a complex mixture of phytoplankton that can change markedly with environmental conditions. In rivers containing significant amounts of phytoplankton, the concentration of algal cells (number per unit volume) is generally highest when flows are lowest, while elevated suspended sediment loads during high flows can lead to reduced light and photosynthesis. Some phytoplankton can cause taste and odor problems in water, and anoxic conditions that can kill fish. Some cyanobacteria produce toxins lethal to various fish, wildlife, and domestic species.

Periphyton and Biofilm

Algae, bacteria, fungi, protozoa, and the breakdown products of dying cells form layers on submerged surfaces, including bottom sediment, rocks, submerged leaves and branches, and macrophytes. The term *periphyton* refers to a layer consisting mainly of algae, but the entire assemblage of layers is often known as *biofilm*. Periphyton is an important food source in shallow, stony rivers with adequate light penetration. Heterotrophic organisms, including larger invertebrates such as snails and

insects, scrape the biofilm from surfaces, while some larger animals, such as fish, also feed on biofilm. Biofilm can be important in absorbing or breaking down chemical contaminants as well. Seasonal changes in the abundance of periphyton reflect fluctuations in river discharge, as layers of algal cells build up in times of low or decreasing flow, and wash away during flood periods.

Lec.13 AIR MICROBIOLOGY

The earth's atmosphere is teeming with airborne microorganisms. These organisms are thought to exhibit correlations with air pollution and weather. Most airborne bacteria originate from natural sources such as the soil, lakes, oceans, animals, and humans. Many 'unnatural' origins are also known, such as sewage treatment, animal rendering, fermentation processes, and agricultural activities which disturb the soil. Viable airborne microorganisms are not air pollutants, but should be considered as a factor affecting air quality. Air is an unfavorable environment for microorganisms, in which they cannot grow or divide. It is merely a place which they temporarily occupy and use for movement.

There are 3 elementary **limiting factors** in the air:

- A lack of adequate nutrients
- Frequent deficit of water (desiccation)
- Solar radiation

The atmosphere can be occupied for the longest time by those forms which, due to their chemical composition or structure, are resistant to desiccation and solar radiation. They can be subdivided into the following groups:

- Bacterial resting forms,
- Bacterial vegetative forms which produce carotenoidal dyes or special protective layers (capsules, special structure of cell wall),
- Spores of fungi,
- Viruses with envelopes

Resting forms of Bacteria

Endospores are the best known resting forms. These structures evolve within cells and are covered by a thick multi-layer casing. Consequently, endospores are unusually resistant to most unfavorable environment conditions and are able to survive virtually endlessly in the conditions provided by the atmospheric air. They are only produced by some bacteria, mainly by Bacillus and Clostridium genera. Because each cell produces only one endospore, these spore forms cannot be used for reproduction.

Another type of resting form is produced by very common soil bacteria, the actinomycetes. Their special vertical, filiform cells, of the so-called air mycelium, undergo fragmentation producing numerous ball-shaped formations. Due to the fact that their production is similar to the formation of fungal, they are also called conidia. Contrary to endospores, the conidia are used for reproduction. There are also other bacterial resting forms, among others, the cysts produced by azotobacters - soil bacteria capable of molecular nitrogen assimilation.

Resistant Vegetative Cells of Bacteria

The production of carotenoidal dyes ensures cells with solar radiation protection. Carotenoids, due to the presence of numerous double bonds within a molecule (-C=C-), serve a purpose as antioxidants, because, as strong reducing agents, they are oxidized by free radicals. Consequently, important biological macromolecules are being protected against oxidation (DNA, proteins etc.). Bacteria devoid of these dyes quickly perish due to the photodynamic effect of photooxidation. That explains why the colonies of bacteria, which settle upon open agar plates, are often colored. The ability to produce carotenoids is possessed especially by cocci and rod-shaped actinomycetes. Rod-shaped actinomycetes, e.g. *Mycobacterium tuberculosis*, besides being resistant to light, also demonstrate significant resistance to drying due to a high content of lipids within their cell wall. High survival rates in air are also a characteristic for the bacteria which possess a capsule, e.g. *Klebsiella* genus, that cause respiratory system illnesses.

Fungal Spores

Spores are special reproductive cells used for asexual reproduction. Fungi produce spores in astronomical quantities, for example the giant puffball (*Calvatia gigantea*) produces 20 billion spores, which get into the air and are dispersed over vast areas. A very common type of spores found in air is that of conidia.

Conidia are a type of spore formed by asexual reproduction. They form in the end-sections of vertical hyphae called conidiophores and are dispersed by wind. The spores of common mould fungi such as *Penicillium* and *Aspergillus* are examples of the above. Spore plants such as ferns, horsetails and lycopods also produce spores. Plant pollen is also a kind of spores.

Resistant Viruses

Besides cells, the air is also occupied by viruses. Among those that demonstrate the highest resistance are those with enveloped nucleocapsids, such as influenza viruses. Among viruses without enveloped nucleocapsids, enteroviruses demonstrate a relatively high resistance.

Factors Affecting Growth of Microorganism in Air

There are several factors which influence the ability of a bioaerosol to survive in air:

- Particular resistance for a given microorganism (morphological characteristics)
- Meteorological conditions (inter alia, air humidity, solar radiation),
- Air pollution,
- The length of time in air.

Infectious Airborne Diseases

The mucous membrane of the respiratory system is a specific type of a 'gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO_2 reacts with water that is present in the respiratory system, creating H_2SO_4, which irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases.

Viral diseases

After penetrating the respiratory system with inhaled air, particles of viruses reproduce inside the cuticle cells of both the upper and lower respiratory system. The most noteworthy viruses are:

Influenza (orthomyxoviruses) Influenza, measles, bronchitis, mumps and pneumonia among newborns (paramyxoviruses)

- German measles (similar to paramyxoviruses)
- Colds (rhinoviruses and koronaviruses)
- Cowpox and true pox (pox type viruses)
- Chickenpox (cold sore group of viruses)
- Foot-and-mouth disease (picorna type viruses)
- Meningitis, pleurodynia (enteroviruses)
- Sore throat, pneumonia (adenoviruses)

Bacterial diseases

Similarly to viruses, some bacteria that find their way to the respiratory system may also cause ailments of other systems. Especially staphylococcus infections assume various clinical forms (bone marrow inflammation, skin necrosis, intestinal inflammation, pneumonia). Bacterial airborne diseases include:

- Tuberculosis (*Mycobacterium tuberculosis*),
- Pneumonia (*Staphylococcus*, *Pneumococci*, *Streptococcus pneumoniae*, less frequently chromatobars of *Klebsiella pneumoniae*),
- Angina, scarlet fever, laryngitis (*Streptococcus*),
- Inflammation of upper and lower respiratory system and meningitis (*Haemophilus influenzae*),
- Whooping cough (chromatobars of *Bordetella pertussis*),
- Diphtheria (*Corynebacterium diphtheriae*),
- Legionnaires disease (chromatobars of *Legionella* genus, among others *L. pneumophila*),
- Nocardiosis (oxygen actinomycetes of *Nocardia* genus).

Fungal diseases

Many potentially pathogenic airborne fungi or the so-called saprophytes live in soil. The following are examples of airborne fungi diseases:

- Mycosis (*Microsporum racemosum*),
- Deep mycosis: aspergillosis (*Aspergillus fumigatus*), cryptococcus (*Cryptococcus neoformans*).

Protozoan diseases

Some protozoa, which are able to produce cysts that are resistant to dehydration and solar radiation, may also infect humans by inhalation. The most common example of the above is *Pneumocystis carinii* which causes pneumoni