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Introduction

microbiology, study of microorganisms, or microbes, a diverse group of generally minute simple life-forms that include bacteria, archaea, algae, fungi, protozoa, and viruses. The field is concerned with the structure, function, and classification of such organisms and with ways of both exploiting and controlling their activities.

Historical background

The 17th-century discovery of living forms existing invisible to the naked eye was a significant milestone in the history of science, for from the 13th century onward it had been postulated that "invisible" entities were responsible for decay and disease. The word *microbe* was coined in the last quarter of the 19th century to describe these organisms, all of which were thought to be related. As microbiology eventually developed into a specialized science, it was found that microbes are a very large group of extremely diverse organisms.

Microbiology essentially began with the development of the microscope. Although others may have seen microbes before him, it was Antonie van Leeuwenhoek, a Dutch draper whose hobby was lens grinding and making microscopes, who was the first to provide proper documentation of his observations. His descriptions and drawings included protozoans from the guts of animals and bacteria from teeth scrapings. His records were excellent because he produced magnifying lenses of exceptional quality.

Introduction to Prokaryotes, Eukaryotes

Microorganisms and all other living organisms are classified as **prokaryotes** or **eukaryotes**.

Prokaryotes and eukaryotes are distinguished on the basis of their cellular characteristics. For example, prokaryotic cells lack a nucleus and other memorane-bound structures known as organelles, while eukaryotic cells have both a nucleus and organelles.



popular microbes

• The most common types are **bacteria**, **viruses and fungi**. There are also microbes called protozoa. These are tiny living things that are responsible for diseases such as toxoplasmosis and malaria.

Definition of Bacteria

 Bacteria are ubiquitous, mostly free-living organisms often consisting of one biological cell. They constitute a large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria were among the first life forms to appear on Earth, and are present in most of its habitats.

Bacteria Diagram

The bacteria diagram given below represents the structure of a typical bacterial cell with its different parts. The cell wall, plasmid, cytoplasm and flagella are clearly marked in the diagram.

Bacterial Cell



1. Flagella (sing. Flagellum)

Flagella are long hair-like filamentous structures of about $4 - 5 \mu m$ long and $0.01 - 0.03 \mu m$ in diameter. They confer motility to the bacteria. Flagella are divided into three parts; filament, hook, and the basal body.

Functions of Flagella

- Responsible for motility
- Aids in chemotaxis
- Aids in bacterial pathogenicity and survival

2. Pili/Fimbriae

They are the short, hollow, non-helical filamentous structure of about 0.5 μ m in length and 0.01 μ m in diameter. They are exclusively found in Gram-Negative bacteria.

They are composed of protein 'pilin' arranged non-helically. They are short, numerous, and straight than flagella.

Sex pili are a special kind of pili that take part in bacterial conjugation. They are larger than usual pili; 10-20 μ m in length. They are few in number, just 1-4 in number. They are further classified into two types; **F-pili and I-pili**.

Functions of Pili/Fimbriae

- Aids in adherence to host cells
- Sex pili helps in bacterial DNA transfer during bacterial conjugation



3. Capsule

It is a viscous outermost layer surrounding the cell wall. It is composed of either polysaccharides or polypeptides of both ($\sim 2\%$) and water ($\sim 98\%$). They are present only in some species of bacteria. The capsule is of 2 types; macro-capsule (capsule with a thickness of 0.2 μ m or more) and micro-capsule (capsule with thickness less than 0.2 μ m).



Streptococcus (Capsulated)

Functions of Capsule

- Aids in adherence
- Prevents from desiccation
- Confer resistance against phagocytosis
- The Slime layer protects from proteolytic enzymes

4. Sheath and Prosthecae

- A sheath is a hollow tube-like structure enclosing chain-forming bacteria, mostly aquatic bacteria. It provides mechanical strength to the chain.
- Prosthecae is a semi-rigid extension of the cell wall and plasma membrane. It increases nutrient absorption and also helps in adhesion.

5. Cell Wall

- The cell wall is a rigid structure made up of peptidoglycan that surrounds the plasma membrane as an external coat. It is 10 -25 µm in thickness.
- Peptidoglycan is a cross-linked polymer of alternately repeating N-Acetylmuramic Acid (NAM) and N-Acetylglucosamine (NAG) polysaccharide sub-units.
- Based on composition, bacterial cell-wall is classified into 2 types; Gram-positive, and Gram-negative cell walls

Gram-positive cell wall

The gram-positive cell wall is a thick cell wall containing a large amount of peptidoglycan, about 40 – 90% of the cell wall, arranged in several layers. This type of cell wall also contains acidic sugars like teichoic acids, teichuronic acids, and neutral sugars like mannose, arabinose, rhamnose, and glucosamine as matrix substances.

- Teichoic acids are made of polyribitol phosphate or polyglycerol phosphate. They are major surface antigens of gram-positive bacteria. They are of two types; wall teichoic acid and lipoteichoic acid.
- Teichuronic acid is a polymer of Nacetylmannuronic acid or D-glucuronic acid.
- This type of cell wall takes up the crystal violet dye and confer the purple color of the grampositive bacteria in Gram staining.



Gram-negative cell wall

The gram-negative cell wall is a thin cell wall with significantly less amount of peptidoglycan. It is comparatively more complex than the gram-positive cell wall. It contains lipoprotein, lipopolysaccharide, and outer membrane in addition to peptidoglycan.



ram-Positive Cell-Wall vs Gram-Negative Cell-Wall

Gram-Positive Cell-Wall	Gram-Negative Cell-Wall
Thick (20 – 80 nm)	Thin (10 – 15 nm)
Higher peptidoglycan content	Lower peptidoglycan content
Lower lipid content (2 – 5%)	Higher lipid content (15 – 20%)
The main components are peptidoglycan, teichoic acid, and teichuronic acid	The main components are peptidoglycan, lipoprotein, lipopolysaccharide, outer membrane
Very few amino acids without any aromatic amino acids	Wide variety of amino acids with different aromatic amino acids

Internal Structure of Bacteria

1. Cell membrane/Plasma membrane

It is the innermost phospholipid bilayer, just beneath the cell wall, enclosing cytoplasm. It is a thin (~ 5 -10 nm) semipermeable layer.

Functions of Cell membrane/Plasma membrane

- Selective permeability regulates the inflow and outflow of nutrients, ions, and metabolites
- Electron transport and oxidative phosphorylation

2. Cytoplasm

- It is a colorless, colloidal, viscous fluid with suspended organic and inorganic solutes enclosed within the plasma membrane.
- Unlike eukaryotic cytoplasm, they lack membrane-bound organelles. They have ribosomes, mesosomes, inclusion bodies, nucleic acids floating in them.

3. Bacterial Nucleus

- They are called nucleoids. Unlike eukaryotic nuclei, they are not enclosed in the nuclear membrane and lack nucleolus and nucleoplasm. It is represented by a dsDNA molecule either in a closed circular form or in coiled form.
- Bacterial DNAs are found either in nucleoid as chromosomal DNA or outside nucleoid as a plasmid.

Endospore of a bacteria

- Some bacteria under stress form a dormant stage called an endospore. They are produced during unfavorable environmental conditions. They grow to vegetative form when the conditions become favorable.
- They have four distinct structural components; (i) core, containing nucleoid and condensed cytoplasm, (ii) spore wall, the innermost wall of peptidoglycan, (iii) cortex, the thickest wall with unusual peptidoglycan, and (iv) protein coat, an outer impermeable layer made of keratin like protein.



Ultrastructure of a Bacteria Cell They are also very versatile organisms, surviving in extremely inhospitable conditions. Such organisms are called extremophiles. Extremophiles are further categorized into various types based on the types of environments they

- 1. Thermophiles
- 2. Acidophiles
- 3. Alkaliphiles
- 4.Osmophiles
- 5.Barophiles
- 6.Cryophiles

Classification of Bacteria

- Bacteria can be classified into various categories based on their features and characteristics. The classification of bacteria is mainly based on the following:
- Shape
- Composition of the cell wall
- Mode of respiration
- Mode of nutrition

Classification of bacteria based on Shape

Type of Classification	Examples
Bacillus (Rod-shaped)	<i>Escherichia coli</i> (E. coli)
Spirilla or spirochete (Spiral)	Spirillum volutans
Coccus (Sphere)	Streptococcus pneumoniae
Vibrio (Comma-shaped)	Vibrio cholerae

Shapes and Arrangement of Bacteria







Classification of bacteria based on the Composition of the Cell Wall

Type of Classification	Examples
Peptidoglycan cell wall	Gram-positive bacteria
Lipopolysaccharide cell wall	Gram-negative bacteria

Classification of bacteria based on the Mode of Nutrition

Type of Classification	Examples
Autotrophic Bacteria	Cyanobacteria
Heterotrophic Bacteria	All disease-causing bacteria
1. Autotrophic bacteria

They are bacteria capable of assimilating inorganic matters into organic matters i.e. capable of preparing their food like plants. They are of 2 types;

Photoautotrophs; They use energy from sunlight for assimilation. It includes cyanobacteria (*Nostoc, Prochlorococcus*, etc.), purple sulfur bacteria (*Nitrosococcus, Thiococcus*)

Chemoautotrophs; They use chemical energy for assimilation. It includes sulfur bacteria (*Thiobacillus, Thiothrix, Sulfolobus,* etc.), nitrogen bacteria (Nitrosomonas, Nitrobacter, etc.), hydrogen oxidizing bacteria (*H. pylori*)

2. Heterotrophic bacteria

They are bacteria that derive energy by consuming organic compounds, but they do not convert organic compounds to inorganics. They are parasitic or symbiotic types. *E. coli, Rhizobium spp*.

3. Saprophytic bacteria

They are bacteria that decompose organic compounds into inorganic and derive energy. They are decomposers and feed on dead plants and animals.

E.g. Cellulomonas, Clostridium



Classification of bacteria based on the Mode of Respiration



1. Aerobic bacteria

They respire aerobically and can't survive in anoxic environments. E.g. *Pseudomonas aeruginosa, Nocardia spp., Mycobacterium tuberculosis, etc.*

2. Facultative aerobes

They survive in very low oxygen levels and can survive in both oxygenic and anoxic environments. They are Microaerophiles. E.g. *E. coli, Lactobacillus spp., Staphylococcus spp., etc.*

3. Anaerobic bacteria

They respire anaerobically and can't survive in an oxygen-rich environment. E.g. *Clostridium perfinges, Campylobacter, Listeria, Bifidobacterium, Bacteroides, etc.*



Classification of Bacteria based on Arrangement of Flagella

1. Atrichous

They are bacteria without flagella. E.g. Lactobacillus spp., Bacillus anthracis, Staphylococcus spp., Streptococcus spp., etc.

2. Monotrichous

They are bacteria with only one flagellum at one pole. E.g. *Campylobacter spp., Vibrio cholerae*, etc.



3. Lophotrichus

They are bacteria with multiple flagella at one end. E.g. *Spirillum, Helicobacter pylori, Pseudomonas fluorescence,* etc.

4. Peritrichous



Figure: Lophotrichous flagella

They are bacteria with multiple flagella projecting in all directions. E.g. *E. coli, Proteus, Salmonella, Typhi*, etc.



Figure: Peritrichous flagella

• 5. Amphitrichous

• They are bacteria with one flagellum at each pole. E.g. *Nitrosomonas*, etc



Figure: Amphitrichous flagella

Size of Bacteria

- Bacteria are microscopic with a wide range of sizes from 0.2 μm to 100 μm.
- Cocci are generally of 0.2 to 1.0 μm.
- Bacilli are generally of 1.0 µm 5 µm in length and 0.5 to 1.0 µm in diameter.
- Spirochetes are generally 20 µm in length and 0.1 to 1.0 µm in diameter.
- The smallest bacilli are *Pelagibacter ubique* (370 890 nm in length and 120 200 nm in diameter).
- The smallest cocci are *Mycoplasma* genitalium with a diameter of 200 – 300 nm.
- The largest bacteria is *Thiomargarita namibiensis* with a diameter of 0.75 mm.

Reproduction in Bacteria

Bacteria have a very short generation time i.e. they reproduce very quickly. Their reproduction is an asexual type and can be classified into the following types.

1. Binary fission

It is the most common type. Under favorable conditions, each bacterium divides into two identical bacteria. The bacterial cells first acquire nutrition grow at their maximum size and replicate their DNA. The new replicated DNA called an incipient nucleus, migrates towards opposite poles. A transverse septum begins to develop and separate the two daughter cells.

Step 1- Replication of DNA

The bacterium uncoils and replicates its <u>chromosome</u>, essentially doubling its content.

Step 2- Growth of a Cell

After copying the chromosome, the bacterium starts to grow larger in preparation for binary fissions. It is followed by an increase in cytoplasmic content. Another prominent trait of this stage is that the two strands migrate to opposite poles of the cell.

Step 3-Segregation of DNA

The cell elongates with a septum forming at the middle. The two chromosomes are also separated in this phase.

Step 4- Splitting of Cells

A new cell wall is formed at this phase, and the cell splits at the centre, dividing the parent cell into two new daughter cells. Each of the daughter cells contains a copy of the nuclear materials as necessary organelles

2. Conidia formation

It is mostly seen in filamentous bacteria like those in actinomycetes, e.g. *Streptomyces, Micromonospora, Rhodomicrobium*, etc.

3. Budding

The bacterial cells develop small swelling, called protuberance or bud, at one side. Bacterial DNA replicates and one copy enters into the bud. The bud eventually separated and develop into a daughter cell. E.g. *Planctomyces spp, Rhodomicrobium vannielia, Hyphomicrobium spp.*, etc

4. Endospore formation

It is seen in some Gram-positive bacteria during unfavorable conditions and environmental stresses. The cytoplasm becomes concentrated around bacterial DNA and a thick, hard, and resistant wall develops around it. E.g. *Bacillus spp., Clostridium spp., Sporosarcina spp.*, etc.

5. Transformation

It is considered a sexual method. In this method, the DNA of one bacterium directly enters into a cell of another bacterium of the same species and forms recombinant DNA. The DNA enters through extracellular environments.

6. Conjugation

It is another sexual method where DNA transformation is by direct contact between donor and recipient bacterium via conjugation tube. Sex pili are responsible for conjugation. Donor cell develops sex pilus and attaches to the recipient cell. A conjugation tube or bridge is formed at the connected point. DNA fragments transform from one bacterium (donor) to another (recipient) through this tube.

7. Transduction

In this method, DNA fragments are transformed from donor bacterium to recipient bacterium by bacteriophages.

Bacterial Identification

This is the method of identifying genera and species of isolated bacteria i.e. to identify which bacteria are isolated. There are several methods designed and used for bacterial identification.

a. Cultural Methods for Bacterial Identification

It is the method of identifying bacteria by studying their cultural characters in a specific <u>culture media</u>. Several selective and indicator media are used for bacterial identification. In this method, we study colonial characters like;

- 1. The shape of colonies (circular, irregular, rhizoid, etc.)
- 2. Size of colonies (micro, small, medium, large, etc.)
- 3. Pigmentation
- 4. Elevation of colonies (concave, convex, flat)
- 5. The margin of colonies (smooth, rough, dented, wavy, etc.)

b. Staining and Microscopy for Bacterial Identification

c. Biochemical Tests for Bacterial Identification

Indole test;

Methyl-Red (MR) test;

Voges Proskauer (VP) test;

Citrate test;

Urease test;

Triple Sugar Iron (TSI) test;



d. Molecular Methods for Bacterial Identification

This test includes the study of a bacterial genome and genomic sequences. This is the most advanced and accurate method used when very precise identification is required. We can classify bacteria into sub-species, strains, serotypes, or pathovar levels using molecular methods. This method includes <u>Polymerase Chain Reactions (PCR)</u>, DNA / RNA probe tests, Microarray, Electrophoresis, Proteomics, etc.

e. Immunological Methods for Bacterial Identification

This method is limited to the identification of pathogenic bacteria only. In this method, we identify bacteria-specific antibodies or antigens in the body of an infected person. The identified antibody or antigen is correlated with the identification of the infecting bacteria.

Enzyme-Linked Immunosorbent Assay (ELISA), Radio-immunoassay (RIA), Fluoro Immuno Assay (FIA), Immuno chromatography tests, etc are commonly used tests.

Useful Bacteria

Not all bacteria are harmful to humans. There are some bacteria which are beneficial in different ways. Listed below are few benefits of bacteria:

- Convert milk into curd Lactobacillus or lactic acid bacteria
- Ferment food products Streptococcus and Bacillus
- Help in digestion and improving the body's immunity system – Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria
- Production of antibiotics, which is used in the treatment and prevention of bacterial infections – Soil bacteria

Harmful Bacteria

There are bacteria that can cause a multitude of illnesses. They are responsible for many of the <u>infectious diseases</u> like pneumonia, tuberculosis, diphtheria, syphilis, tooth decay. Their effects can be rectified by taking antibiotics and prescribed medication.

Bacterial Streak and Black Chaff

Bacterial streak and black chaff is a bacterial disease of wheat common in irrigated fields or in areas with abundant rainfall during the growing season. It is also known as bacterial stripe or bacterial leaf streak. The disease also occurs on barley, oats, rye, triticale, and many grasses. It is the most important and most widely distributed bacterial disease of small grains and can cause yield losses of up to 40%

Cause and Symptoms

Black chaff is caused by the bacterium Xanthomonas campestris. The disease derives its name from the darkened glumes of infected plants (*Figure 1*). This symptom is similar to that caused by genetic melanism (darkening of tissue) and glume blotch, a fungal disease incited. Black chaff can be distinguished from other diseases by the appearance of cream to yellow bacterial ooze in the form of slime or viscous droplets produced on infected plant parts during wet or humid weather. This ooze appears light colored and scale-like when dry. Bands of necrotic and healthy tissue on awns ("barber's pole") (*Figure 1*) are indicative of black chaff.

A dark brown to purple discoloration may appear on the stem below the head and above the flag leaf. On leaves, symptoms start as small water-soaked spots or streaks that turn brown after a few days. Lesions are irregularly shaped and elongate and may extend the length of the leaf blade (*Figure 2*). In wheat, a diffuse lime-green halo may surround lesions. Leaf symptoms give plants an overall orange cast.

Conditions that Favor Epidemics

- Warm temperatures
- Wet conditions (free moisture and high humidity)
- Frost damage and ice nucleation
- Injury from hail, sandblasting, or other causes
- Root rot and leaf spot diseases caused by fungi
- Insects, which may cause injury and also disseminate the bacterium

Management

The most effective management strategy for black chaff is use of certified, pathogen-free seed.

1- Seed producers should consider testing their seed lots for black chaff before planting.

2-Controlling volunteer cereals and grassy weeds can help to reduce primary inoculum.

3-Irrigation management is critical in creating an environment that is less favorable for disease development and spread.

4-Irrigation should be managed in such a way as to allow the plant canopy to dry completely between irrigations.

Bacterial Blight On Wheat, Oats And Barley

Bacterial blight infestations only occur after prolonged periods of wet weather. They are usually localized within the field and therefore do not usually cause widespread damage.

Host Crops

Wheat, oats, barley

Symptoms Of Damage

The initial lesions appear as small watersoaked spots. The lesions expand and then appear as dry dead tissue. The spots often join within a few days to form large blotches. The entire surface area of an infected leaf may be affected. During wet periods, bacterial ooze may develop within the lesions. The head does not usually develop symptoms.

Control Tips

- Avoid infesting clean fields by following good production practices. Use seed from crops that are free of bacterial disease. Use a crop rotation of three or four years.
- Mow infected wild grass along headlands before sowing winter rye. No chemical controls exist.

1. What are the different types of bacteria??

Bacteria can be divided into several types based on several characteristics such as shape, cell wall composition, mode of respiration, and mode of nutrition.

2. What is bacteria? How do you define bacteria?

Bacteria are prokaryotic unicellular organisms. They have a relatively simple cell structure compared to eukaryotic cells. They also do not possess any membrane-bound organelles such as a nucleus. However, do they possess genetic material (DNA or RNA) in the intracellular space called the nucleoid

3. How do bacteria reproduce?

Bacteria reproduce through a process called binary fission. In this process, a single bacterium divides into two daughter cells. These daughter cells are identical to the parent cell as well as to each other.

- 4. State 4 examples of bacteria.
- •Streptococcus
- •Bacillus
- •Actinobacteria
- Proteobacteria
- 5. The study of bacteria is called?
- The study of bacteria is called bacteriology.
- 6. What are the examples of acidophilic bacteria?

Acetobacter aceti and Alicyclobacillus acidiphilus are two examples of acidophilic bacteria.

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Fungi

 A fungus (plural: fungi) is a type of eukaryotic organism belonging to the kingdom Fungi, alongside plants, animals, protozoa, and monera. Fungi are incredibly diverse, with commonly encountered forms including yeast, molds, truffles, and mushrooms. Most fungi are microscopic, consisting of thread-like structures less than 10 µm in diameter named hyphae. These branching structures grow into a root-like vegetative mass named a mycelium, which acts to absorb nutrients from the environment rather than relying on photosynthesis. A wide variety of nutrient sources are utilized by fungi, from soil and water to decaying matter and other living organisms
Importance of fungi

• Humans have been indirectly aware of fungi since the first loaf of leavened bread was baked and the first tub of grape must was turned into <u>wine</u>. Ancient peoples were familiar with the <u>ravages</u> of fungi in agriculture but attributed these diseases to the wrath of the gods. The Romans designated a particular deity, Robigus, as the god of <u>rust</u> and, in an effort to appease him, organized an annual festival, the <u>Robigalia</u>, in his honour.

• While scientists have identified about 100,000 species of fungi, this is only a fraction of the 1.5 million species of fungus likely present on Earth. Edible mushrooms, yeasts, black mold, and the producer of the antibiotic penicillin, *Penicillium notatum*, are all fungi. And currently the largest (and perhaps, oldest) living organism on Earth's surface is a fungus!

Characteristics of fungi

fungi can be multicellular or unicellular, all fungi have two things in common:

 cell walls made of a tough polysaccharide, called **chitin**, which provides structure
external digestion of food

Reproductive life cycle

• Fungi can reproduce sexually and/or asexually. 'Perfect' fungi reproduce both sexually and asexually, while the so-called 'imperfect' fungi reproduce only asexually (by mitosis). Because of the variety of reproductive methods, the specific structures produced by a fungal for reproduction help to classify it among fungal phyla (subgroups), such as Basidiomycota, Ascomycota, Glomeromycota, and Chytridiomycota. We will cover these groups more in-depth in class.

Types of fungi

Fungi are subdivided on the basis of their life cycles, the presence or structure of their fruiting body and the arrangement of and type of spores (reproductive or distributional cells) they produce.

The three major groups of fungi are:

- Multicellular filamentous moulds.
- Macroscopic filamentous fungi that form large fruiting bodies. Sometimes the group is referred to as 'mushrooms', but the mushroom is just the part of the fungus we see above ground which is also known as the fruiting body.
- Single celled microscopic yeasts.