# FE 305 FOOD MICROBIOLOGY
# Characteristics of Microorganisms

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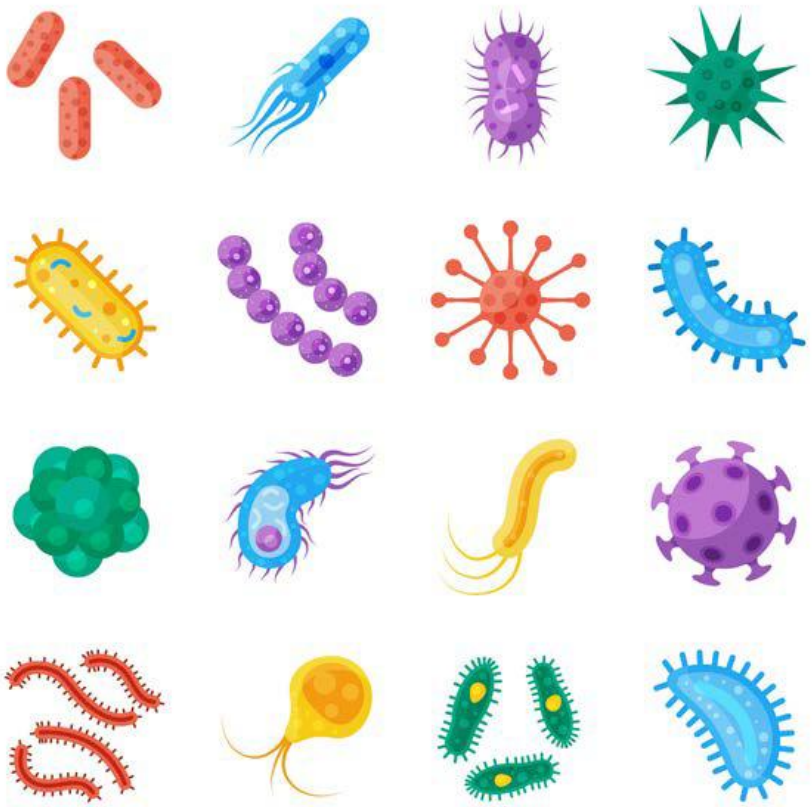
# Introduction

- Microbiology is a compound word and is formed by the combination of the Greek words **micro**, **bio** and **logy**. Micro of these words; too small to be seen with the naked eye, bio; living, logy means science.
- Simple and single-celled organisms that cannot be seen with the naked eye but can be seen with a microscope are called “microorganisms”.
- In microbiology, the shapes, structures, reproduction, physiological and biochemical activities of microorganisms are examined.
- The scope of microbiology;
  - The distribution of microorganisms in nature,
  - their relations with each other and with other living things,
  - their harmful and beneficial effects, and
  - the naming and classification of microorganisms.
- All microorganisms studied in microbiology;
  - Reproductive
  - Digesting and metabolizing nutrients
  - Dispose of waste
  - Responding to environmental changes have common characteristics
- These features differ only in viruses. Viruses can only reproduce inside living cells.

# Introduction

- Microorganisms live in the entire biosphere:
  - In water:
    - oceans, lakes, ponds, waterfalls,
    - In swamps, even in rain clouds!
  - On land:
    - On soil and rocks (both inside and outside)
  - In interlayers:
    - all water bottoms, groundwater
  - In and out of animals and plants



# Food Microbiology

- **Food** is all of the raw, semi or fully processed foods that can be eaten or smoked, excluding drugs and tobacco, that constitute the source of nutrients (fat, carbohydrates, vitamins, etc.) necessary for people to continue their lives.
- **Food microbiology** is a sub-discipline that studies the microorganisms naturally found in foods and the microorganisms that contaminate foods during storage, transportation and processing.
- Microorganisms have two different effects in the food industry: beneficial and harmful.
- **Classification of Microorganisms According to the Roles They Play in the Food Industry**
  - Pathogenic Microorganisms
  - Spoilage Microorganisms
  - Beneficial Organisms
  - Indicator Microorganisms

# Advances in Food Microbiology

- B.C. 6000-8000: Using cooking
- B.C. 3000: Salting, drying, using oil
- B.C. 1000: Burial of the fish-shrimp
- 943 Claviceps purpurea, 40,000 deaths from ergotism (France)
- 10-11. century: Diseases occur through invisible beings (Ibn Sina) (and Akşemseddin; 15th century)
- 1658: Invisible worms are responsible for spoiling food (Kircher)
- 1683: Leeuwenhoek invented the microscope
- 1765: Spallanzani disproves the theory of "spontaneous generation".
- 1837: Schwann laid the foundations for the preservation of food by heat treatment.
- 1810: Appert finds that keeping meat in glass in hot water extends its shelf life.
- Pasteur was the microbiologist who first described the existence and role of microorganisms in food.

# Advances in Food Microbiology

- Antony van Leeuwenhoek (1632 – 1723) invented the first microscope and was the first to observe and describe microorganisms.
- Berkeley and Pasteur – microorganisms cause disease.
  - Joseph Lister – ensured that surgical operations were performed sterile.
- Robert Koch (1843 – 1910) demonstrated the relationship between *Bacillus anthracis* and anthrax and isolated the tuberculosis bacillus.
- Charles Chamberland (1851-1908) discovered viruses and demonstrated their role in disease.

# Advances in Food Microbiology

**Louis Pasteur (1822 – 1895)**

- He developed vaccines for chickenpox, anthrax, and rabies.
- It showed that all fermentations depended on specific yeast and bacteria.
- Developed Pasteurization for wine storage.
- He showed that the fermenting microorganisms were anaerobic and could not survive in the presence of oxygen.
- It showed that the fermentation was done by special yeast and bacteria.

# Advances in Food Microbiology

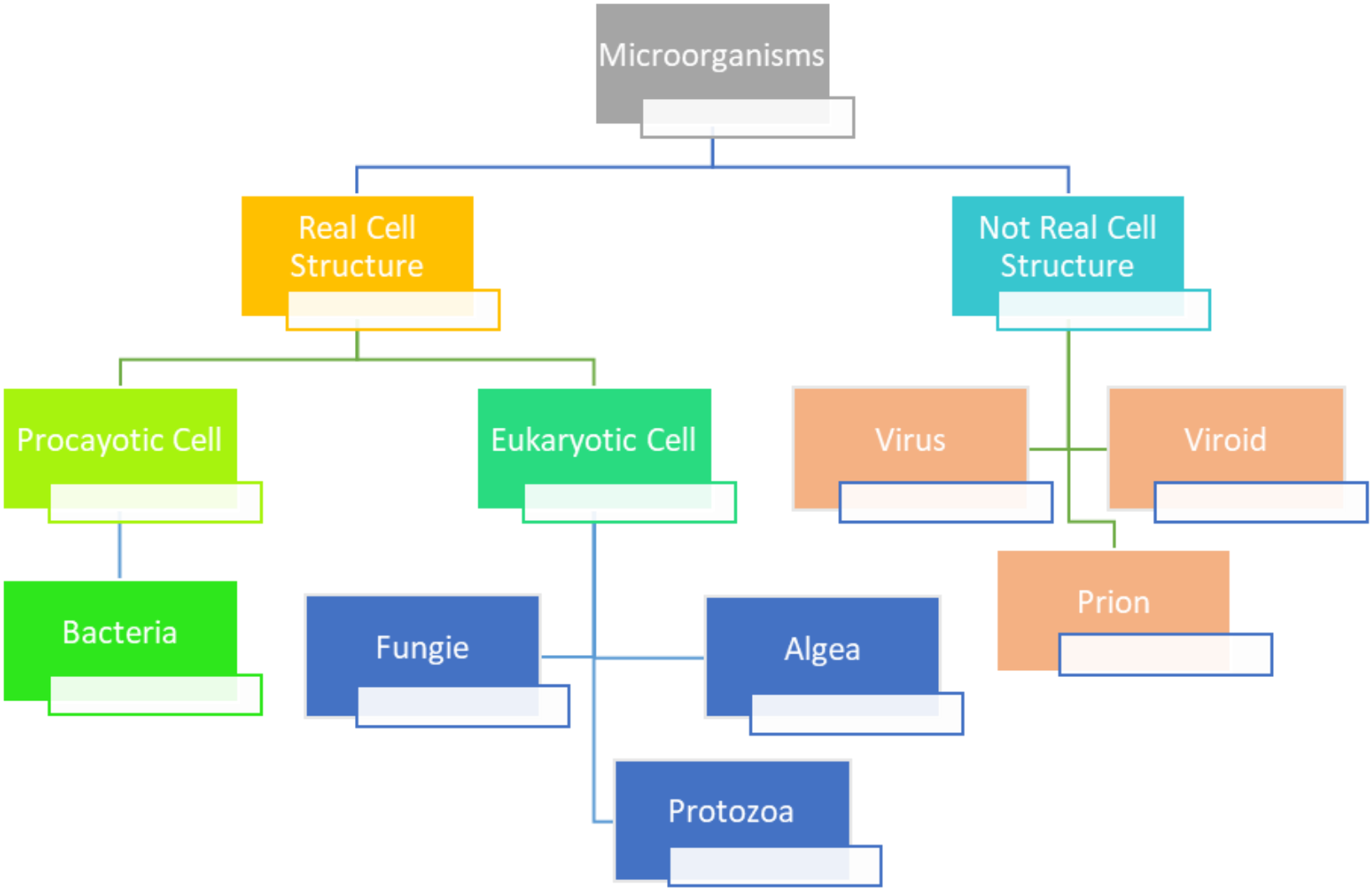
The branches of science that examine living things that are in the group of microorganisms and have different characteristics have emerged. These:

- **Bacteriology**: It is the specialty in which bacteria are studied.
- **Mycology**: It is the specialty that examines yeast and molds together.
- **Virology**: It is the specialty that studies viruses.
- **Parasitology**: It is the science that studies the life and disease-causing properties of parasites.
  - The following branches of science have emerged in different areas of specialization.
- **Medical microbiology**: It studies the relationship of microorganisms to humans and the harm they cause to humans.
- **Food microbiology**: It aims to prevent the harmful effects of microorganisms in food production technology and to use them in food production by taking advantage of their beneficial effects.
- **Technical and industrial microbiology**: It covers the production of different nutrients using microorganisms, the culture methods used in production (such as cheese, yoghurt yeast) and techniques, the development of new methods and techniques in production, the applications to be used in protecting microorganisms from harmful effects, the evaluation of wastes, water and waste treatment processes.

# Microorganisms

- Although living things called microorganisms spread on the earth before advanced creatures, they were very small, so it was necessary to wait for the development of microscope and microscopy techniques so that they could be observed and studied directly.
- The beings that make up the living realm are grouped into three major groups, taking into account their structures and other characteristics. These;
  - plants,
  - animals,
  - protista (microorganisms).
- Scientists have found a separate classification as protista (Greek primitive animal) due to the differences in the metabolic activities of microorganisms as well as their nutrition, movement and cell structures.

# Classification of microorganizms According to Cell Structure



# Microorganisms

- Protista are also divided into two according to their cell structure:
  - **Higher protists**: They are **eukaryotic** organisms similar to animals and plants in terms of cell structure. Fungi (molds and yeasts), algae and protozoa are examples of these microorganisms.
  - **Simple protists**: Microorganisms that are surrounded by a specific nuclear membrane and do not have a true nucleus are defined as prokaryotes, that is, primitive nuclei. Bacteria, blue-green algae and viruses fall into this group.
- Among these groups of microorganisms, there are bacteria, fungi (molds and yeasts) and viruses, which are important microorganisms in foods.
- Many bacteria, molds and yeasts have biological activity in foods.
- While some types of bacteria, molds and yeasts multiply in plant and animal foods, they cause the deterioration of these foods and sometimes the emergence of infectious diseases or food poisoning in humans and animals who consume these foods, while others take part in the production of fermented foods and alcoholic beverages.

# Nomenclature of Microorganisms

- The binomial system is used in naming of microorganisms and Latin naming is done. It consists of two words.
  - The first word is the genus name and begins with a capital letter. The genus name originates from the name of the person who first discovered the microorganism; It takes it from the morphological, physiological, ecological and biochemical characteristics of the microorganism.
  - The second word is the species name and starts with a lowercase letter. This last name reflects the various characteristics of the microorganism (colony color, location, disease, form, etc.).
- These names are written in italics.
  - *Bacillus anthracis*
  - *Bacillus cereus*
  - *Bacillus subtilis*
  - *Staphylococcus aureus*
  - *Salmonella typhi*
- The first word is ***Escherichia***, the name of the scientist **Escherich** who first described this bacterium; ***coli***, which forms the species name, is the name of the large intestine (colon) where this bacterium lives.

# Characteristics of Microorganisms

- The simplest cellular forms of life consists of individual, small cell.
- Such microscopic units are typical of microbial groups:
  - bacteria, yeasts, molds, viruses, algae, and protozoa.
- Microorganisms differ from one another in
  - size,
  - shape,
  - energy-yielding biochemical reactions and
  - internal organization.
- Bacteria, yeasts, molds and viruses are important in:
  - food spoilage,
  - food production and
  - foodborne diseases (not yeasts).

# TYPES OF MICROORGANISMS

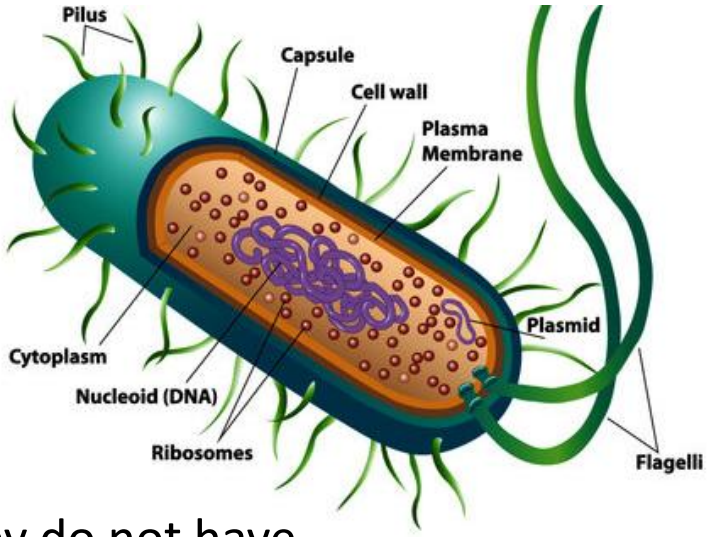
- bacteria
- yeasts
- molds
- viruses,
- algae and
- protozoa

# Bacteria

- Different of structures is present in procaryotic cells (rod, coccus, spiral, etc.).
- Not all structures are found in every genus.
- Cells: unicellular, about 0.2x10 μm in size.
- motile or nonmotile.
- a rigid wall on the surface.
- The membrane also contains energy-generation compounds.
- The ribosomes are 70S type
- Genetic materials (structural and plasmid DNA) are circular, not enclosed in nuclear membrane, do not contain basic proteins such as histones.
- Cells divide by binary fission.
- flagella, capsule, surface layer proteins and pili for specific functions.
- endospores (one per cell).
- Gram-stain behavior: bacterial cells are grouped as Gram-positive and Gram-negative.

# Cell Structure of Bacteria

- Bacteria with prokaryotic characteristics can be found everywhere in nature such as soil, water, air, plants and animals.
- Most bacteria are single-celled and reproduce by division. Its nutritional requirements are simple and it is able to adapt quickly to environmental conditions. Their lengths vary between 2 – 10 µm and diameters 0.5 – 2.5 µm.




**Bacterial main structures**:

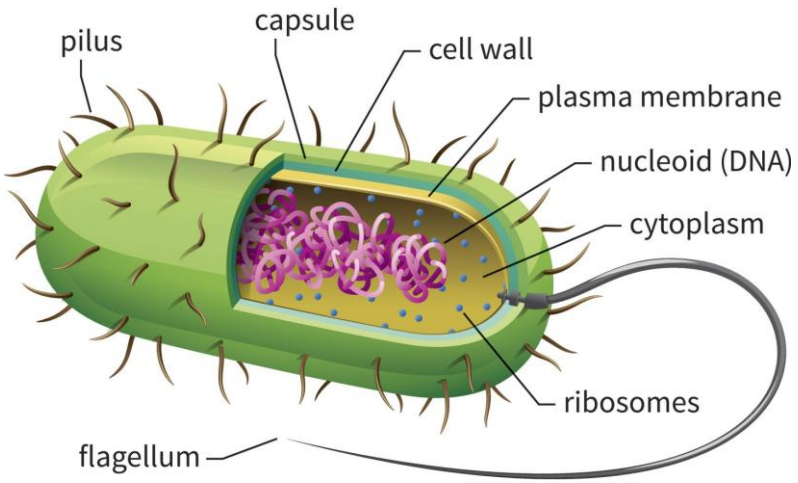
- Capsule
- Cell wall
- Cytoplasm membrane (Cell membrane)
- Flagella and (filament) pleats (whips)
- Endospore
- Storage nutrients
- Ribosomes and bacterial chromosome (They do not have a true nucleus surrounded by a specific membrane. Condensed nuclear material is found anywhere in the cytoplasm.)

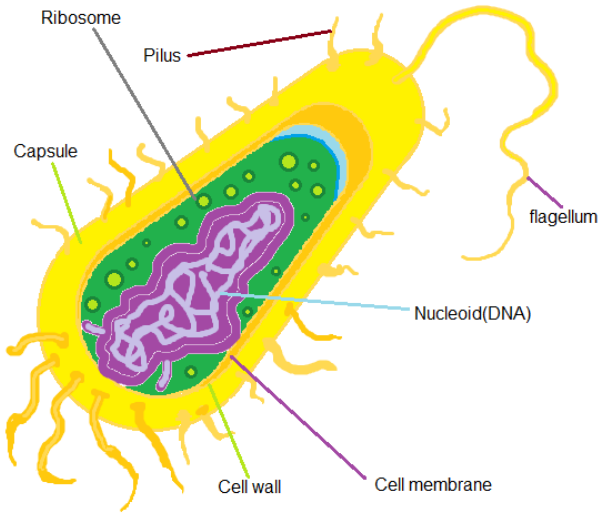
# Bacterial Main Structure

**Cell wall**: It is a thin, semi-solid and strong layer that surrounds the cytoplasm and protects the weak inner parts and organelles from external influences.

- This layer, which is responsible for the characteristic shape of the cell, is semi-permeable.
- This structure plays a role in the passage of food substances in the environment into the cell, and the elimination of metabolic wastes.

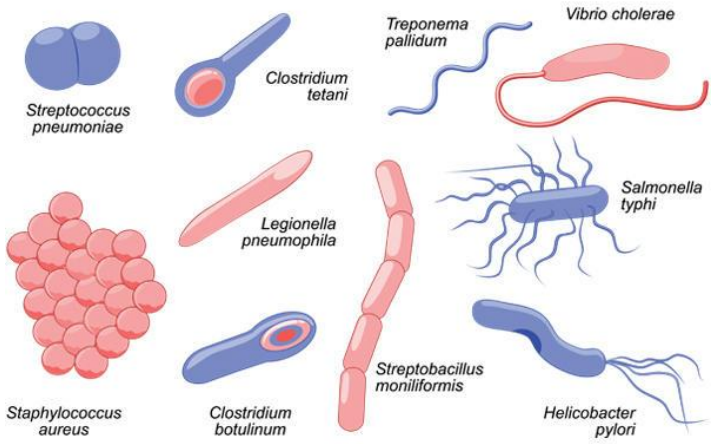
- The cell wall is effective in gram staining, which is of particular importance for bacteria. The reason for the reaction of bacteria against gram staining is related to the structure of the cell wall (murain network).

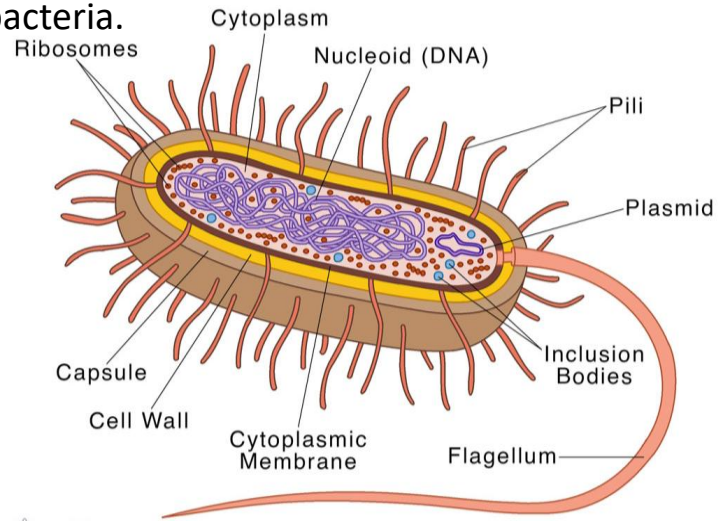


# Cell Structure of Bacteria

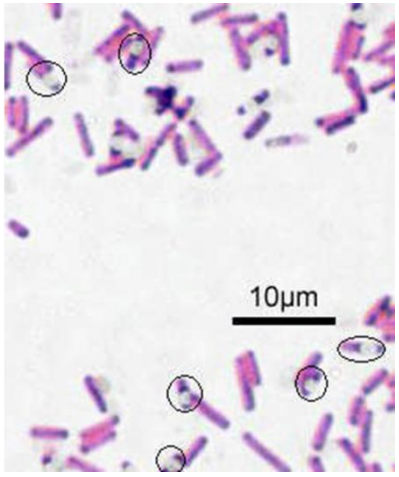
- **Cytoplasmic membrane**: Located just below the cell wall, this membrane surrounds and protects the cytoplasm. It is thinner than the cell wall. Any damage to this membrane leads to the death of the bacterium.
- **Cytoplasm**: Physically it is a clear, slightly sticky liquid. In terms of function, it is the place where chemical reactions occur in the cell and where the building materials of the cell are synthesized.
- **Nucleus**: The nucleus of the bacterial cell consists of DNA (deoxyribonucleic acid) and a small amount of RNA (ribonucleic acid). The places where these are found in excess in the cytoplasm are called the nuclear region. The nuclear membrane and nucleolus are absent in bacteria.
- **Ribosome**: It is the only organelle found in bacteria. It does protein synthesis.
- **Flagella**: It is a structure specific to bacteria and provides movement in the cell. Flagella may be single or multiple.
- **Capsule**: Not necessary for cellular functions. However, it plays a role in the accumulation of storage nutrients and the excretion of metabolic wastes in the bacteria it is found in. As a result of the growth of capsule-forming bacteria in the food, stickiness (rop) occurs. In addition, the presence of capsules increases the infection efficiency of bacteria.

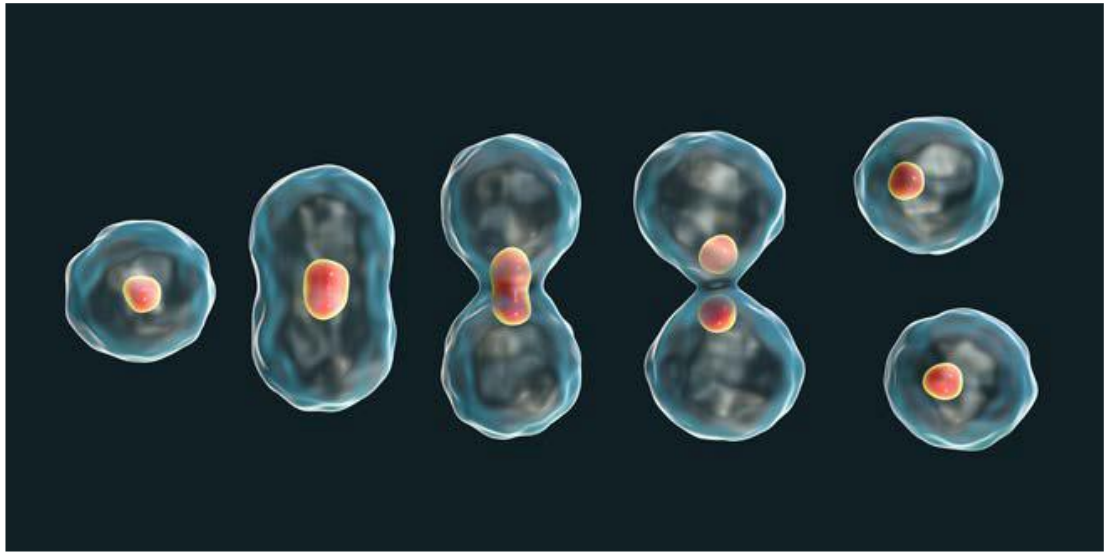


# Growing of Bacteria

- Bacteria and all microorganisms grow and multiply when they are in suitable environments. The creation of new organisms similar to themselves in order to ensure the continuation of their generation is called reproduction.
- Bacteria reproduce by fission, which is a form of vegetative reproduction.
- Microorganisms grow and multiply in a time specific to their species in appropriate nutrient media and environmental conditions. During development, factors such as the amount of nutrients in the environment, the amount of waste material, and the temperature of the environment also affect the growth rate of the bacteria. Therefore, different growth stages of bacteria occur in the culture medium.



# Growing of Bacteria

- In reproduction by division into two;
  - Copying of genetic material (DNA),
  - Separation of DNA by fragmentation,
  - The cell membrane moves into the cytoplasm and forms a septum (division) between the cell walls,
  - There are stages in the separation of the cell from the septum-formed parts.
- It is the division of the first cell into two new cells, which are a genetically true copy.
- When examining the developmental period of bacterial populations, it is seen that the structural components of a bacterial cell double during division. The two cells that arise as a result of the division of the bacterial cell and consist of the parent cell are called the **generation**, and the time taken for a cell to divide into two is called the **generation time**. The generation time differs between microorganism species. For example, while the generation time for *E. coli* is 20 minutes, it is 10-12 minutes for *V. parahaemolyticus*.
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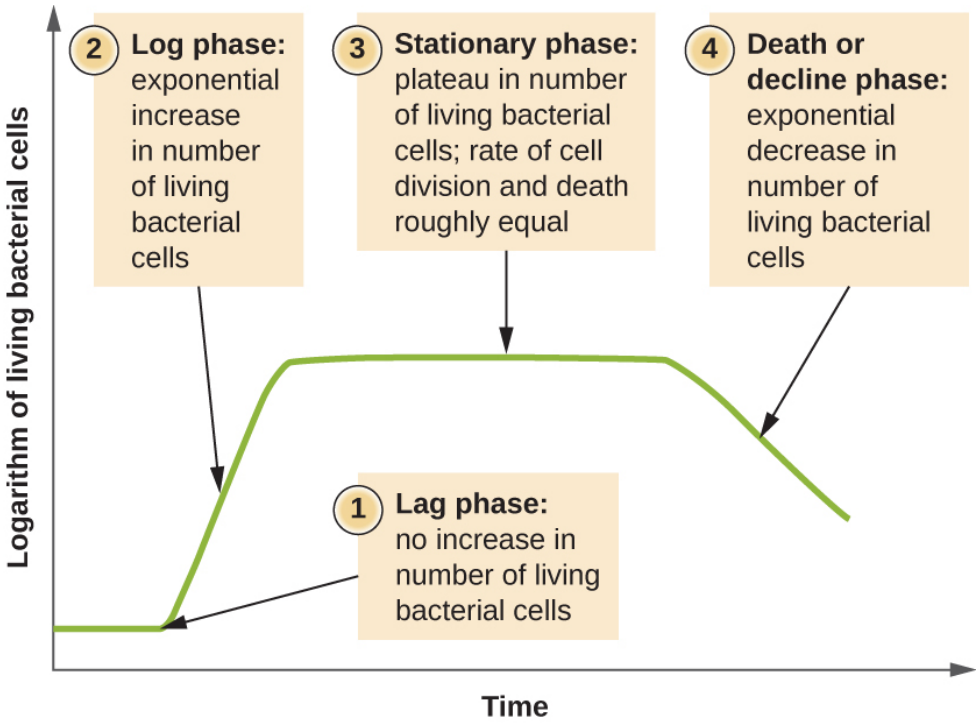
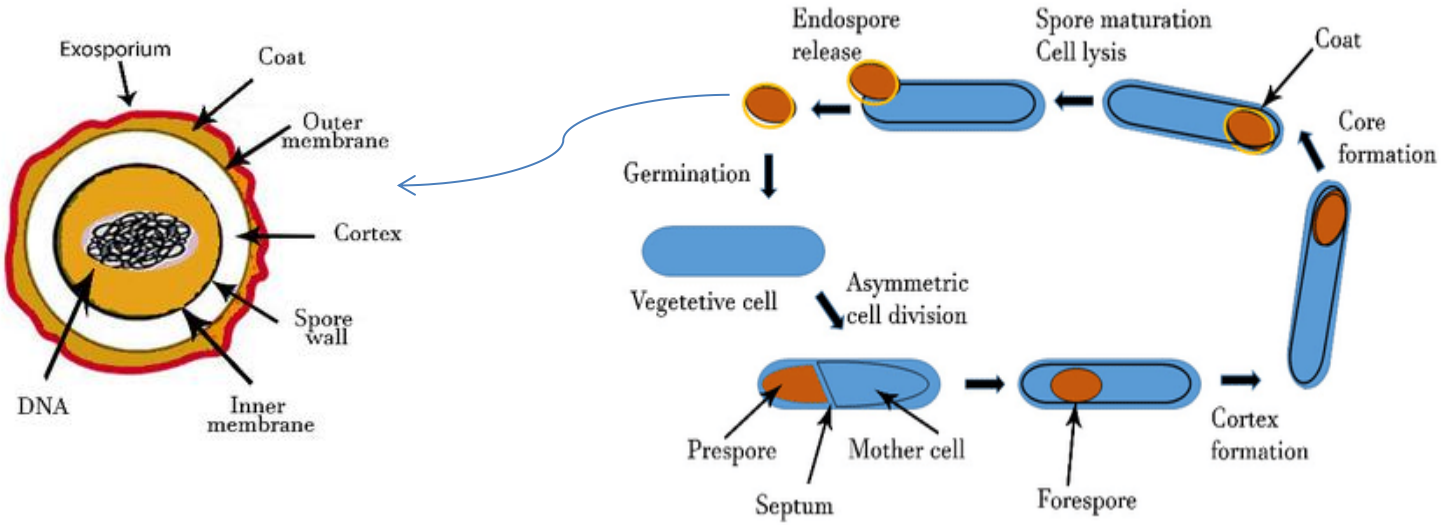
# Growing of Bacteria

- These developmental periods are:
  - **Lag Phase**: It is the period in which the population does not change, the cells grow, and the mass of the bacteria increases.
  - **Logarithmic Growth Phase**: It is the period in which the number of the population increases as the cells in the population divide and multiply.
  - **Stagnant Phase** (**Stationary Period**): As a result of the decrease in the amount of nutrients in the environment and the accumulation of waste material, a small number of cells proliferate while a small number of cells die. This keeps the number of living populations constant.
  - **Death Phase**: After the stationary phase, the death rate of the cells becomes higher than the proliferation rate. Therefore, there is a significant decrease in the number of cells.

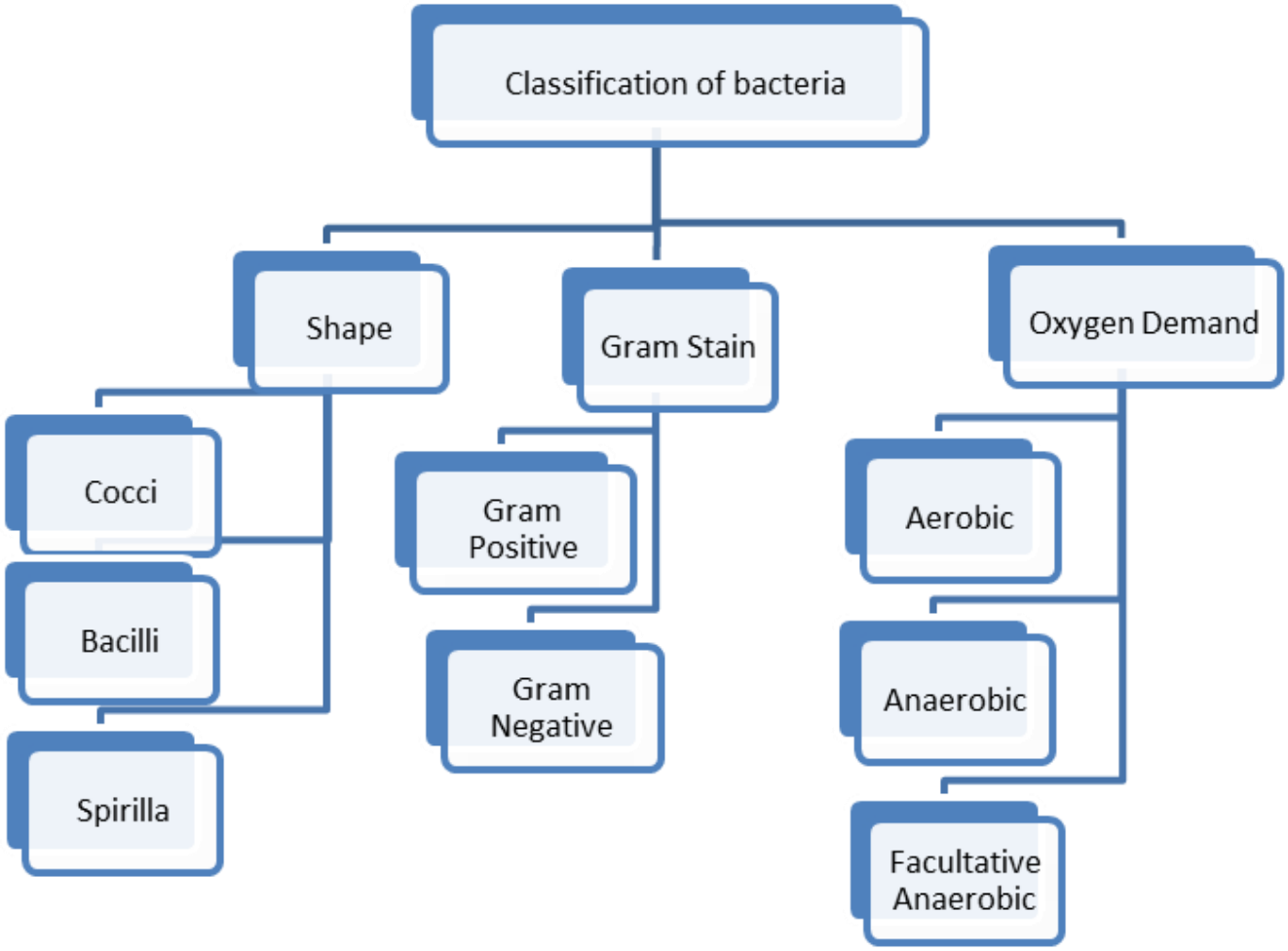


# Growing of Bacteria

- *Bacillus, Clostridium etc.* Some bacteria use heat, light, frost, drying, radiation, etc. according to the vegetative bacterial form in order to survive in these difficult conditions when the environmental conditions they are in become difficult. It forms a thick-walled and resistant, round object, which is a more resistant form to environmental conditions. This is called an **endospore**. The spore or endospore is the living but dormant form of the bacterium. Endospore formation is not a form of reproduction, but it ensures the protection of the generation. When the environmental conditions improve, the endospore can turn into a vegetative form by germination.
- The **endospore** can form in different parts of the cell, mostly at the ends of the bacterium. In other words, it is located in the **middle (central), near the tip (sub-terminal) or at the tip (terminal) of the bacterium**.

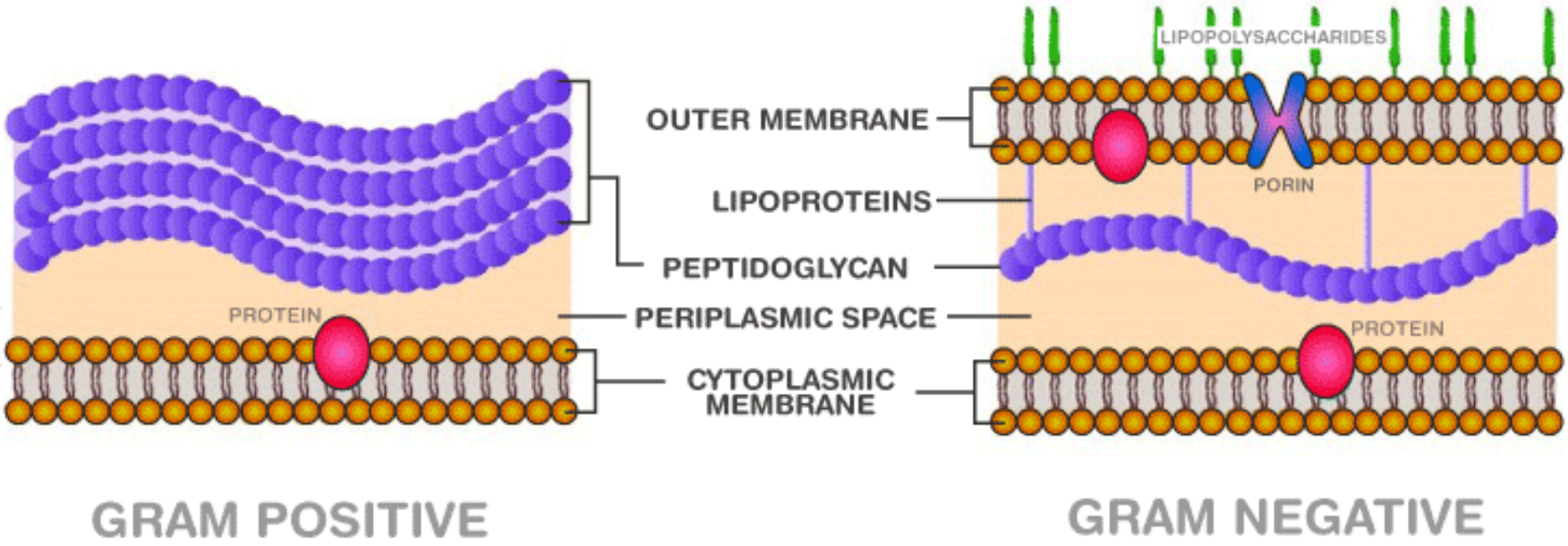


# Classification of Bacteria

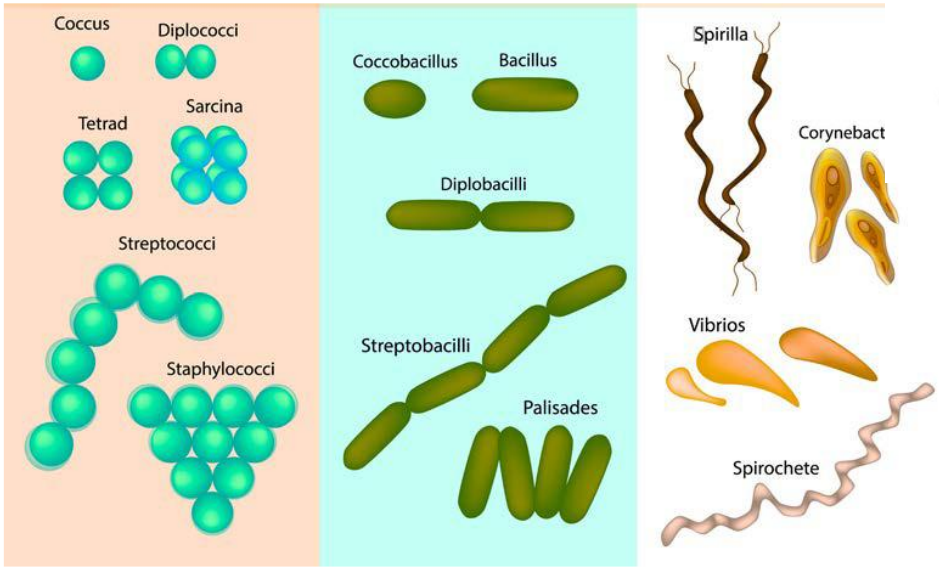


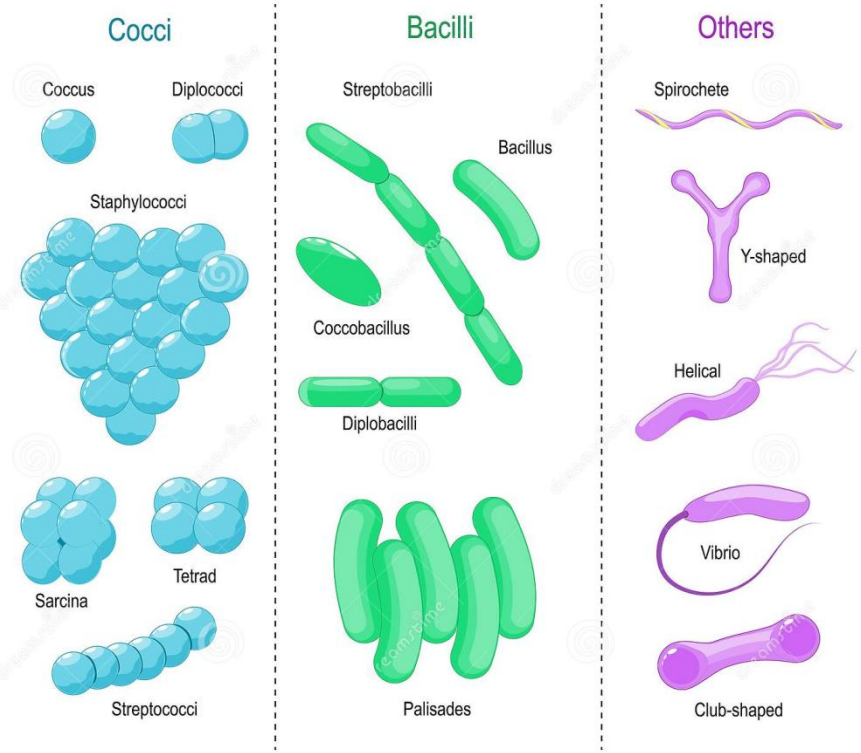
# Classification of bacteria based on gram staining

- The cell wall is effective in gram staining, which is of particular importance for bacteria. The reason for the reaction of bacteria against gram staining is related to the structure of the cell wall (murain network).
  - Bacteria that are stained with crystal violet and appear purple in Gram staining are gram-positive and take on a blue-purple color under the microscope.
  - Bacteria staining red-pink with saffron are classified as gram negative..



# Cellular morphology

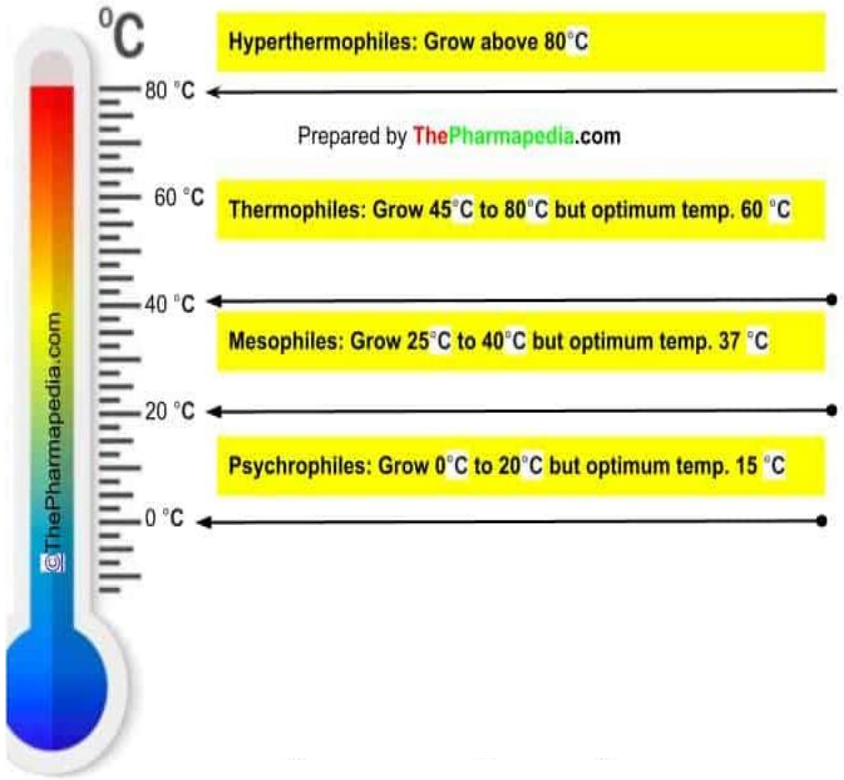


# Classification of bacteria based on temperature

Bacteria are examined in three groups based on their temperature requirements: psychrophilic, mesophilic and thermophilic:

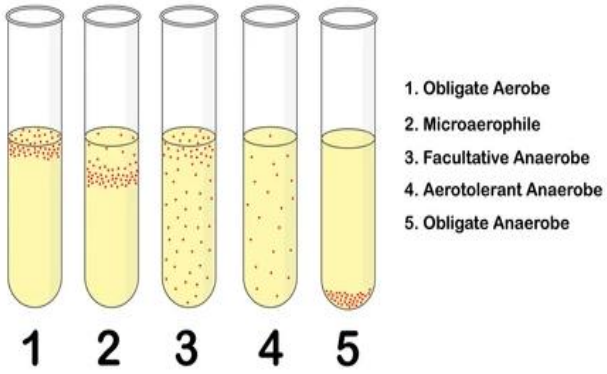
- **Psychrophilic Bacteria**: Bacteria in this group are called cold-loving bacteria. It grows at a minimum of 0 °C, an optimum of 15 °C and a maximum of 20 °C. For example; *Listeria monocytogenes, Pseudomonas fluorescens* bacteria etc.
- **Mesophilic Bacteria**: Bacteria group with optimum growth temperature between 25 °C and 45 °C. Saprophytic bacteria, which decompose plant or animal remains in nature into simple components, and many bacterial species that play an important role in the food industry are included in this group. For example; *Escherichia coli, Staphylococcus* bacteria etc.
- **Thermophilic Bacteria**: Bacteria that can withstand relatively high temperature conditions and survive are included in this group. Thermophilic bacteria grow at a minimum of 35-45 °C, an optimum of 55 °C and a maximum of 60-90 °C. For example; *Bacillus, Clostridium* bacteria etc.

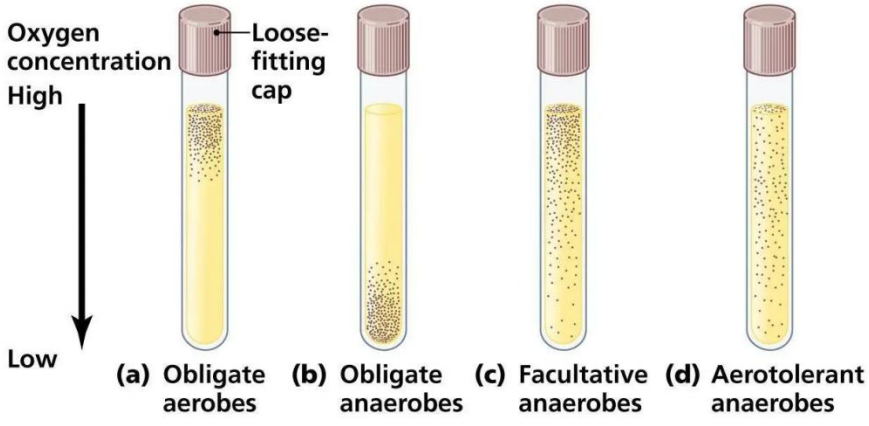


# Classification of bacteria based on oxygen demand

Bacteria are classified to five groups based on the oxygen demand;

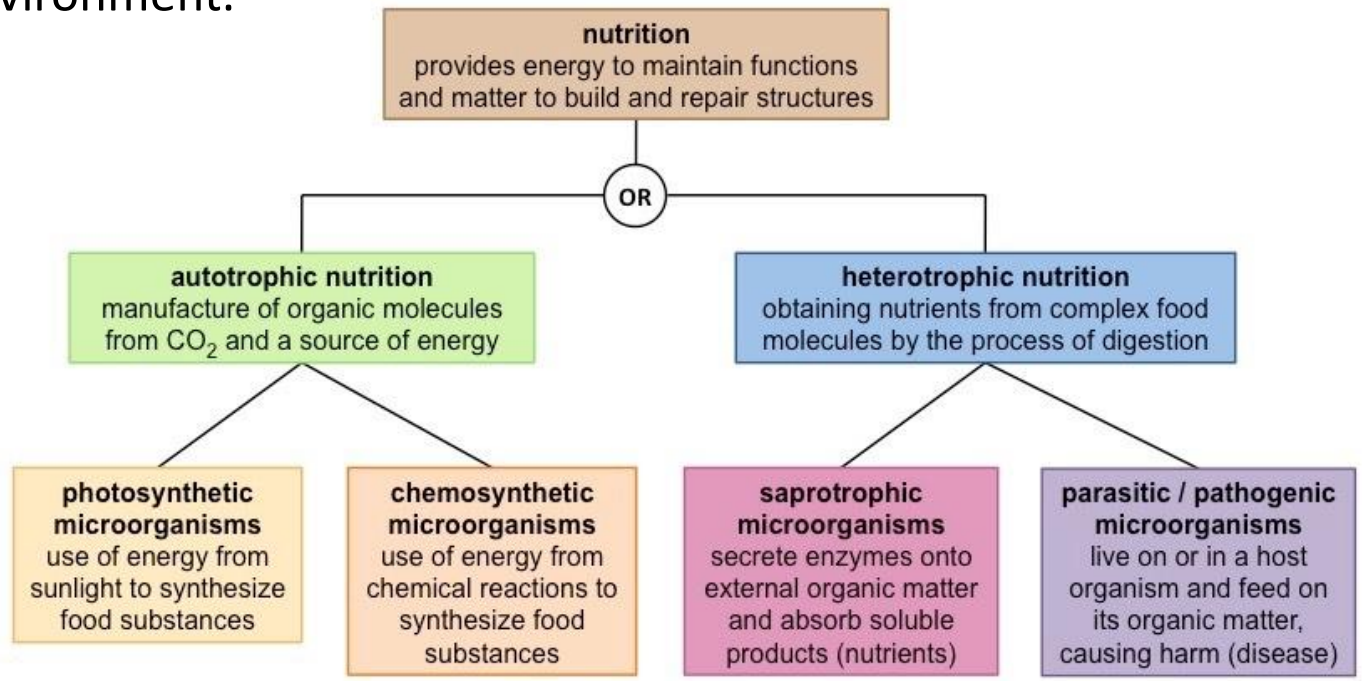
- **Obligate aerobes**: Bacteria that need oxygen in order to survive, reproduce and develop. For example; *M. tuberculosis, B. subtilis* etc.
- **Obligate anaerobes**: Bacteria that thrive in an oxygen-free environment, and use anaerobic respiration and/or fermentation to generate ATP. Oxygen has a toxic effect on anaerobic bacteria. For example, *Clostridium* sp. etc.
- **Facultative Anaerobes**: They are microorganisms that can use oxygen but survive by fermentation or anaerobic respiration. Grows in the best where most oxygen is presented,Utilize oxygen and aerobic respiration whenever possible and They can undergo anaerobic respiration and /or fermentation to survive without oxygen.For example, *Lactobacillus bulgaricus* etc.
- **Aerotolerant anaerobes**: Bacteria that grow evenly, oxygen has no toxic effect and utilize anaerobic respiration and/or fermentation to produce ATP.
- **Microaerophiles**: Bacteria that can grow only where a low concentration of oxygen present in the medium



# Classification of bacteria based on nutrition

- Bacteria, according to their nutrition, are divided into two groups as;
  - **Autotrophic bacteria** that can make their own food.
  - **Heterotrophic bacteria** that take their nutrients readily from their environment.

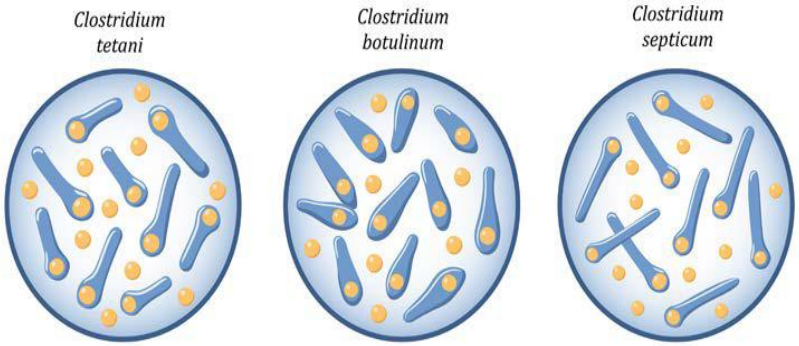


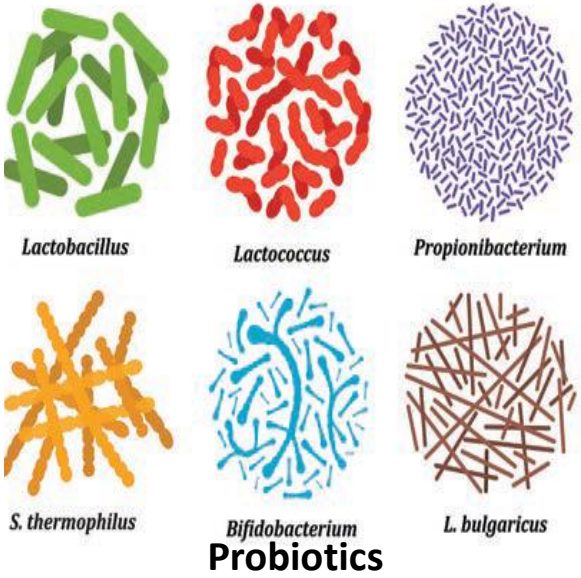
# Importance of Bacteria in Food Industry

Bacteria have a great importance in the food industry with their features such as being used in the production of various foods (performing fermentation, etc.), causing some foods to deteriorate and affecting the quality characteristics of the food (appearance, taste, texture, etc.). For this reason, some bacteria that are important in the food industry should be known.

- For example: *E. coli, Clostridium, Lactobacillus, Staphylococcus, Salmonella, Streptococcus*,

**Starter Culture**: Yogurt, cheese, kefir, pickles, sausage, etc., under certain conditions. in the production of foods to develop aroma, texture development and acid formation, etc. used for microorganisms.



# Fungi (Yeasts and Molds)

- Yeasts and molds: less susceptible to osmotic pressure than bacteria.
- Some molds and yeasts grow in the presence of as much as 60 % sucrose
  - while most bacteria are inhibited by much lower levels.
- Microorganisms that are able to grow in high concentrations of sugars: called osmophiles.
- Osmoduric microorganisms are unable to grow at high level of sugar
  - but able to grow at a level of sugar concentration.

# YEAST

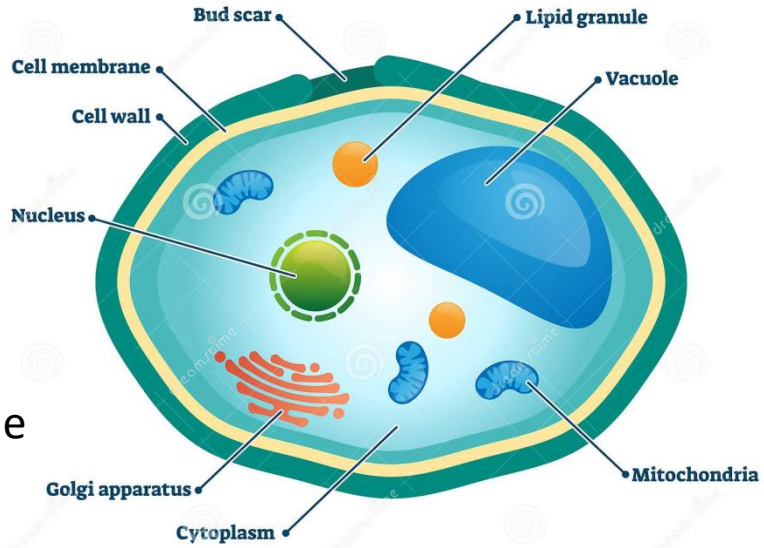
Yeasts with eukaryotic cell structure are filamentless and incapable of photosynthesis. It grows very well under aerobic conditions. It is widely found in nature. The most important feature that distinguishes them from molds is that they are single-celled. The important features that distinguish yeast from bacteria are:

- Yeast cell shapes (ellipse, oval, etc.) are different from bacteria (comma, spirochete, round, rod, etc.)
- Yeast cells are larger than bacterial cells
- Reproduction of yeast by budding
- To be able to distinguish nuclei and cytoplasmic vacuoles in yeast cells under microscope.

# Cell Structure of Yeast

- Yeast cells have eukaryotic cell structure, are unicellular and are larger than bacterial cells. Sometimes during budding, the daughter cell cannot separate from the parent cell and the cells come together to form pseudohyphae (pseudohyphae) with a multicellular appearance.
- The size of the cells varies according to the species and environmental conditions. Cells can be round, oval or cylindrical.

**Nucleus**: A cellular structure with a membrane around it, nucleolus and chromosomes.
**Cytoplasm and Organelles**: In the cytoplasm of a yeast cell; endoplasmic reticulum, vacuoles, granules, mitochondria, Golgi apparatus and many ribosomes.
**Cell Membrane**: Under the cell wall in yeast cell, in sterol structure, permeable has dice. The membrane is rich in enzyme content.
**Cell Wall**: The cell wall is composed of polysaccharide and chitin**.** It gives the yeast cell its shape.



# Properties of Yeast

- In the food industry, it has important functions such as dough fermentation, synthesis of some vitamins and enzymes. However, since it causes deterioration of foodstuffs, it causes negative consequences such as loss of product and cost.
  - It is unicellular.
  - Their cells have membrane-enclosed organelles.
  - Some yeast strains are pathogenic.
  - It is used as a starter culture in food production.
  - The pH development range is wide and some of them can survive in highly acidic conditions such as pH: 1.5.
  - Some yeast strains can grow at high alcohol concentrations such as 18%, while some osmophilic strains can grow at high sugar concentrations such as 55-60%.
  - Yeast generally grows in the temperature range of 0-40 °C. This range is between 25-37 °C for pathogenic species.
  - It mostly uses carbohydrates as an energy source.
  - Forms cream colored colonies on solid media.

# Reproduction of Yeasts

- Depending on reproduction, yeasts can be divided into two groups:
  - sexual reproduction of "**true**" yeasts (Ascomycotina) reproduce by ascospores.

    such as *Saccharomyces*
  - "**False**" yeasts, fungi imperfecti, do not produce by sexual spores;

    such as *Candida*, *Rhodotorula*, and *Cryptococcus*.

Asexual reproduction of the Yeasts

- Reproduction by Division:
- Reproduction by Budding
- Reproduction by Spore

# Importance of Yeast in Food Industry

Yeasts can have both beneficial and harmful effects in the food industry. Yeasts are involved in the production of some foods and additives, and they can also cause spoilage of some foods. Some types of yeast of importance in the food industry are:

- ***Candida***: There are about 200 species belonging to the genus. It can grow in environments with high acid and salt content. It forms white colonies in foods. There are types found in fresh minced meat and poultry meat.
- ***Kluyveromyces***: Used in the manufacture of fermented products and low-lactose dairy products. It can produce lactase from whey. It can spoil the cheeses.
- ***Saccharomyces***: It reproduces by multiple budding. It cannot ferment lactose. The most well-known species of this genus is *Saccharomyces cerevisiae*, also called baker's yeast. It plays an important role in the rise of dough in bread production, in the production of invertase enzyme, in the alcoholic beverage industry such as beer, wine and champagne, in the production of ethanol and glycerol.
- ***Pichia***: Some species belonging to the genus *Pichia* cause deterioration in olive brine, fresh fish, acidic foods such as pickles. *Pichia kluyveri* species produces toxins in food and poses a danger to the food industry. The *P. pastoris* species is used in enzyme production. The *P. anomala* species protects the stored grains and prevents spoilage.

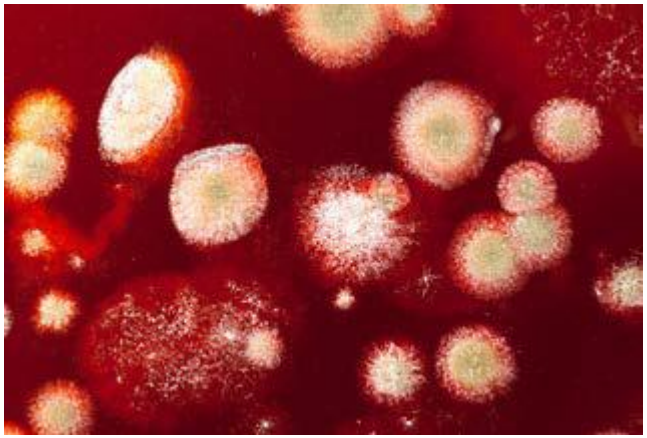
# Molds

- Molds are multicellular, filamentous and eukaryotic microorganisms in the fungi kingdom (including unicellular or multicellular fungi).


# Properties of Molds

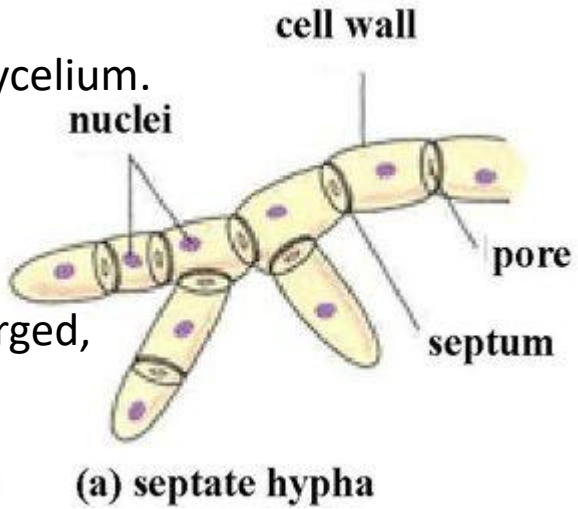
- Molds are very common in nature and can be found almost everywhere. It is easily recognized by its cottony appearance on foods. Usually it is white but black, green, yellow, orange etc. can also form colored colonies.

# Mold colonies

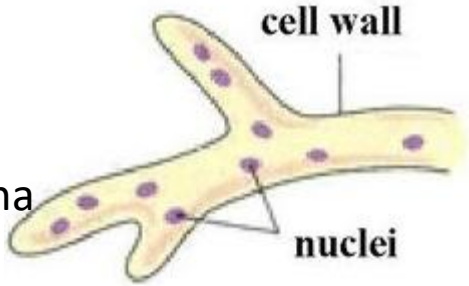


# Cell Structure of Molds

- Molds are filamentous fungi with eukaryotic cell structure. Each of the filaments is called hyphae.
- The hair-like mass formed by the hyphae is called the mycelium.
- Hyphae are classified in several ways:
  - The hyphae that develop on the medium are called aerial,
  - the hyphae that enter the medium are called submerged,
  - the hyphae that take part in nutrition are called vegetative, and
  - the hyphae that reproduce and reproduce are called fertile hyphae.
- In most molds, the fertile hyphae are overhead. Air micelles form the visible part of the colony.
- In some hyphae, the cell membrane between cells dissolves and disappears during the fusion of cells, and while the hypha takes the shape of a tube (nonseptate), in some hyphae, the cell membrane between the cells does not dissolve, and a compartmentalized (septate) structure is formed.



(a) septate hypha



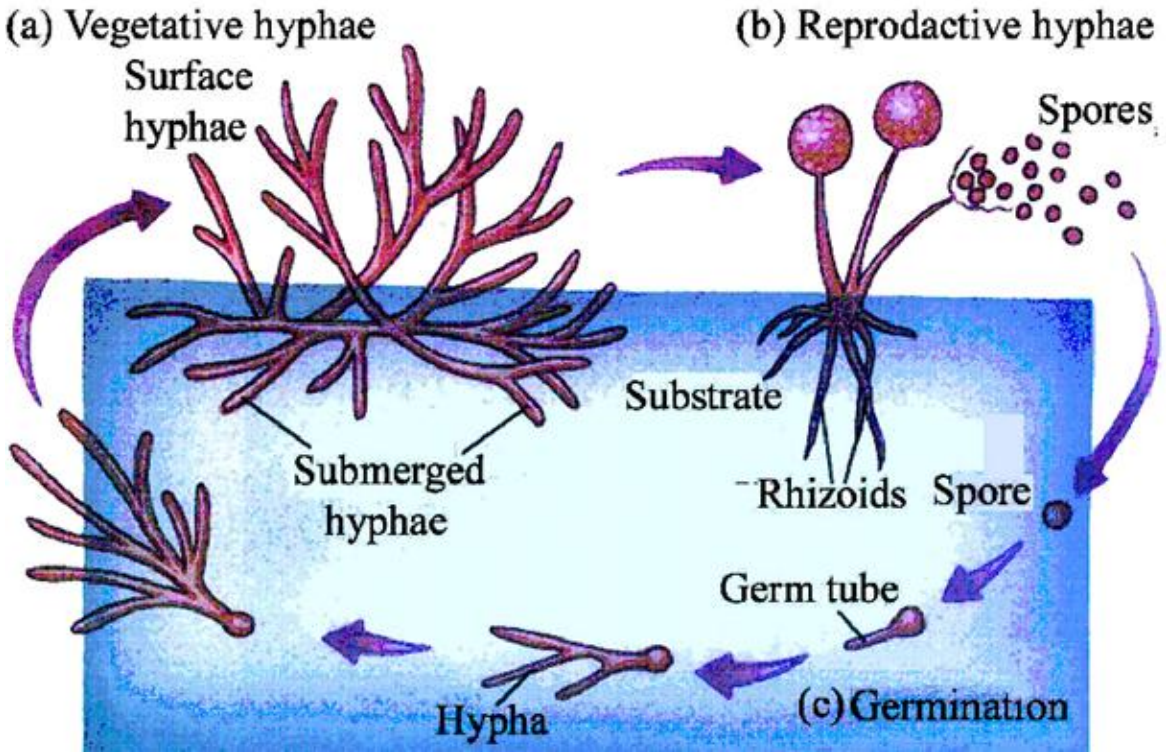
(b) nonseptate hypha

# Properties of molds

- They are aerobic microorganisms and they are mostly mesophilic, although they can grow in a wide temperature range such as 0-60 °C. Optimum temperature demands are between 22-32 °C. Although the optimum pH requirements of molds vary between 5-6, there are species that can grow in highly acidic environments such as pH 2.5. It has a rich enzyme system. In this way, it is fed by diffusion.
- Molds have become industrially important because of the positive and negative changes they cause in foods.
- The benefits of molds can be listed as follows:
  - Cultivated mushrooms are consumed by living things as food.
  - It increases soil fertility by breaking down organic matter in the soil.
  - It provides the development of taste, smell and structure in the production of some cheeses such as Roquefort and Camembert.
  - It is used in the production of antibiotics (penicillin).
  - Molds are used in the production of some vitamins, enzymes, pigments, ethyl alcohol and glycerin.

# Reproduction of molds

- Molds reproduce by asexual and sexual spores;
  - called **perfect molds** and classified as
    - » *Oomycetes, Zygomycetes, Ascomycetes, and Basidomycetes*.
- Molds reproduce by only with asexual spores
  - called **imperfect molds** and classified as
    - » *Conidia, Oidia, Sporangia and Chlamydia*



# Harms of Molds

Some types of mold are pathogenic to humans and animals.

- There are species that produce toxic mycotoxins.
- It causes the loss of nutrients by creating undesirable color, odor, bitterness in foods.
- In humid environments, it damages the structures of organic materials such as wood, fabric, leather.
- In addition to many benefits of molds, there are also harmful aspects.

# Importance of molds in Food Industry

Molds have an important place in the industrial sense due to the positive and negative changes they cause in foods. Some mold types that are important in the food industry are listed as follows:

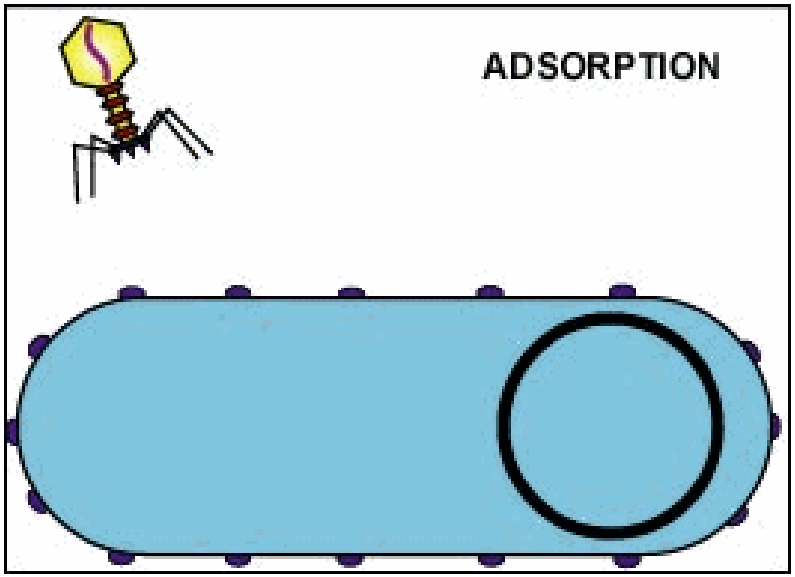
- ***Alternaria***: There are nearly fifty species of the genus *Alternaria*, which are saprophytes and plant pathogens. These species cause decay in plants before and after harvest. Many *Alternaria* species are saprophytes. Depending on the disease, a significant loss of product is observed in vegetables such as apples, potatoes, tomatoes, zucchini and carrots.
- ***Aspergillus***: It is a genus of mold with a very wide distribution in nature. *Aspergillus* species can be found in colors ranging from yellow to green to black. It has segmented hyphae. They cause spoilage in fruits and vegetables, meat, grain products and many other foods. It is the cause of black rot on plums, figs and citrus fruits.
- ***Fusarium***: Produces cotton-like mycelium, usually in light pink, white, yellow color. *Fusarium* mold containing many common soil saprophytes and plant pathogens; grain, sugar beet, chickpea, corn etc. wilt, blight or rot in parts of plants such as seedlings, roots, throats, stems, and cobs; Causes Fusarium wilt or rot on fruits and vegetables such as figs, citrus fruits, bananas, pineapples, pomegranates, potatoes.
- ***Penicillium***: *Penicillium* molds form colonies in foods (citrus, etc.) with colors ranging from blue to blue-green.

# Virus

- **Viruses**; It is an intracellular parasite that can pass through filters that the smallest bacteria cannot pass, and cannot be observed under a simple light microscope. Viruses reproduce only in living cells. It cannot show a complete cell organization. Some viruses only infect animal, plant or bacterial cells.
  - For example, the hepatitis virus only infects the human liver.
  - Rabies virus infects nerve cells of both humans and animals.
- Viruses cannot grow in food, but water and food serve as vectors for the transport of viruses.
- Viruses that cause infectious diseases in humans when taken through the digestive tract are called **enteric viruses**.
  - **Enteric viruses** multiply in the intestines and spread to the environment with feces. The most important enteric viruses transmitted to humans through food are Hepatitis-A virus (the causative agent of jaundice; sarılık) and poliovirus (the causative agent of polio, Çocuk felci).

# Reproduction of viruses

- A bacteriophage
  - attaches itself to the surface of a host bacterial cell
  - inoculates its nucleic acid into the host cell.
  - many phages form in the host and
  - released outside following lysis of the cell.
- Viruses cause diseases of humans, plants, and animals.



# Parasites

- Parasites: roundworms, flatworms, tapeworms and protozoa.
- cause human illness and associate with foods.
- Trichinosis is caused by *Trichinella spiralis* present in meat.
- Anisakiasis is caused by *Anisakis simplex* present in many marine foods (fish).
- Taeniasis is caused by *Taenia saginata* from beef meat and *Taenia solium* from pork meat.
- Toxoplasmosis is caused by *Toxoplasma gondi* present in meat and milk.
- Giardiasis is caused by *Giardia lamblia* present in raw vegetables.
- Ascarisis is caused by *Ascaris lumbricoides* present in raw vegetables.

# Important Bacterial Groups

**1) Lactic acid bacteria**

- ferment carbohydrates and producing relatively large quantities of lactic acid.
- Major genera: *Lactococcus*, *Lactobacillus*, *Leuonostoc*, *Pediococcus*, *Streptococcus thermophilus*.

**Table 2.2.** Principle genera of the lactic acid bacteria

| Genus | Cell morphology | Fermentation | Lactate isomer |
|---|---|---|---|
| *Lactococcus* | cocci in chains | homo | L |
| *Pediococcus* | cocci | homo | DL |
| *Streptococcus* | cocci in chains | homo | L |
| *Lactobacillus* | rods | homo/hetero | DL, D, L |
| *Leuconostoc* | cocci | hetero | D |

## i) Characteristics of LAB

- LAB are a group of Gram-positive, nonmotile, nonsporeforming, rods or cocci, catalase- and oxidase-negative, need carbohydrate to produce energy and lactic acid as the major end product without the need for oxygen.
- LAB are microaerophilic (most are aerotolerant anaerobes).
  - require small amounts of oxygen to function.
    - some LAB use oxygen to produce hydrogen peroxide.
  - require amino acids, B-vitamins, nucleic acid bases (purine and pyrimidine),
  - some grow at high temperature (as high as 45°C),
  - some grow at different pH but mostly well at 4.0-4.5.
- Generation time of LAB ranges from 30 to 90 min.
- Lactic acid produced by LAB inhibits the growth of other bacteria that may decompose or spoil the food.
- They can be divided into different groups depending on glucose metabolism, growth temperature and other characteristics.

**i) Groups of LAB depending on glucose metabolism**

- LAB use one of two pathways; based on type of end products of glucose metabolism.
- This provides a classification: homofermentative and heterofermentative.
- Lactic acid production pathway differ between two groups.

**Homolactic fermentation**

- Homofermentative LAB produce lactic acid as the major product (70-90 %) of glucose fermentation.
  - use Embden-Meyerhof-Parnas (EMP) pathway (glycolysis) to ferment sugar.
  - have key enzymes ALDOLASE and HEXOSE ISOMERASE
    - but lack phosphoketolase.
  - In EMP, six carbon molecule glucose is hydrolyzed to glyceraldehyde-3-phosphate.
  - Then it is converted to pyruvate with production of two ATP molecules by substrate level phosphorylation.
  - Fermentation of 1 mole of glucose yields two moles of lactic acid.
- All members of genera *Lactococcus*, *Pediococcus* and Streptococcus, and some members of *Lactobacillus* are homofermenters,
  - while all members of *Leuonostoc* and some members of *Latobacillus* are heterofermenters.

**Heterolactic fermentation**

- Heterofermentative LAB produce equal amounts of lactate (50 %) and ethanol, acetic acid, $CO_2$, etc. from glucose.
- They have PHOSPHOKETOLASE
  - but lack aldolase and hexose isomerase.
- They use pentose pathway in the conversion of hexose (glucose) to pentose and ribose.
- Heterofermenters and homofermenters can be easily distiquished in the laboratory;
  - by the ability of heterofermenters to produce $CO_2$.
- Fermentation of 1 mole of glucose yields 1 mole each of lactic acid and other end products such as ethanol, acetate and $CO_2$ through pentose pathway.
- Heterolactics are more important than homolactics in the production of flavor and aroma compounds such as acetaldehyde and diacetyl.

- Glycolytic pathway is used by all LAB except *Leuconostoc*, group III *Lactobacillus*, *Oenococci* and *Weissella*.
  - Glycolytic pathway pathway use excess sugar and limited oxygen.
- Homolactics produce twice as much energy from glucose than heterolactics.

**ii) Groups of LAB depending on growth temperature**

**Mesophilic LAB**

- Mesophilic LAB are used for the production of fermented milk products (such as fresh cheese, semi-hard cheese, butter) and sucuk.
- Optimum growth temperature of mesophilic bacteria ranges from 20 to 30°C.
- *Lactococcus lactis* subsp. *lactis* and *Lac. lactis* subsp. *cremoris* are mesophilic LAB.
- *Lac. lactis* subsp. *cremoris* is more salt (<4 %) and temperature sensitive (<40°C) than *Lac. lactis* subsp. *lactis*.
- Mesophilic aroma cultures: *Lac. lactis* subsp. *lactis* biovar. diacetylactis and *Leu. mesenteroides* subsp. *cremoris*,
  - Their growth temperature range from 5 to 38°C,
  - They produce diacetyl flavor from 18 to 25°C and
  - They produce $CO_2$.

**Thermoduric LAB**

- Their optimum growth temperature range from 40 to 45°C with temperature range from 20 to 52°C.
- They are mainly used for the production of yoghurt, hard cheese and soft cheese.
- They are *Str. thermophilus*, *Lb. bulgaricus*, *Lb. helveticus*, *Lb. lactis*, *Lb. casei* subsp. *casei* and *Lb. acidophilus*.
- They may be single strain (containing only one strain of bacteria) or multiple strain (a mixture of several microbial strains, each with its own specific effect).

**Miscellaneous LAB**

- *Bifidobacterium longum*, *Bifidobacterium infantis* and *Propionibacterium shermanii* have optimum growth temperature ranging from 37 to 41°C.
- *Bifidobacterium* spp. are used as probiotic cultures.
- *Propionibacterium* are used in the production of some type of cheeses.

## 2) Acetic acid bacteria

- Acetic acid-forming bacteria: *Acetobacter* and *Gluconobacter*.
- Characteristics of acetic acid forming bacteria:
  1. They are able to oxidize ethyl alcohol to acetic acid,
     - useful in vinegar manufacture and
     - harmful in alcoholic beverages.
  2. *Acetobacter* further oxidize acetic acid to $CO_2$.
  3. They oxidize D-sorbitol to L-sorbose in the preparation of ascorbic acid.
  4. Some species can cause excessive sliminess, e.g., *Acetobacter aceti* subsp. *suboxydans*.

**3) Butyric acid bacteria**

- produce butyric acid in higher amounts under anaerobic condition from carbohydrates.
- Such as *Clostridium* (*C. butyricium*).

**4) Propionic acid-forming bacteria**

- Most of the bacteria in this group present in the genus *Propionibacterium:*
  - *P. jensenii*, *P. freundenreichii*, *P. shermanii*.
- They
  - are cocci,
  - produce propionic acid and
  - are used in dairy fermentation; some types of cheese.

## 5) Proteolytic bacteria

- They hydrolyze proteins due to production of extracellular and intracellular proteinases;
  - to amino acids and small polypeptides.
- Proteolytic species in genera: *Micrococcus*, *Streptococcus*, *Bacillus*, *Clostridium* (*Clostridium sporogenes* and *C. putrefaciens*), *Pseudomonas* (*P. fluorescens*), *Alteromonas*, *Flavobacterium*, *Alcaligenes*, *Enterobacteriaceae*, *Brevibacterium*.
- Some bacteria termed as "acid-proteolytic"
  - carry out acid fermentation and proteolysis simultaneously.
  - *Streptococcus faecalis* var. *liquefaciens* and *Micrococcus caseolyticum* are acid proteolytic bacteria.
- Putrefactive bacteria decompose proteins to produce foul-smelling compounds such as hydrogen sulphide, mercaptanes, amines, indole.

**6) Lipolytic bacteria**

- produce extracellular lipases
- hydrolyzee fats to fatty acids and glycerol.
- *Pseudomonas* (*Pseudomonas fluorescens*, *P. aeroginosa*), and *Alcaligenes* (*A. viscolactis*) are strongly lipolytic.
- *Serratia*, *Staphylococcus*, *Flavobacterium* and *Micrococcus* also contain lipolytic species.
- Many microbial lipases are resistant to food processing techniques; such as UHT treatments of milk.

**7) Saccharolytic bacteria**

- hydrolyze disaccharides and polysaccharides to simple sugars.
- produce extracellular amylase to hydrolyze starch.
- *Bacillus subtilis*, *Clostridium butyricum*, *Aeromonas*, *Pseudomonas* and *Enterobacter*.

**8) Pectolytic bacteria**

- Pectins are complex carbohydrates present in vegetables and fruits.
- produce a mixture of pectolytic enzymes, called pectinase.
  - Hydrolyze pectin and cause softening plant tissues.
- Species of *Bacillus*, *Clostridium*, *Achromobacter*, *Aeromonas*, *Arthrobacter* and *Flavobacterium* include in this group.

**9) Thermophilic bacteria (or thermophiles)**

- are able to grow at 55°C and above.
- are important in foods held at high temperatures;
  - cause spoilage.
- *Geobacillus stearothermophilus* causes flat-sour spoilage in low-acid canned foods.
- *Thermoanaerobacterium thermosaccharolyticum* causes gaseous spoilage in canned foods.

**10) Thermoduric bacteria**

- can survive at heat treatment such as pasteurization;
  - cause spoilage.
- optimum growth temperature is 45°C.
- Some species of *Bacillus* (spores), *Micrococcus*, *Lactobacillus*, *Pediococcus*, *Clostridium* (spores), *Microbacterium* and *Enterococcus* present in this group.

**11) Psychrophilic bacteria (or psychrophile)**

- grow optimally at about 12 to 15°C
- have growth temperature ranging from –5 and 22°C.
- are able to grow in refrigerated and chilled foods.

**12) Psychrotrophic bacteria (or psychrotrophs)**

- are able to grow at refrigerator temperature (≤5°C)
- are important in refrigerated foods.
- have optimum growth temperature between 25 and 30°C;
  - can not grow above 35°C.
- Species of this group present in the genera: *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Alteromonas*, *Brochothrix thermosphacta*, *Arthrobacter*;
- less frequently in the genera *Listeria*, *Yersinia*, *Aeromonas*, *Escherichia*, *Serratia mercescens*, *Proteus*, *Vibrio*, *Bacillus*, *Leuconostoc*, *Clostridium*, *Citrobacter*, *Micrococcus*, *Lactobacillus* and *Enterobacter*.

**13) Osmophilic bacteria**

- can grow at higher osmotic environment than other bacteria.
- can grow in high concentrations of sugar
  - such as species of *Leuconostoc*.
- Some species from genera *Staphylococcus*, *Leuconostoc* and *Lactobacillus* are included in this group.

**14) Halophilic bacteria (or halphiles)**

- important in highly salted foods and brine.
- *Halobacterium*, *Sarcina*, *Micrococcus*, *Pseudomonas*, *Vibrio*, *Bacillus*, *Pediococcus*, *Alcaligenes*. *Micrococcus*, *Staphylococcus*, *Pediococcus*, *Vibrio* and *Corynebacterium*.
- require a concentration of dissolved NaCl for growth.
- Bacteria growing either with or without salt are called salt tolerant bacteria (*Micrococcus*).
- Bacteria can survive, but not grow in high concentrations of salt are called halotolerant.
  - usually grow in foods containing 5 % NaCl or more.
  - bacterial species present in the genera *Bacillus*, *Micrococcus*, *Corynebacterium*, *Streptococcus* and *Clostridium* spp.

- **Groups of halophilic** bacteria depending on salt requirements for growth:
  - Some bacteria grow best at 0.5 to 3 % NaCl called slightly halophilic,
    - bacterial species present in the genera *Pseudomonas*, *Moraxella*, *Flavobacterium*, *Acinetobacter*, and *Vibrio*.
    - They survive in fish and shelfish.
  - Bacteria growing at 3 to 15 % NaCl are called moderate halophiles.
    - bacterial species present in the genera *Bacillus*, *Micrococcus*, *Vibrio*, *Acinetobacter* and *Moraxella*.
  - Some other bacteria growing 15 to 30 % NaCl are called extremely halophilic.
    - bacterial species present in the genera *Halobacterium* (such as *Halobacterium halobium*) and *Halococcus* species are extremely halophiles.
    - survive in heavily brined foods.

**15) Pigmented bacteria**

- produce pigment from black to white on/in foods.
- All species of *Flavobacterium* (yellow to orange) and *Serratia* (red), and many species of *Micrococcus* are pigmented bacteria.
- *Halobacterium* and *Halococcus* produce pigments from red to orange color.
- *Lactobacillus plantarum* discolors cheese.

**16) Slime or rope-forming bacteria**

- produce slime due to synthesis of polysaccharides.
- Some species in the genera *Klebsiella*, *Xanthomonas*, *Leuconostoc*, *Alcaligenes*, *Enterobacter*, *Lactococcus*, and *Lactobacillus* are slime forming bacteria.
- *Leuconostoc* spp. produce slime in sucrose solutions and on foods.
- Some *Bacillus* species are responsible for ropiness in bread.
- *Alcaligenes viscolactis* and *Enterobacter aerogenes* cause ropiness in milk.
- Some species of *Streptococcus* and *Lactobacillus* make milk slimy or ropy.
- *Micrococcus* spp. cause ropiness on meat.
- Strains of *Lactobacillus plantarum* can cause ropiness in various fruits, vegetables and grain products.

**17) Gas-forming bacteria**

- They produce gas ($CO_2$, $H_2$, $H_2S$) from carbohydrates.
- Species in the genera *Leuconostoc* and *Lactobacillus* (heterofermentative) produce only one type of gas; $CO_2$.
- *Propionibacterium* and *Lactobacillus* (heterofermentative), *Escherichia*, *Enterobacter*, *Desulfotomaculum*, *Proteus*, *Bacillus*, and *Clostridium* produce both $CO_2$ and $H_2$.

**18) Sporeformers**

- Rod shaped *Bacillus*, *Clostridium*, *Sporolactobacillus* and *Desulfotomaculum* and *Sporosarcinia* species are able to produce spores.
- Only first two genera are important in food industry.

## 19) Indicator bacteria

- Indicator organisms:
  - coliforms,
  - fecal coliforms,
  - enteric pathogens (*Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia*, *Vibrio*, *Listeria*),
  - enterococci and
  - total counts
    - can be used as an indicator bacteria in the detection of sanitary quality of foods.

# Important Yeast Genera

*Brettanomyces*

- produce high amounts of acid and
- are used in the late fermentation of Belgian and English beers.
- may present in spoiled pickles.
- *B. bruxellansis* and *B. lambicus* are typical species.

*Debaromyces*

- form pellicles on meat brines.
- *D. kloeckeri* grows on cheese and sausage.

*Kluyveromyces*

- ferment sugars including lactose.
- *K. marxianus* produce ethanol from D-xylose,
- *K. fragilis* and *K. lactis* produce β-galactosidase (lactase).

*Mycoderma*

- usually grow on the surface of beers, pickle, wines, fruit juices, vinegar and others.
- produce a heavy film or pellicle.
- *M. vini* associate with wine, vinegar and related products.

*Saccharomyces*

- produce round, oval or elongated cells
- may form a pseudomycelium.
- reproduce by budding and ascus formation.
- used in baking for the leavening of bread and in alcoholic fermentation.
- also involved in bioprocessing (brewing, baking and distilling industries)
- cause spoilage of food with the production of alcohol and $CO_2$.

*S. cerevisiae* is used in many food industrial area:

- special strains used for the leavening of bread; bread production
- Alcoholic beverages:
  - as top yeasts.
    - Top yeasts (film yeasts) grow rapidly at 20°C.
    - Clumping of cells due to rapid production of $CO_2$; swim cells on surface; called top yeast.
    - *S. cerevisiae* var. *ellipsoideus* is a high-alcohol-yielding top strain used to produce industrial alcohol, wines and distilled liquors.
  - as bottom yeasts,
    - Bottom yeast do not clump, grow more slowly, due to evaluation of lower amount of $CO_2$.
    - This permits the yeast to settle to the bottom.
    - are best fermenters at low temperatures (10 to 15°C).
    - *S. cerevisiae* var. *carlbergensis* is used in beer production.
- used for commercial alcohol, glycerol and invertase production.
- *S. fragilis* and *S. lactis* ferment lactose in milk or milk products.

*Pichia*

- form pellicle in beer, wine and brine to cause spoilage.
- Some are also used in oriental food fermentation.
- *P. membranaefaciens* grows as pellicle on beers and wines.

*Torulapsis*

- cause spoilage of milk due to the ability to ferment lactose (*Torulapsis versatilis* and *T. sphaerica*).
- also spoil fruit juice concentrates and acid foods.
- spoil sweetened condensed milk, fruit-juice concentrates and acid foods.

*Candida*

- A few species are of both industrial and medical importance.
- spoil foods with high acid, salt, and sugar and
- form film and pellicle on the surface of liquids.
- cause rancidity in butter (such as *Candida lipolytica*) and other in dairy products. (such as *C. krusei* and *C. utilis*).

*Zygosaccharomyces*

- able to grow in high concentrations of sugar, are called osmophilic.
- spoil honey, syrups and molasses
- are used for the fermentation of soy sauce and some wines.
- *Z. nussbaumeri* grows in honey.

# Groups of yeasts

- Film yeasts (top yeasts): *Pichia*, *Hansenula*, *Debaromyces*, *Candida*, and *Trichosporan*,
  - grow on the surface of acid products such as sauerkraut and picles.
  - oxidize the organic acids, and enable less acid-tolerant organisms to continue the spoilage.
  - *Hansenula* and *Pichia* tolerate high levels of alcohol and may oxidize it in alcoholic beverages.
  - *Debaromyces* is very salt tolerant and can grow on cheese brines with as much as 24 % salt.
  - Film yeasts produce little or no alcohol from sugars.
- **Osmophilic yeasts**
  - grow well in
    - an environment of high osmotic pressure;
    - high concentrations of sugars and salts (such as *Zygosaccharomyces rouxii* and *Z. mellis*).
    - cause spoilage on dry fruits, concentrated juices, honey, and other high-sugar foods.
    - *Debaromyces* species are most salt-tolerant osmophilic film yeasts.

# Important Mold Genera

***Aspergillus***

- xerophilic (able to grow in low $a_w$),
- appear yellow to black color on a large number of foods.
- can grow in grains, jams, cured ham, nuts, and fruits and vegetables.
- useful in the preparation of certain foods.
- cause spoilage in foods: *A. glaucus* and *A. repens*
- Some species produce mycotoxin (e.g., *Aspergillus flavus* produces aflatoxin).
- used in food and food additive processing.
  - *Aspergillus oryzae*: used to hydrolyze starch by $\alpha$-amylase in the production of sake.
  - *Aspergillus niger*: used in the commercial production of:
    - citric and gluconic acids from sucrose and starch, and
    - enzymes such as beta-galactosidase.

*Alternaria*

- cause food spoilage,
- dirty gray green,
- defects on foods:
  - rot in tomatoes and citrus fruits
  - rancid flavor in dairy products and
- Some species produce mycotoxins.
- *Alternaria citri* (rotting on citrus fruits),
- *A. tenuis*, and *A. brassicae* are other common species.

*Byssochlomyse*

- produce heat resistant spores; important in heat treated foods.
- can cause spoilage in high acid canned foods.
- present in soils, ripening fruits (especially grapes).
- *B. fulva*

*Geatrichum*

- produce cotton-cream colony.
- associate with equipment (called machinery mold)
- often grow on dairy products (dairy mold).
- *Geotrichum candidum* often called the “dairy mold,” give white to cream-colored growth.

*Mucor*

- produce cottony colonies.
- appear blue to blue-green color on foods.
- used in food fermentation and as a source of enzymes.
- *Mucor rouxii* is used for the saccharification of starch
- *Mucor* help to ripen some cheeses and are used in making certain oriental foods
- cause spoilage of some foods such as vegetables.

*Penicillium*

- used in food production
  - *Penicillium roquefortii* (with bluish-green conidia) and *Penicillium camembertii* (with grayish conidia) in cheese.
- cause food spoilage of grains, breads, and meat.
  - *P. expansum* with blue-green-spore cause soft rods of fruits, and
  - *P. digitatum* with yellow-green conidia cause soft rods of citrus fruits.
- produce mycotoxins
  - e.g., *P. ochraceum*=ochratoxin, *P. patulum* =patulin.
- produce antibiotics (such as, penicillin).

*Rhizopus*

- usually black in color.
- widespread in nature and present on foods such as fruits, cakes, preserves and bread.
- involved in the spoilage of many fruits and vegetables.
- *Rhizopus stalonifer* is the common black bread mold.
- Some species ferment starch to produce alcohol.