

FE 305 FOOD MICROBIOLOGY

Characteristics of Microorganisms

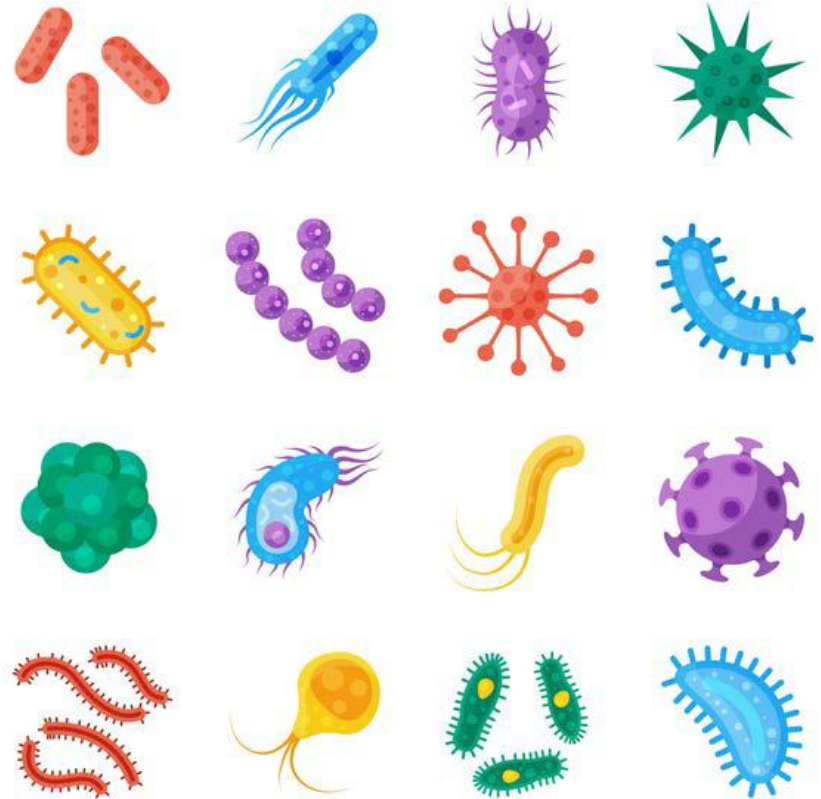
Dr. Hüseyin BOZKURT

Introduction

- The word “microbiology” is formed from the Greek words micro (too small to be seen with the naked eye), bios (life) and logos (science).
- Simple organisms that cannot be seen with the naked eye but can be observed with a microscope are called “microorganisms”.
- Microbiology examines the morphology, structure, reproduction and physiological and biochemical activities of microorganisms.
- The scope of microbiology covers:
 - the distribution of microorganisms in nature;
 - their relationships with each other and with other living things;
 - their harmful and beneficial effects; and
 - the nomenclature and classification of microorganisms.
- All cellular microorganisms studied in microbiology share the following common properties:
 - they reproduce;
 - they digest and metabolise nutrients;
 - they excrete waste products;
 - and they respond to changes in their environment.
- Viruses are the exception: they are acellular and can replicate only inside a living host cell.

Introduction

- Microorganisms live throughout the biosphere:
 - in water:
 - oceans, lakes, ponds and waterfalls,
 - in marshes and even in rain clouds;
 - on land:
 - on and within soil and rocks;
 - in intermediate layers:
 - sediments of all water bodies and groundwater;
 - and on and inside animals and plants.



Food Microbiology

- **Food comprises all raw, semi-processed and processed materials, excluding drugs and tobacco, that are eaten or drunk and that supply the nutrients (protein, fat, carbohydrate, minerals, vitamins) people need to maintain life.**
- **Food microbiology is the sub-discipline that studies the microorganisms naturally present in foods and those that contaminate foods during storage, transport and processing.**
- Microorganisms have two kinds of effect in the food industry: beneficial and harmful.
- **Classification of microorganisms according to the role they play in the food industry:**
 - pathogenic microorganisms
 - spoilage microorganisms
 - beneficial (technologically useful) microorganisms
 - indicator microorganisms

Advances in Food Microbiology

- 6000–8000 BC: preservation by cooking
- 3000 BC: salting, drying and preservation under oil
- 1000 BC: burial and fermentation of fish and shrimp
- 944–945 AD: about 40 000 deaths from ergotism caused by *Claviceps purpurea* (France)
- 10th–11th century: Ibn Sina (Avicenna) suggests that diseases are transmitted by invisible agents (also Akşemseddin, 15th century)
- 1658: Kircher proposes that invisible “worms” are responsible for food spoilage
- 1676–1683: Leeuwenhoek, using the microscopes he built himself, observes and describes bacteria for the first time
- 1765: Spallanzani provides evidence against the theory of “spontaneous generation”
- 1810: Appert shows that keeping foods in sealed glass jars heated in hot water extends their shelf life
- 1837: Schwann lays the foundations for the preservation of foods by heat treatment
- 1857–1876: Pasteur is the first to describe the existence and role of microorganisms in foods and fermentations

Advances in Food Microbiology

- Antonie van Leeuwenhoek (1632–1723) built his own single-lens microscopes and was the first to observe and describe microorganisms.
- Berkeley and Pasteur showed that microorganisms cause disease.
 - Joseph Lister introduced antiseptic technique into surgical operations.
- Robert Koch (1843–1910) demonstrated the relationship between *Bacillus anthracis* and anthrax and isolated the tubercle bacillus; he also formulated Koch's postulates.
- Charles Chamberland (1851–1908) developed the porcelain filter that retains bacteria; using such filters, Ivanovsky (1892) and Beijerinck (1898) demonstrated the existence of viruses.

Advances in Food Microbiology

Louis Pasteur (1822 – 1895)

- He developed vaccines against fowl cholera, anthrax and rabies.
- He showed that each type of fermentation depends on a specific yeast or bacterium.
- He developed pasteurisation for the preservation of wine and beer.
- He showed that the microorganisms responsible for fermentation are anaerobic and that some of them cannot grow in the presence of oxygen.
- He disproved the theory of spontaneous generation with his swan-necked flask experiments.

Advances in Food Microbiology

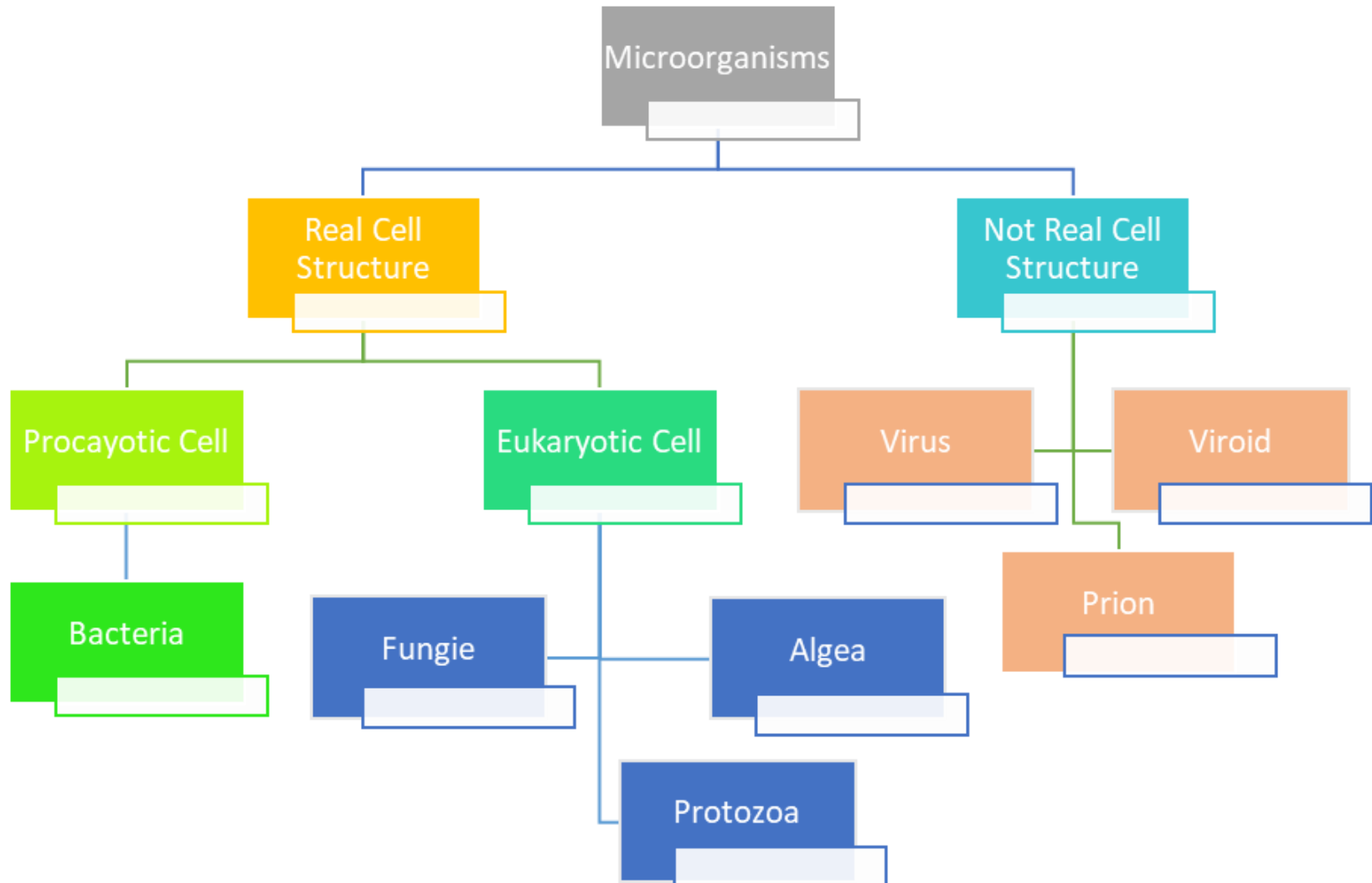
Separate branches of science have developed for the different groups of microorganisms. These are:

- **Bacteriology:** the discipline that studies bacteria.
- **Mycology:** the discipline that studies yeasts and moulds (fungi).
- **Virology:** the discipline that studies viruses.
- **Parasitology:** the science that studies parasites and their disease-causing properties.
 - In addition, the following applied branches have developed:
- **Medical microbiology:** studies the relationship between microorganisms and humans and the harm they cause.
- **Food microbiology:** aims to prevent the harmful effects of microorganisms in food technology and to exploit their beneficial effects in food production.
- **Industrial (technical) microbiology:** covers the production of foods, additives and metabolites with microorganisms, the starter cultures and techniques used (for cheese, yoghurt, baker's yeast, etc.), the development of new production methods, the protection of cultures, the utilisation of wastes and water and wastewater treatment.

Microorganisms

- Although microorganisms appeared on earth long before higher organisms, they are so small that their direct observation and study had to await the development of the microscope and of microscopic techniques.
- In the older classification systems, living things were divided into three large groups on the basis of their structure and other characteristics:
 - plants,
 - animals, and
 - protista (microorganisms).
- Because of the differences in the nutrition, motility, cell structure and metabolic activities of microorganisms, Haeckel placed them in a separate kingdom, the Protista. Today, however, the three-domain system proposed by Woese (1990) is used: Bacteria, Archaea and Eukarya. Viruses are acellular and are not included in this system.

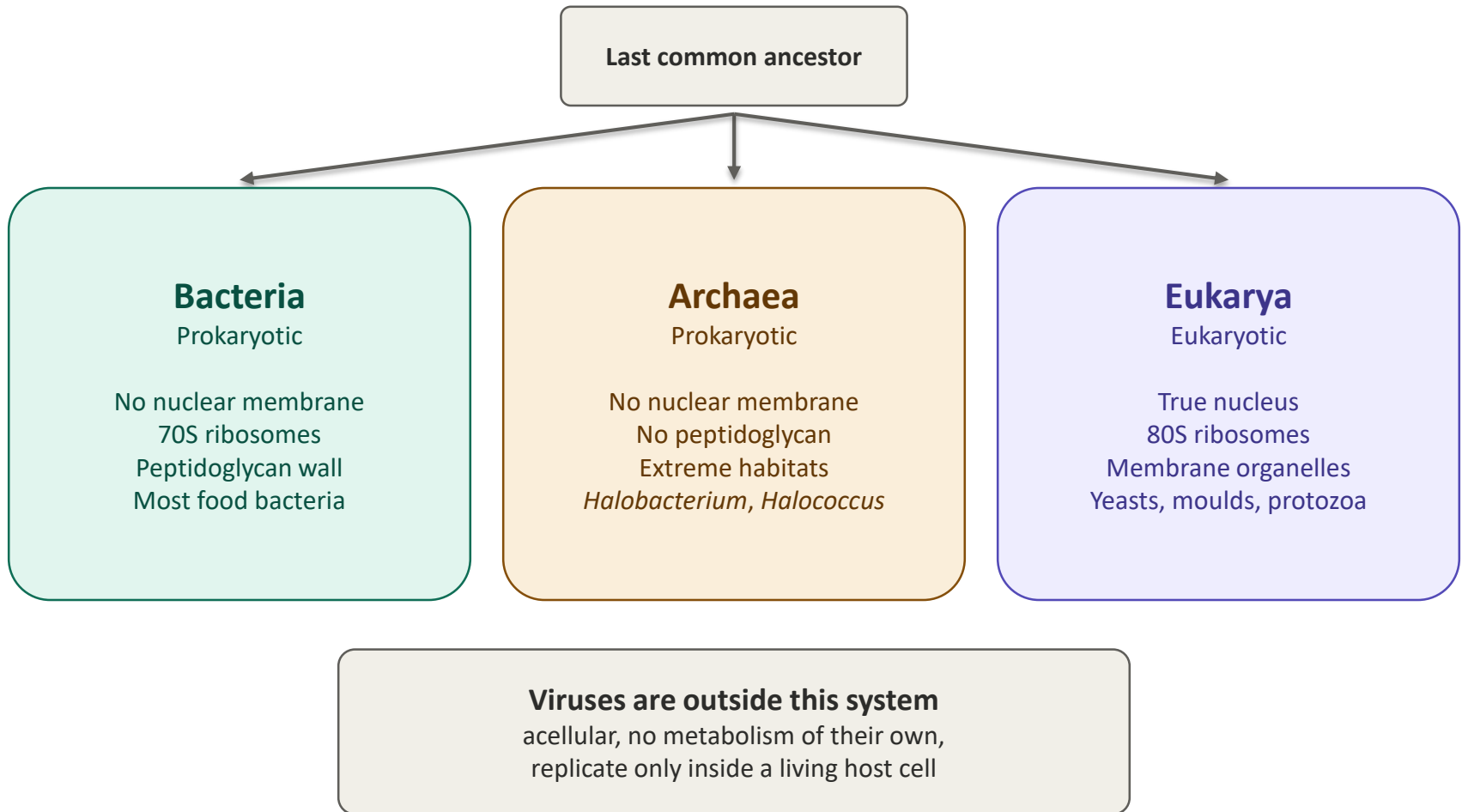
Classification of Microorganisms According to Cell Structure



Microorganisms

- According to their cell structure, microorganisms are divided into two main groups:
 - **Eukaryotic microorganisms:** organisms whose cell structure resembles that of animals and plants, with a true nucleus surrounded by a nuclear membrane. Fungi (moulds and yeasts), algae and protozoa belong to this group.
 - **Prokaryotic microorganisms:** organisms that have no true nucleus surrounded by a nuclear membrane. Bacteria (including the cyanobacteria, formerly called blue-green algae) and the Archaea belong to this group. Viruses are acellular and belong to neither group.
- Of these groups, the microorganisms important in foods are the bacteria, the fungi (moulds and yeasts) and the viruses.
- Many bacteria, moulds and yeasts are biologically active in foods.
- Some bacteria, moulds and yeasts growing in foods of plant and animal origin cause spoilage and may cause infection or poisoning in the people and animals that consume them, while others are used in the production of fermented foods and alcoholic beverages.

Classification of Living Organisms



Three-domain system proposed by Woese (1990); it replaced the older division into plants, animals and Protista.

Nomenclature of Microorganisms

- Microorganisms are named according to the binomial system, using Latinised names consisting of two words.
 - The first word is the genus name and begins with a capital letter. It may be derived from the name of the person who first described the microorganism or from its morphological, physiological, ecological or biochemical characteristics.
 - The second word is the species epithet and begins with a small letter. It reflects a characteristic of the organism (colony colour, habitat, disease caused, shape, etc.).
- Both words are written in italics; in handwriting they are underlined.
 - *Bacillus anthracis*
 - *Bacillus cereus*
 - *Bacillus subtilis*
 - *Staphylococcus aureus*
 - *Salmonella enterica* subsp. *enterica* serovar Typhi (serovar names are not italicised)
- In *Escherichia coli*, the first word commemorates the scientist Escherich, who first described the bacterium, and the species epithet *coli* refers to the colon, the part of the large intestine in which it lives.

Characteristics of Microorganisms

- The simplest cellular forms of life consist of single, small cells.
- Such microscopic units are typical of the microbial groups:
 - bacteria, yeasts, moulds, viruses, algae and protozoa.
- Microorganisms differ from one another in
 - size,
 - shape,
 - energy-yielding biochemical reactions and
 - internal organisation.
- Bacteria, yeasts, moulds and viruses are important in:
 - food spoilage,
 - food production, and
 - foodborne diseases (yeasts are not involved in foodborne disease).

TYPES OF MICROORGANISMS

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

Bacteria

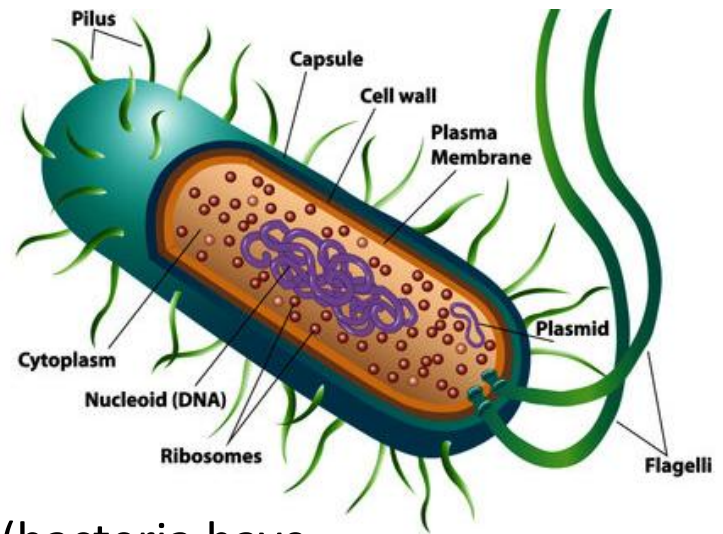
- Prokaryotic cells occur in different shapes (rod, coccus, spiral, etc.).
- Not all of the structures listed below are found in every genus.
- Cells: unicellular, generally 0.5–2.5 μm in diameter and 2–10 μm long.
- motile or non-motile;
- surrounded by a rigid cell wall;
- the cytoplasmic membrane also carries the components of energy generation;
- the ribosomes are of the 70S type;
- the genetic material (chromosomal and plasmid DNA) is circular, is not enclosed in a nuclear membrane and is not associated with basic proteins such as histones;
- the cells divide by binary fission;
- flagella, capsule, surface-layer proteins and pili serve specific functions;
- some genera form endospores (one per cell);
- on the basis of their Gram-staining behaviour, bacterial cells are grouped as Gram-positive and Gram-negative.

Cell Structure of Bacteria

- Bacteria, which have a prokaryotic cell structure, are found everywhere in nature: in soil, water and air and on plants and animals.
- Most bacteria are unicellular and reproduce by binary fission. Their nutritional requirements are simple and they adapt rapidly to environmental conditions. Their length varies between 2 and 10 μm and their diameter between 0.5 and 2.5 μm .

The main structures of a bacterial cell are:

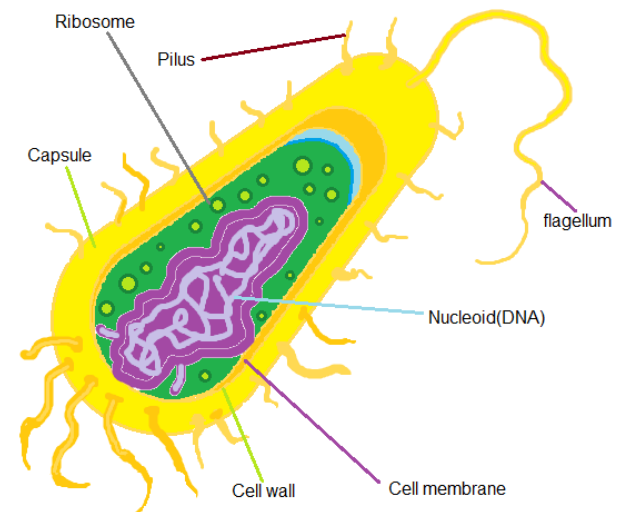
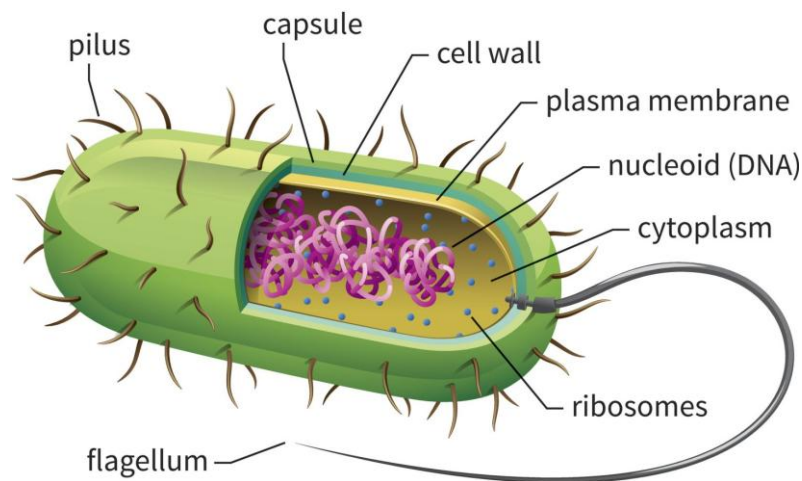
- Capsule
- Cell wall
- Cytoplasm membrane (Cell membrane)
- Flagella and pili (fimbriae)
- Endospore
- Storage granules (reserve nutrients)
- Ribosomes and the bacterial chromosome (bacteria have no true nucleus surrounded by a membrane; the condensed genetic material lies free in the cytoplasm as the nucleoid)



Bacterial Main Structure

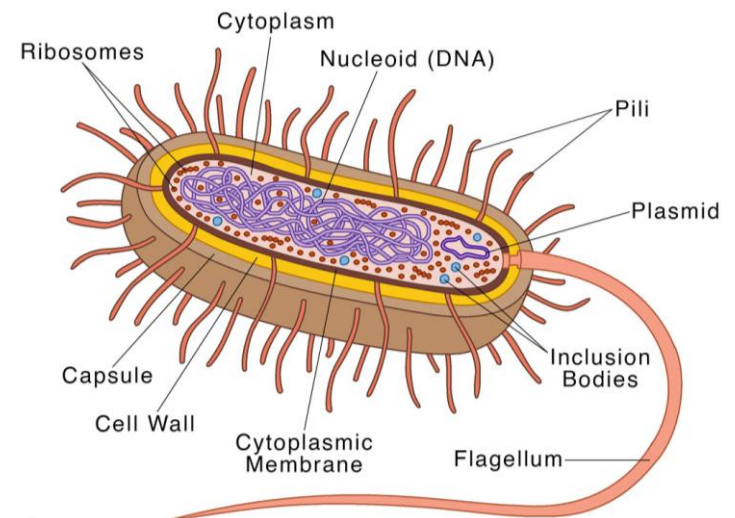
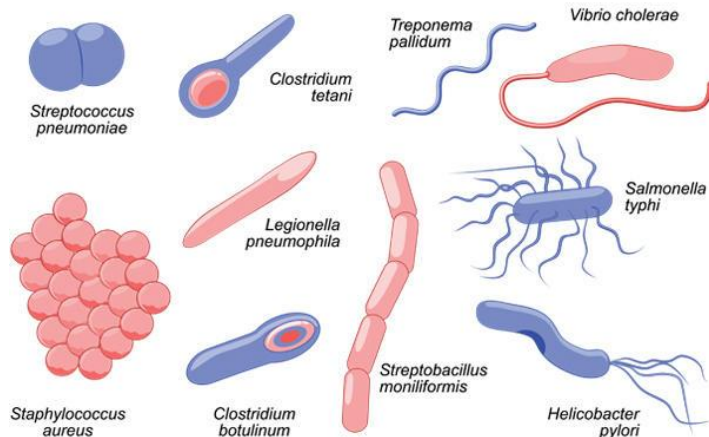
Cell wall: a thin, semi-rigid and strong layer that surrounds the cytoplasmic membrane and protects the delicate inner structures from external effects.

- This layer, which is responsible for the characteristic shape of the cell, is freely permeable to small molecules.
- It plays a role in the passage of nutrients into the cell and in the removal of metabolic wastes.
- The cell wall determines the Gram-staining reaction, which is of particular importance for bacteria; this reaction is related to the peptidoglycan (murein) network of the wall.

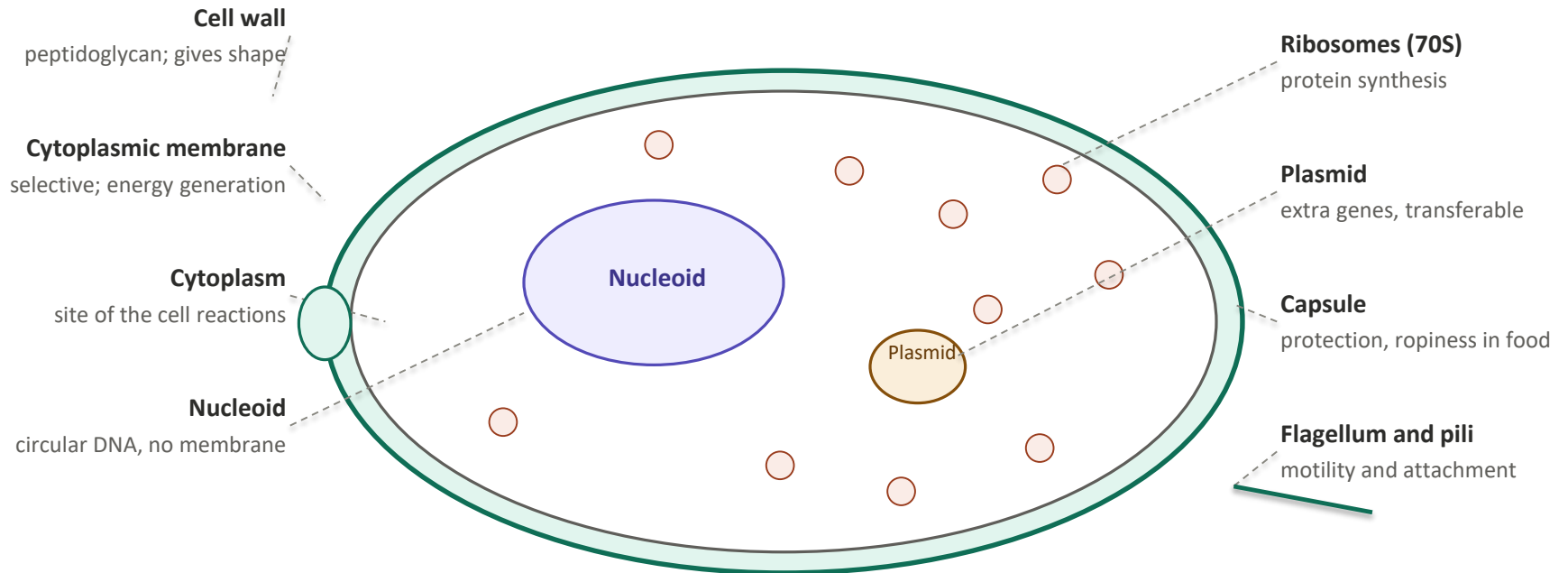


Cell Structure of Bacteria

- Cytoplasmic membrane: this membrane lies immediately beneath the cell wall and surrounds and protects the cytoplasm. It is thinner than the cell wall, and any damage to it leads to the death of the bacterium.
- Cytoplasm: physically it is a clear, slightly viscous fluid. Functionally it is the site where the chemical reactions of the cell take place and where the building blocks of the cell are synthesised.
- Nucleoid: the genetic material of the bacterial cell consists of DNA (deoxyribonucleic acid) together with a small amount of RNA (ribonucleic acid). The region of the cytoplasm in which it is concentrated is called the nucleoid. Bacteria have neither a nuclear membrane nor a nucleolus.
- Ribosomes: the only organelles found in bacteria; they carry out protein synthesis.
- Flagella: structures that provide motility. They may be single or multiple and differ in their arrangement on the cell (monotrichous, lophotrichous, amphitrichous, peritrichous).
- Capsule: not essential for cellular function. However, it protects the cell against desiccation and phagocytosis and increases the infectivity of pathogenic bacteria. When capsule-forming bacteria grow in a food, ropiness (sliminess) develops.



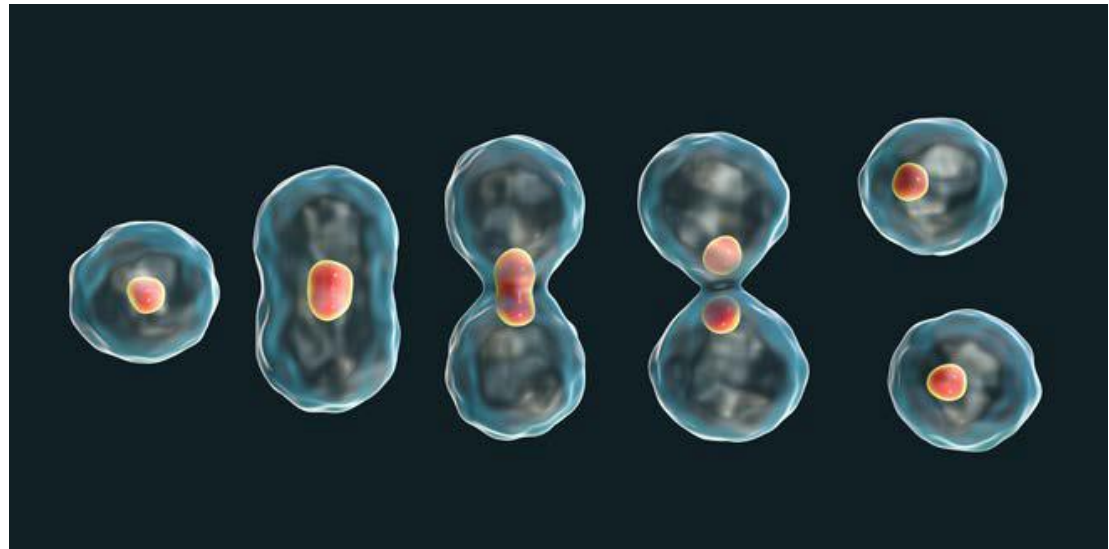
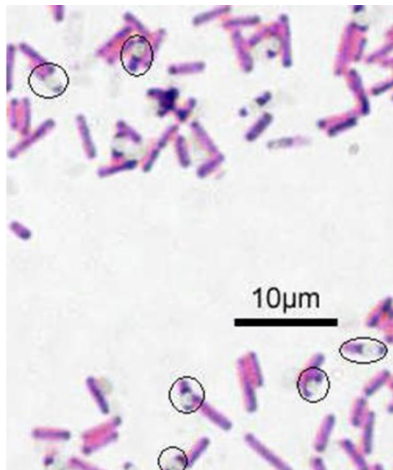
Structure of a Bacterial Cell



*Bacteria have no nuclear membrane, no mitochondria and no endoplasmic reticulum. Endospores, formed by *Bacillus* and *Clostridium*, are not shown here - they are survival structures, not a means of reproduction.*

Growing of Bacteria

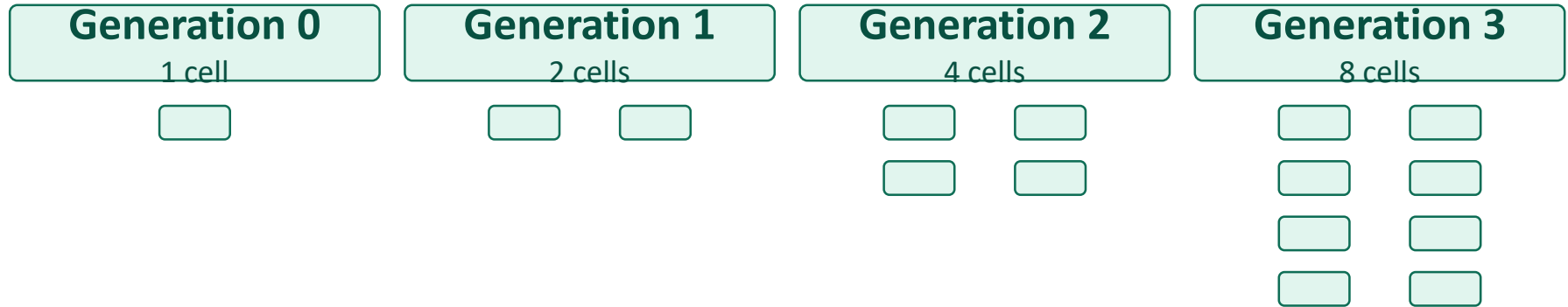
- Bacteria, like all microorganisms, grow and multiply when the environment is suitable. The formation of new individuals similar to themselves in order to continue the species is called reproduction.
- Bacteria reproduce by binary fission, a form of vegetative reproduction.
- In a suitable medium and under suitable environmental conditions, microorganisms grow and multiply at a rate characteristic of the species. Factors such as the amount of nutrients, the accumulation of waste products and the temperature also affect the growth rate. Therefore, distinct growth phases are observed in a culture medium.



Growing of Bacteria

- Binary fission takes place in the following stages:
 - replication of the genetic material (DNA);
 - separation of the two DNA copies towards the poles of the cell;
 - invagination of the cytoplasmic membrane and formation of a septum between the two halves;
 - separation of the two daughter cells at the septum.
- The result is the division of the mother cell into two genetically identical daughter cells.
- During division, the structural components of the bacterial cell are doubled. The two cells arising from the division of the mother cell are called a generation, and the time required for a cell to divide into two is the generation (doubling) time. The generation time differs between species; under optimum conditions it is about 20 minutes for *Escherichia coli* and only 10–12 minutes for *Vibrio parahaemolyticus*.

Binary Fission and Exponential Growth



With a generation time of 20 minutes (*E. coli* under optimum conditions):

time	0 h	1 h	2 h	4 h	7 h	10 h
generations	0	3	6	12	21	30
cells	1	8	64	4 096	about 2 million	about 1 billion

$$N = N_0 \times 2^n$$

$$n = t / g$$

t = time, g = generation time

Why this never goes on for long in food

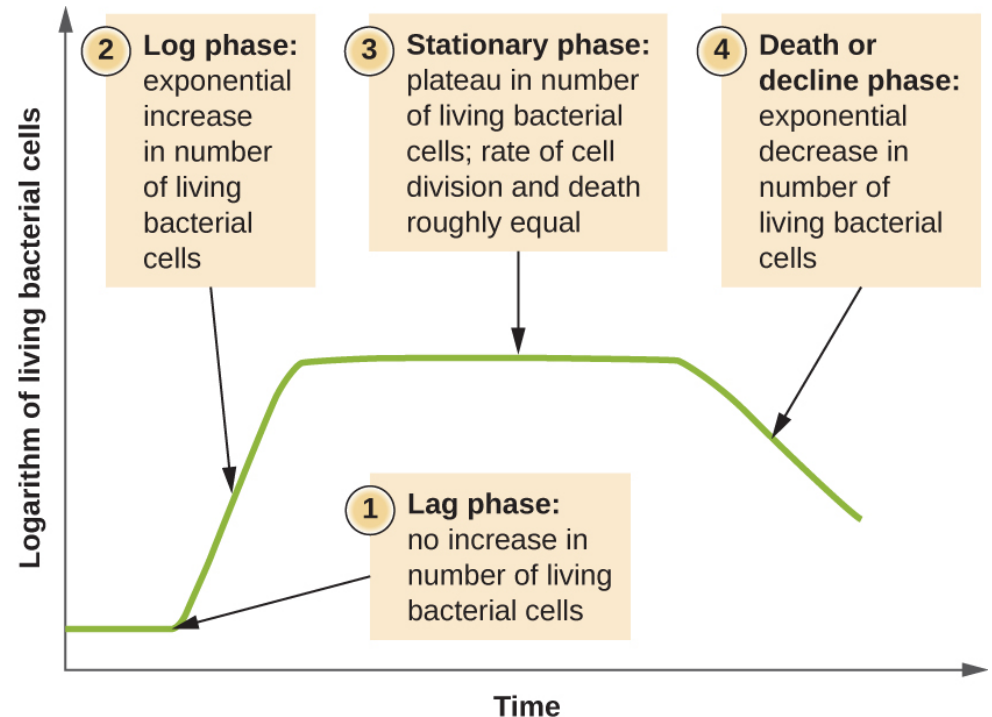
nutrients run out and waste products

accumulate - growth reaches the

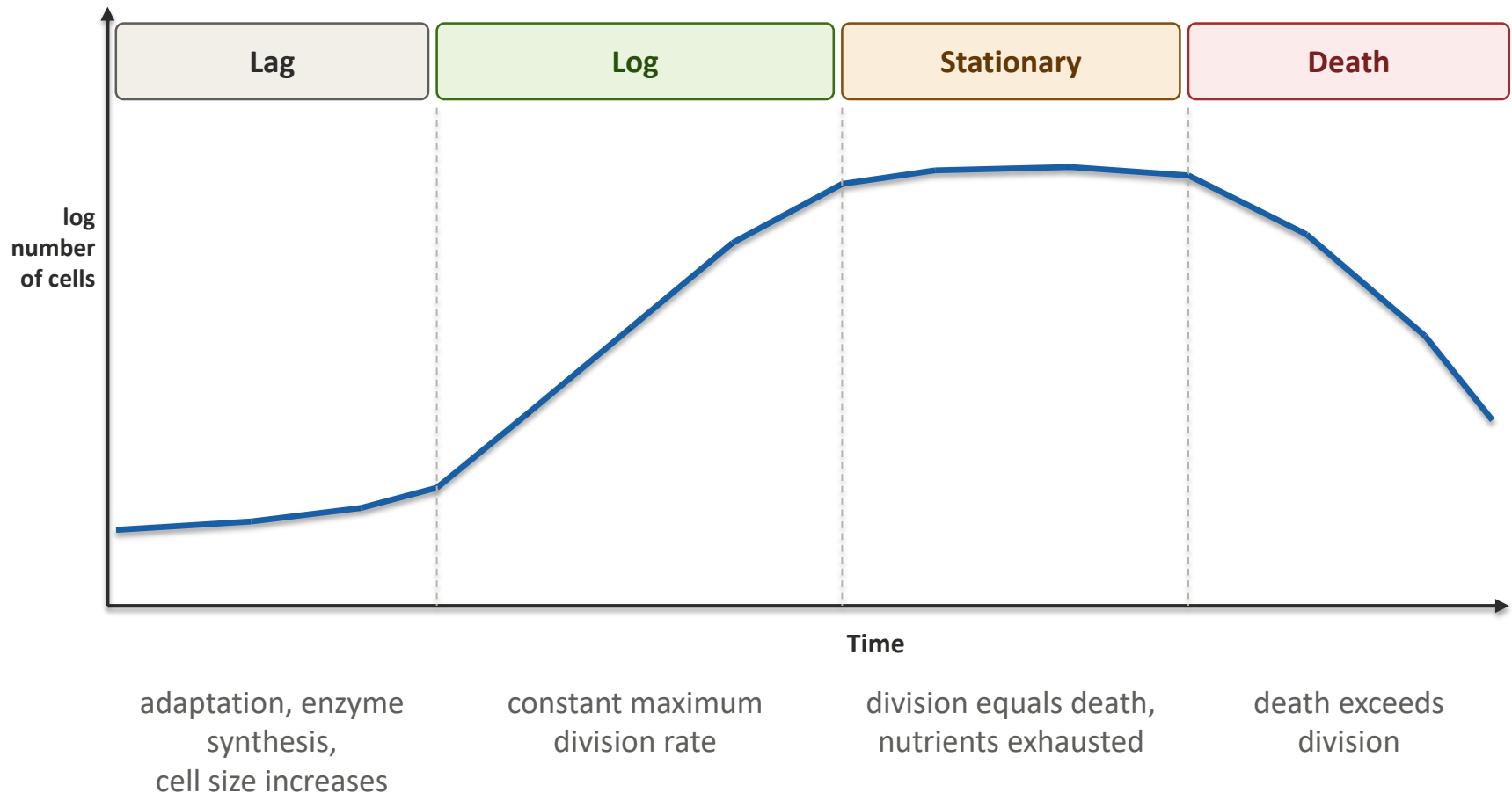
stationary phase (about 10^9 - 10^{10} cells/g)

Growing of Bacteria

- These growth phases are:
 - **Lag phase:** the number of cells does not change; the cells adapt to the medium, increase in size and synthesise the enzymes they need.
 - **Logarithmic (exponential) phase:** the cells divide at a constant maximum rate, so that the number of cells increases logarithmically.
 - **Stationary phase:** as nutrients are depleted and waste products accumulate, the number of dividing cells equals the number of dying cells, so the viable count remains constant.
 - **Death phase:** after the stationary phase the death rate exceeds the division rate and the number of viable cells falls markedly.



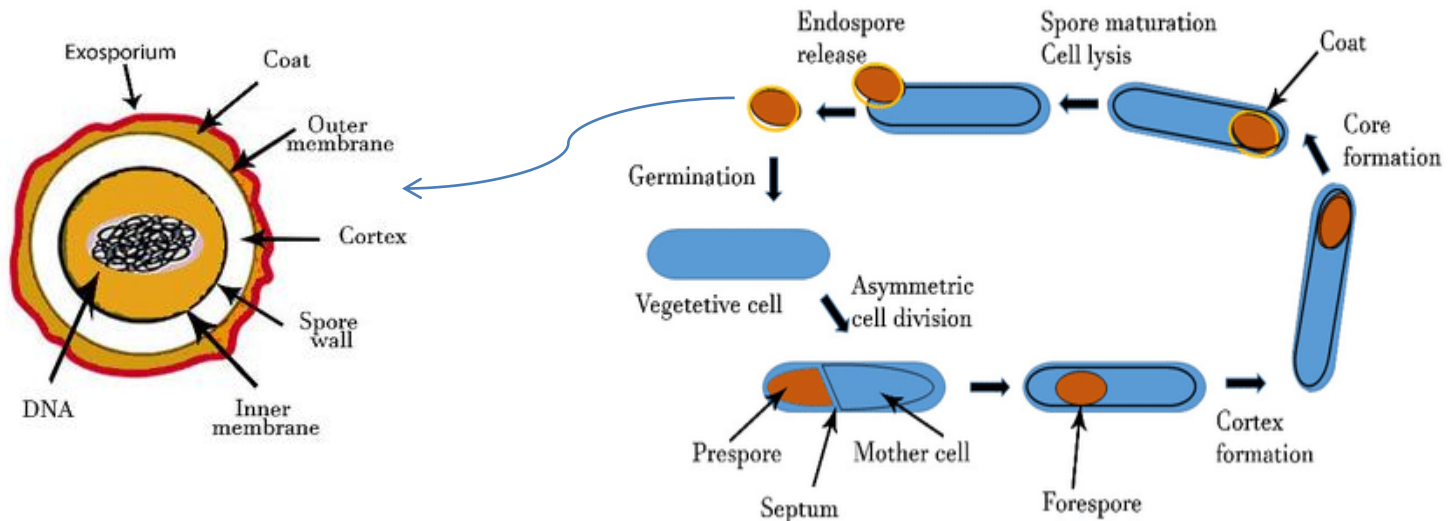
The Bacterial Growth Curve



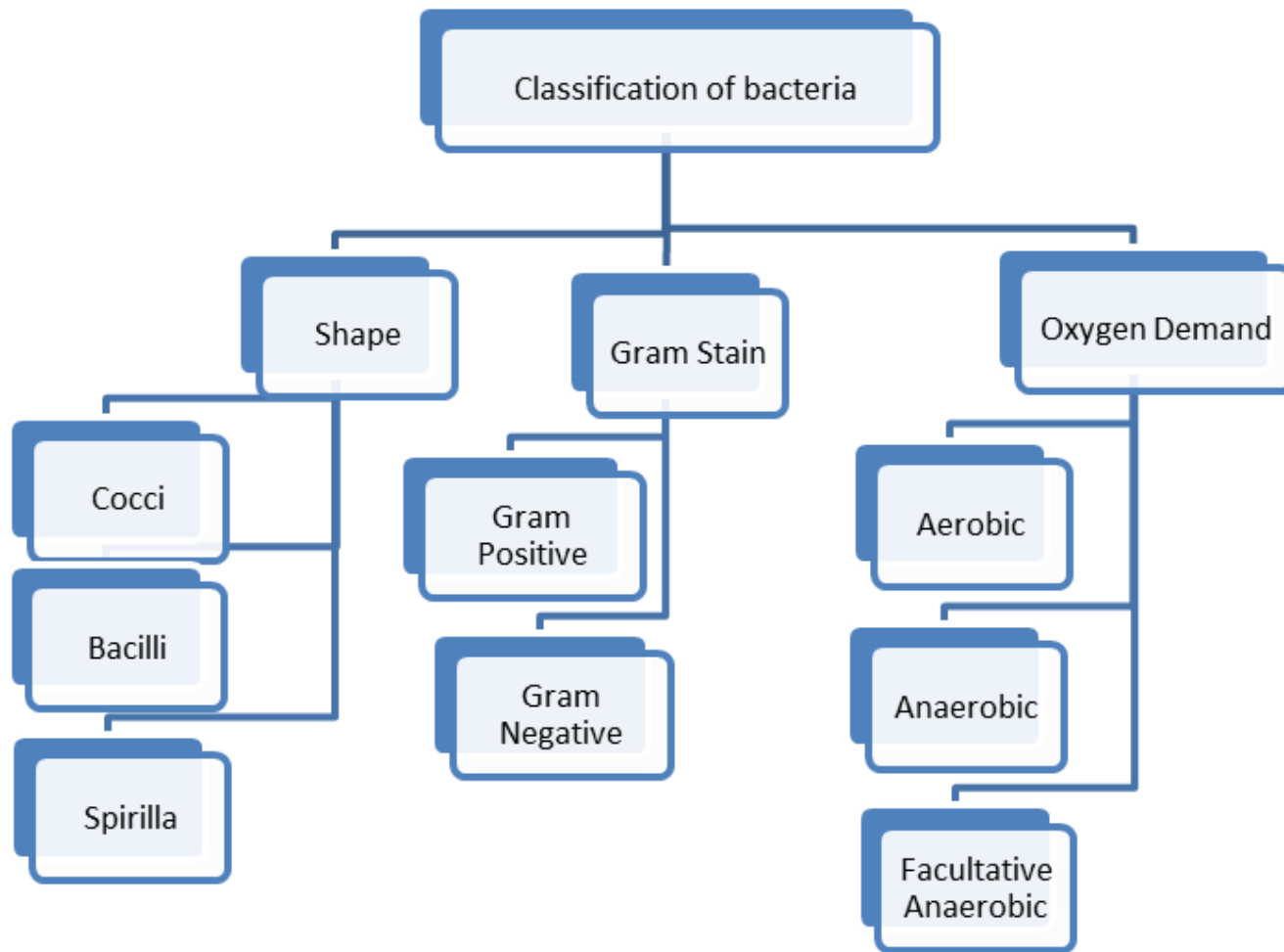
Generation time is measured in the log phase. Spoilage usually becomes detectable only late in this phase, at about 10^7 cells per gram - long after growth has begun.

Growing of Bacteria

- Some bacteria, such as those of the genera *Bacillus* and *Clostridium*, form a thick-walled, round, highly resistant structure when environmental conditions (heat, light, freezing, drying, radiation, lack of nutrients) become unfavourable. This structure, which is far more resistant than the vegetative cell, is called an endospore. The endospore is the living but dormant form of the bacterium. Its formation is not a means of reproduction but a means of survival. When conditions improve, the endospore germinates and returns to the vegetative form.
- The endospore may form in different parts of the cell: in the middle (central), near one end (subterminal) or at the end (terminal); it may or may not swell the cell.

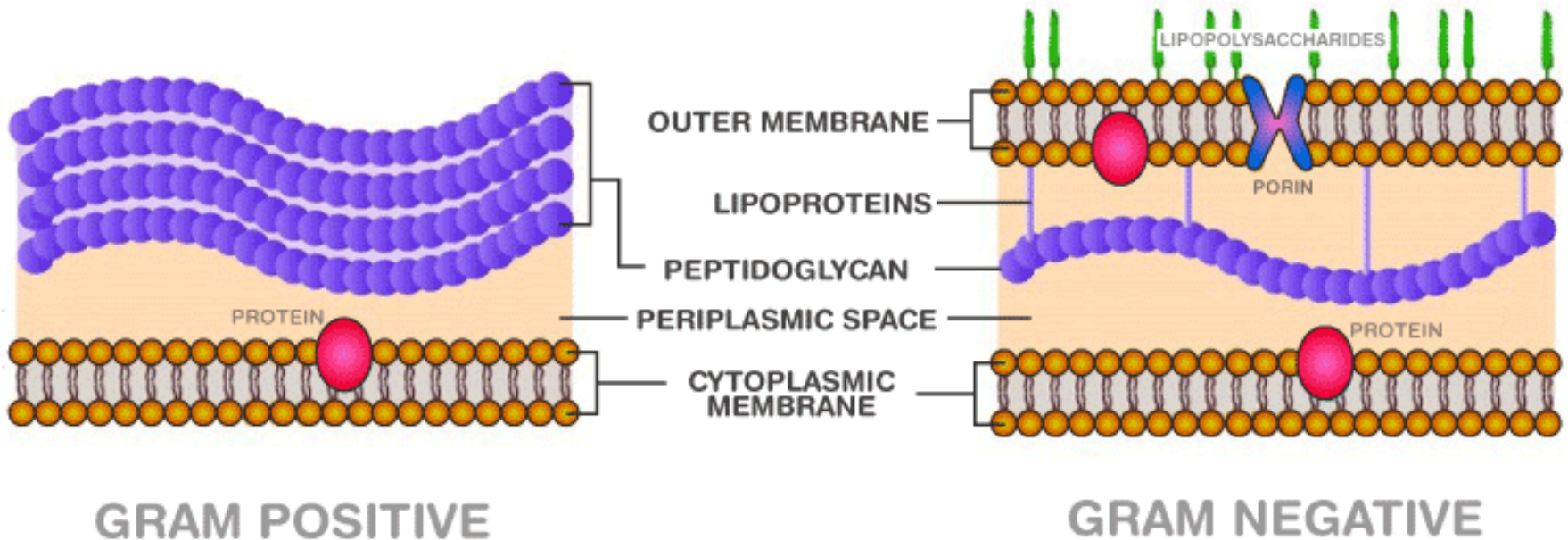


Classification of Bacteria



Classification of bacteria based on gram staining

- The Gram reaction depends on the peptidoglycan (murein) layer of the cell wall.
 - Gram-positive: thick peptidoglycan layer; retain crystal violet and appear blue-purple.
 - Gram-negative: thin peptidoglycan plus an outer membrane; stained red-pink by safranin.



Gram-Positive and Gram-Negative Cell Walls

Gram-positive

Thick peptidoglycan (20 - 80 nm)

Teichoic acids

Cytoplasmic membrane

Consequences

Retains crystal violet - stains blue-purple
No outer membrane, no lipopolysaccharide
More sensitive to penicillin and to lysozyme
Examples: *Staphylococcus*, *Bacillus*, LAB

Gram-negative

Lipopolysaccharide (endotoxin)

Outer membrane with porins

Thin peptidoglycan (2 - 7 nm)

Periplasmic space

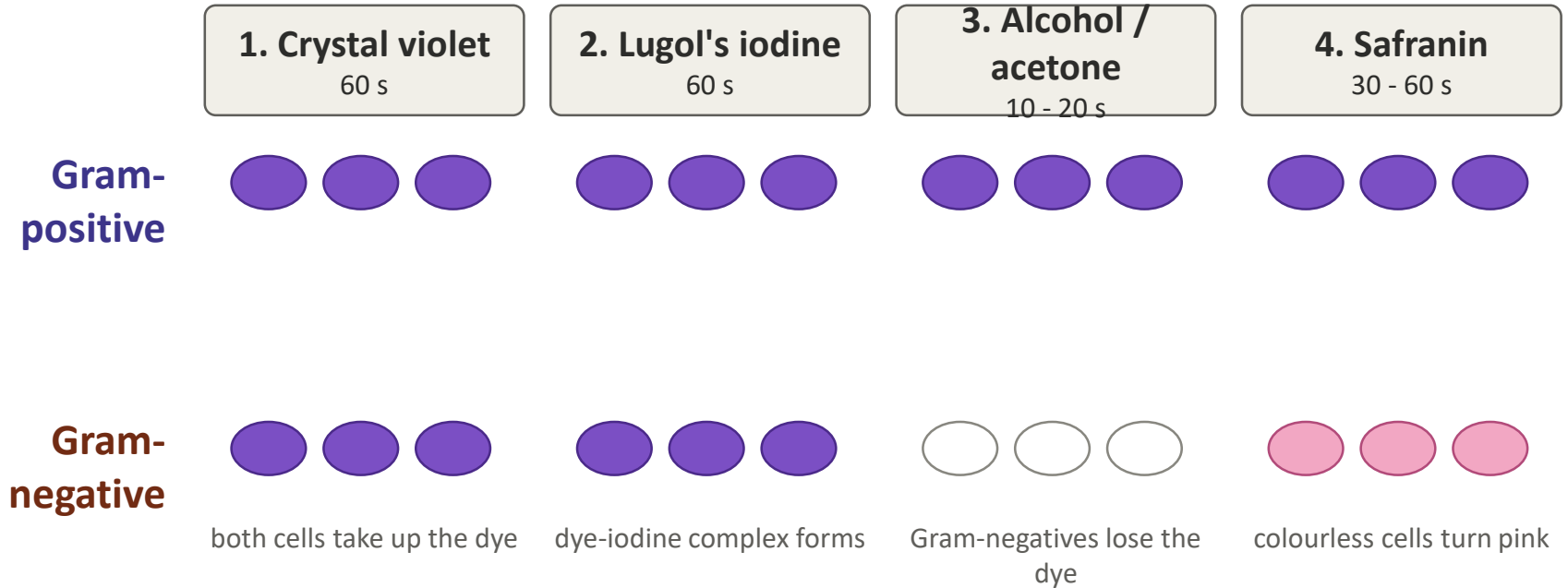
Cytoplasmic membrane

Consequences

Loses the dye, counterstained red-pink by safranin
Outer membrane resists bile salts and many antibiotics
More sensitive to CO₂ and to drying
Examples: *Salmonella*, *E. coli*, *Pseudomonas*

The difference in the wall explains the staining reaction and much of the behaviour of these bacteria in foods.

Gram Staining Step by Step

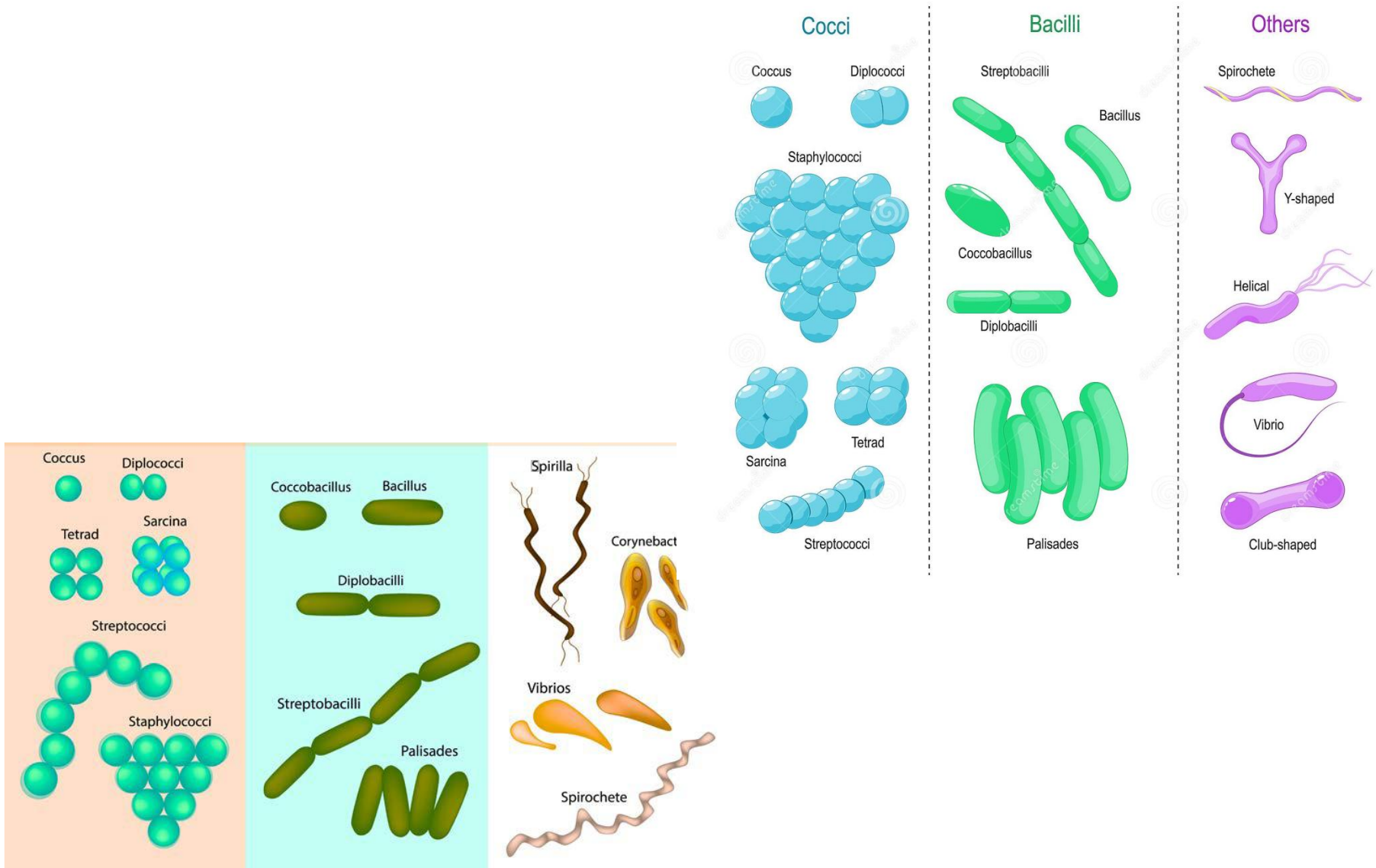


Why the results differ

Gram-positive: thick peptidoglycan traps the dye-iodine complex; alcohol dehydrates and tightens the wall, so the cells stay violet.

Gram-negative: alcohol dissolves the outer membrane and the thin peptidoglycan cannot hold the complex, so the cells are decolourised and take up the pink counterstain.

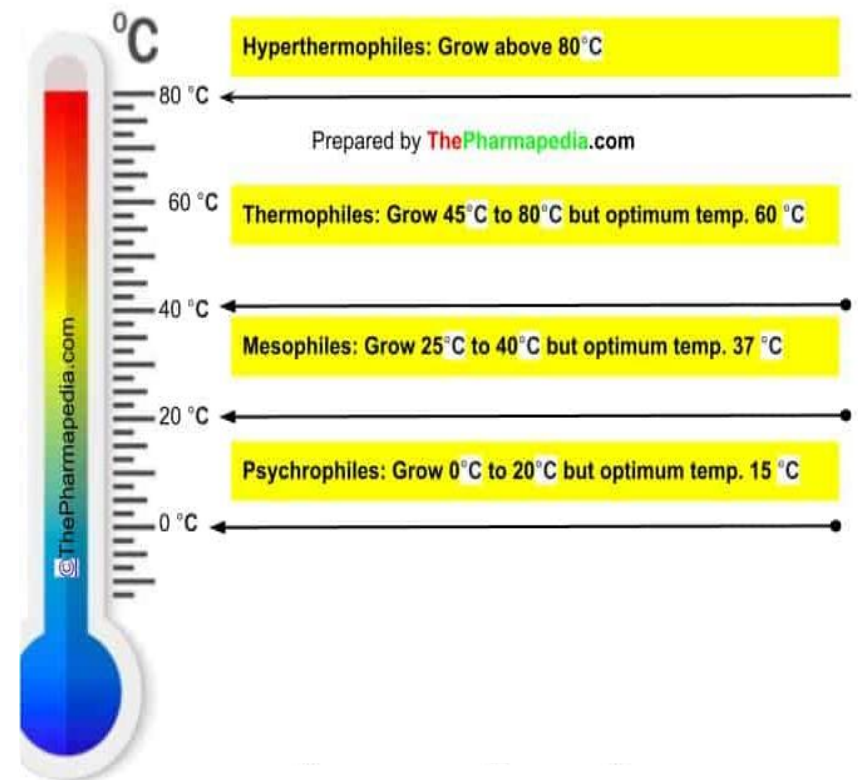
Cellular morphology



Classification of bacteria based on temperature

Bacteria are examined in three groups according to their temperature requirements:

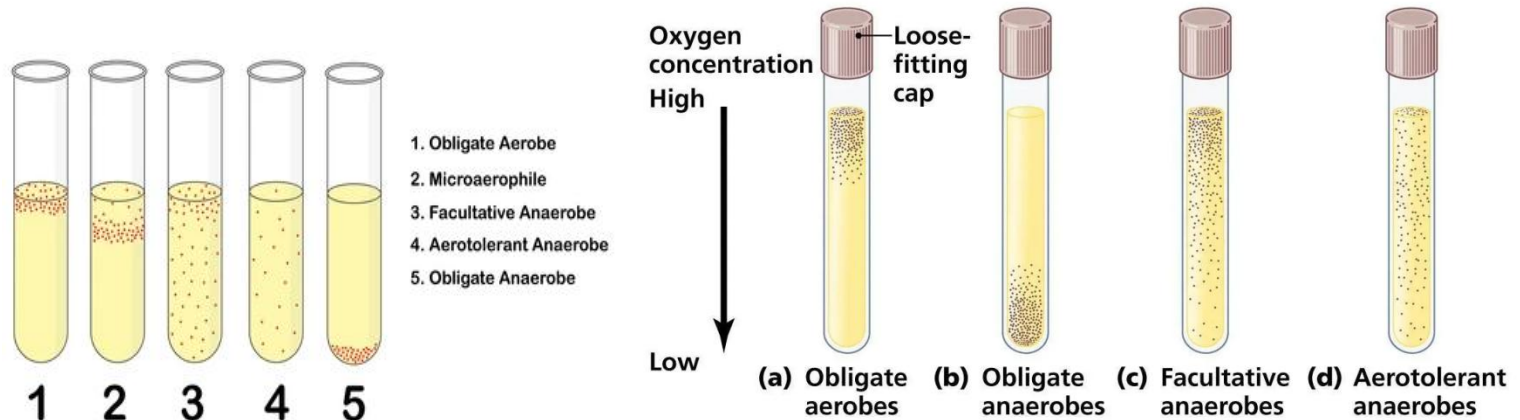
- **Psychrophilic bacteria:** the cold-loving bacteria. They grow at a minimum of about 0 °C, with an optimum near 15 °C and a maximum of about 20 °C; for example *Psychrobacter* and some *Shewanella* species. *Listeria monocytogenes* and *Pseudomonas fluorescens*, which grow in the cold but have a higher optimum, are psychrotrophs.
- **Mesophilic bacteria:** optimum growth temperature between 25 °C and 45 °C. This group includes the saprophytes that degrade plant and animal residues and most of the bacteria important in the food industry; for example *Escherichia coli* and *Staphylococcus aureus*.
- **Thermophilic bacteria:** they grow at a minimum of 35–45 °C, with an optimum near 55 °C and a maximum of 60–90 °C; for example *Geobacillus stearothermophilus*.



Classification of bacteria based on oxygen demand

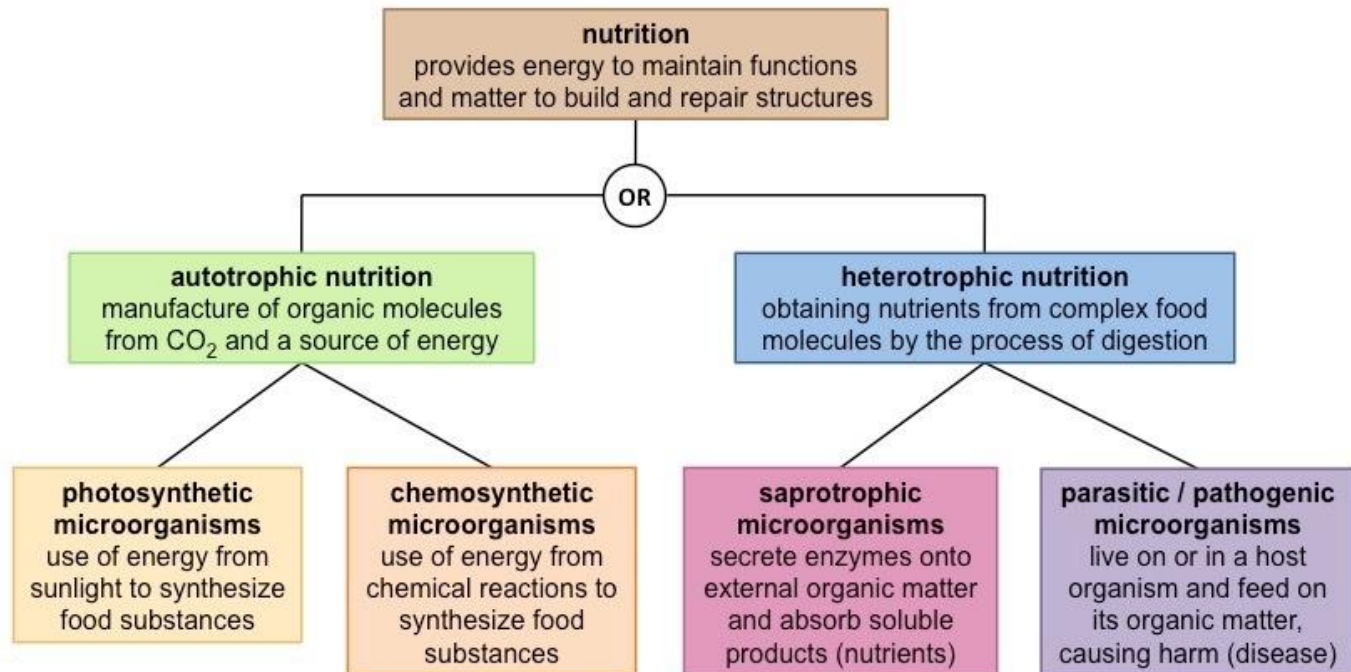
According to their oxygen requirement, bacteria are classified into five groups:

- **Obligate aerobes:** bacteria that require oxygen in order to grow and survive; for example *Mycobacterium tuberculosis*, *Bacillus subtilis* and *Pseudomonas* species.
- **Obligate anaerobes:** bacteria that grow only in the absence of oxygen and generate ATP by anaerobic respiration and/or fermentation. Oxygen is toxic to them; for example *Clostridium* species.
- **Facultative anaerobes:** microorganisms that grow both in the presence and in the absence of oxygen. They use aerobic respiration whenever oxygen is available and switch to anaerobic respiration or fermentation when it is not; for example *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*.
- **Aerotolerant anaerobes:** bacteria for which oxygen is not toxic but which do not use it; they obtain ATP only by fermentation. Most lactic acid bacteria belong to this group.
- **Microaerophiles:** bacteria that grow only at reduced oxygen concentrations (about 3–10%); for example *Campylobacter jejuni*.



Classification of bacteria based on nutrition

- According to their source of carbon and energy, bacteria are divided into two main groups:
 - **autotrophic bacteria**, which synthesise their own organic compounds from CO_2 ;
 - **heterotrophic bacteria**, which take ready-made organic nutrients from the environment.

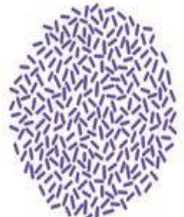
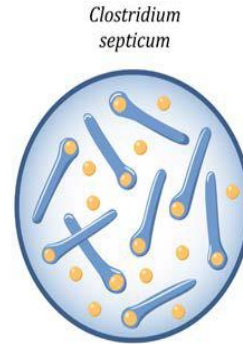
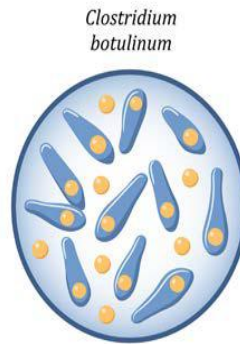
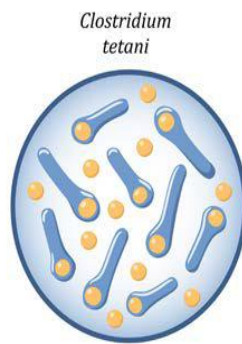


Importance of Bacteria in Food Industry

Bacteria are of great importance in the food industry: they are used in the production of many foods (fermentation, etc.), they cause spoilage in others and they affect quality characteristics such as appearance, taste and texture. For this reason the bacteria of importance in the food industry must be known.

- For example: *Escherichia coli*, *Clostridium*, *Lactobacillus* and the related genera, *Staphylococcus*, *Salmonella*, *Lactococcus* and *Streptococcus thermophilus*.

Starter culture: the microorganisms added to foods such as yoghurt, cheese, kefir, pickles and fermented sausage in order to produce acid and to develop aroma and texture.



Probiotics

Fungi (Yeasts and Molds)

- Yeasts and moulds are less sensitive to osmotic pressure than bacteria.
- Some moulds and yeasts grow in the presence of as much as 60% sucrose,
 - whereas most bacteria are inhibited at much lower concentrations.
- Microorganisms able to grow at high sugar concentrations are called osmophiles.
- Osmoduric microorganisms cannot grow at high sugar concentrations
 - but are able to survive them.

YEAST

Yeasts are unicellular, eukaryotic, non-filamentous fungi that cannot photosynthesise. They grow well aerobically and are widespread in nature. Yeasts differ from bacteria in that:

- yeast cell shapes (ellipsoidal, ovoid, cylindrical) differ from those of bacteria;
- yeast cells are considerably larger than bacterial cells;
- yeasts mostly reproduce by budding;
- the nucleus and the vacuoles can be seen under the light microscope.



Cell Structure of Yeast

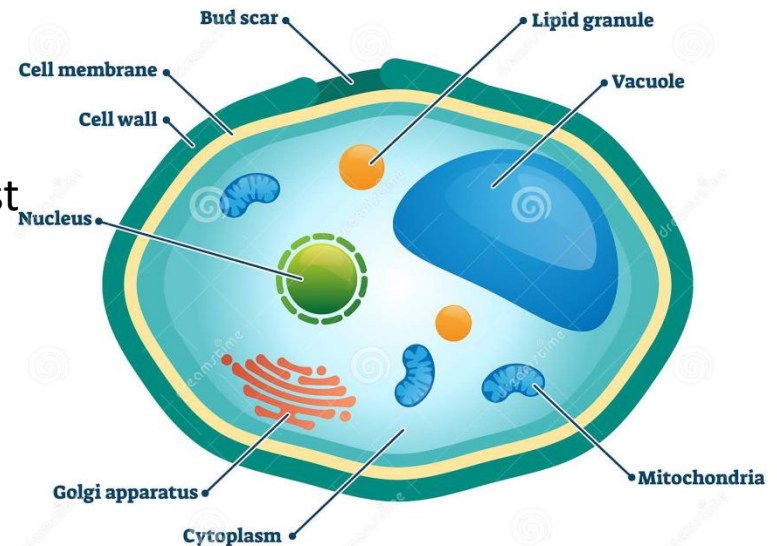
- Yeast cells have a eukaryotic structure, are unicellular and are larger than bacterial cells. During budding the daughter cell sometimes fails to separate from the mother cell, and the cells remain attached to form pseudohyphae with a multicellular appearance.
- Cell size varies with the species and with environmental conditions. Cells may be spherical, ovoid or cylindrical.

Nucleus: a true nucleus surrounded by a nuclear membrane and containing a nucleolus and chromosomes.

Cytoplasm and organelles: the cytoplasm of a yeast cell contains endoplasmic reticulum, vacuoles, granules, mitochondria, a Golgi apparatus and numerous ribosomes.

Cell membrane: lies beneath the cell wall, is rich in sterols and enzymes and is selectively permeable.

Cell wall: composed mainly of the polysaccharides glucan and mannan together with chitin; it gives the yeast cell its shape.



Properties of Yeast

- In the food industry, yeasts perform important functions such as leavening dough and synthesising certain vitamins and enzymes. However, they also cause spoilage of foods and thus product and cost losses.
 - They are unicellular.
 - Their cells contain membrane-bound organelles.
 - A few yeast species are opportunistic pathogens.
 - They are used as starter cultures in food production.
 - Their pH range for growth is wide, and some can survive at pH values as low as 1.5.
 - Some strains grow at alcohol concentrations as high as 18%, while osmophilic strains grow at sugar concentrations of 55–60%.
 - Yeasts generally grow between 0 and 40 °C; for the pathogenic species this range is 25–37 °C.
 - They mostly use carbohydrates as an energy source.
 - On solid media they form cream-coloured, smooth colonies.

Reproduction of Yeasts

- On the basis of their mode of reproduction, yeasts are divided into two groups:
 - “true” yeasts (Ascomycota), which reproduce sexually by forming ascospores;
for example *Saccharomyces*;
 - “false” yeasts (anamorphic yeasts, formerly called Fungi imperfecti), which do not form sexual spores;
for example *Candida*, *Rhodotorula* and *Cryptococcus*.

Asexual reproduction of yeasts occurs by:

- fission (for example *Schizosaccharomyces*);
- budding (the most common form);
- the formation of asexual spores.

Importance of Yeast in Food Industry

Yeasts have both beneficial and harmful effects in the food industry: they are used in the production of some foods and additives, and they also cause spoilage. The yeast genera of importance in the food industry are:

- ***Candida***: the genus contains about 200 species. They grow in media with high acid and salt contents and form white colonies on foods. Some species occur in fresh minced meat and poultry meat; *C. lipolytica* has been transferred to the genus *Yarrowia*.
- ***Kluyveromyces***: used in the production of fermented and low-lactose dairy products. It ferments lactose and produces lactase (β -galactosidase) from whey, but it can also spoil cheese.
- ***Saccharomyces***: reproduces by multilateral budding and cannot ferment lactose. The best-known species is *Saccharomyces cerevisiae*, baker's yeast, which is important in the leavening of bread dough, in the production of invertase, in the beer, wine and sparkling-wine industries and in the production of ethanol and glycerol.
- ***Pichia***: some species cause spoilage in olive brine, fresh fish and acid foods such as pickles. *Pichia kluyveri* produces killer toxins. *Komagataella phaffii* (formerly *Pichia pastoris*) is widely used for enzyme and recombinant protein production, and *Wickerhamomyces anomalus* (formerly *Pichia anomala*) is used as a biocontrol agent to protect stored grain from spoilage.

Molds

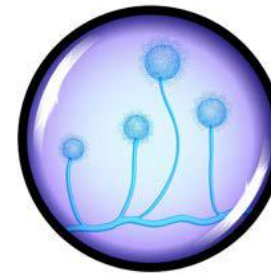
- Moulds are multicellular, filamentous, eukaryotic microorganisms belonging to the kingdom Fungi.



Aspergillus



Penicillium

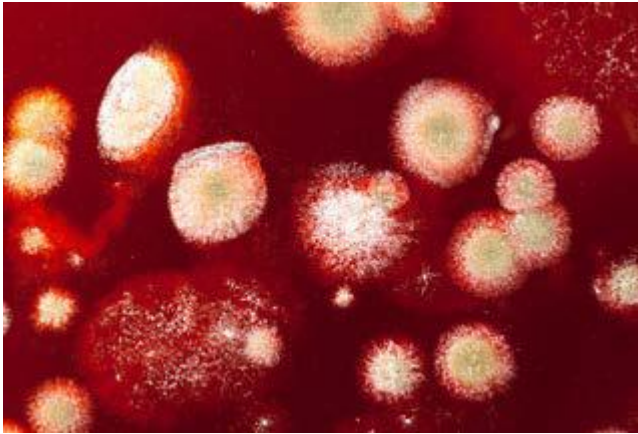


Mucor

Properties of Molds

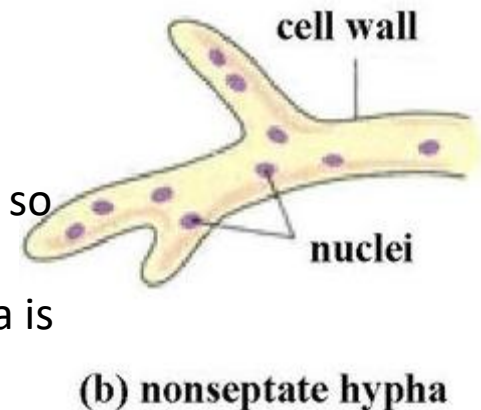
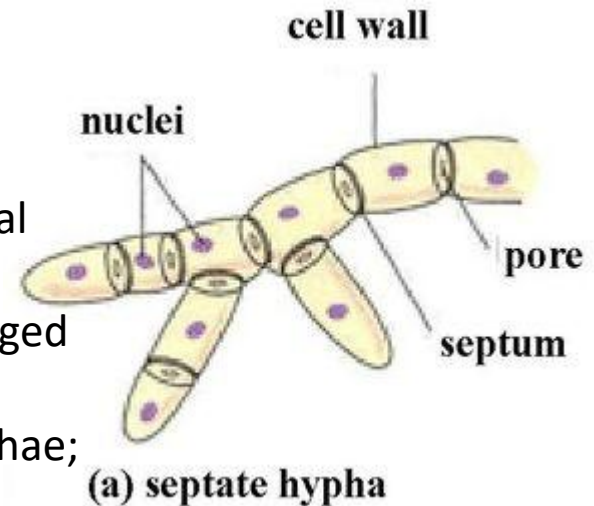
- Moulds are very common in nature and can be found almost everywhere. They are easily recognised on foods by their cottony appearance. Their colonies are usually white at first but may later become black, green, yellow, orange or other colours.

Mold colonies



Cell Structure of Molds

- Moulds are filamentous fungi with a eukaryotic cell structure. Each filament is called a hypha.
- The mass formed by the hyphae is called the mycelium.
- Hyphae are classified in several ways:
 - hyphae that grow above the medium are called aerial hyphae;
 - those that penetrate the medium are called submerged hyphae;
 - those involved in nutrition are called vegetative hyphae; and
 - those that bear the reproductive structures are called fertile hyphae.
- In most moulds the fertile hyphae are aerial. The aerial mycelium forms the visible part of the colony.
- In some moulds there are no cross-walls between the cells, so that the hypha forms a continuous tube (non-septate, coenocytic); in others cross-walls are present and the hypha is divided into compartments (septate).



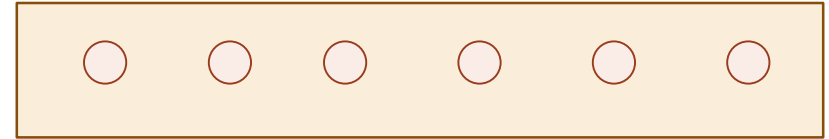
Structure of a Mould

Septate hypha



Cross-walls divide the hypha into cells; each compartment has one or more nuclei.

Non-septate (coenocytic) hypha



No cross-walls; nuclei lie in a continuous tube. Typical of *Rhizopus* and *Mucor*.

How the parts are named

Hypha	a single filament
Mycelium	the mass formed by the hyphae
Vegetative hypha	grows into the substrate and takes up nutrients
Aerial hypha	grows above the substrate
Fertile hypha	bears the reproductive spores; forms the visible colony
Conidia and sporangiospores	asexual spores that spread the mould

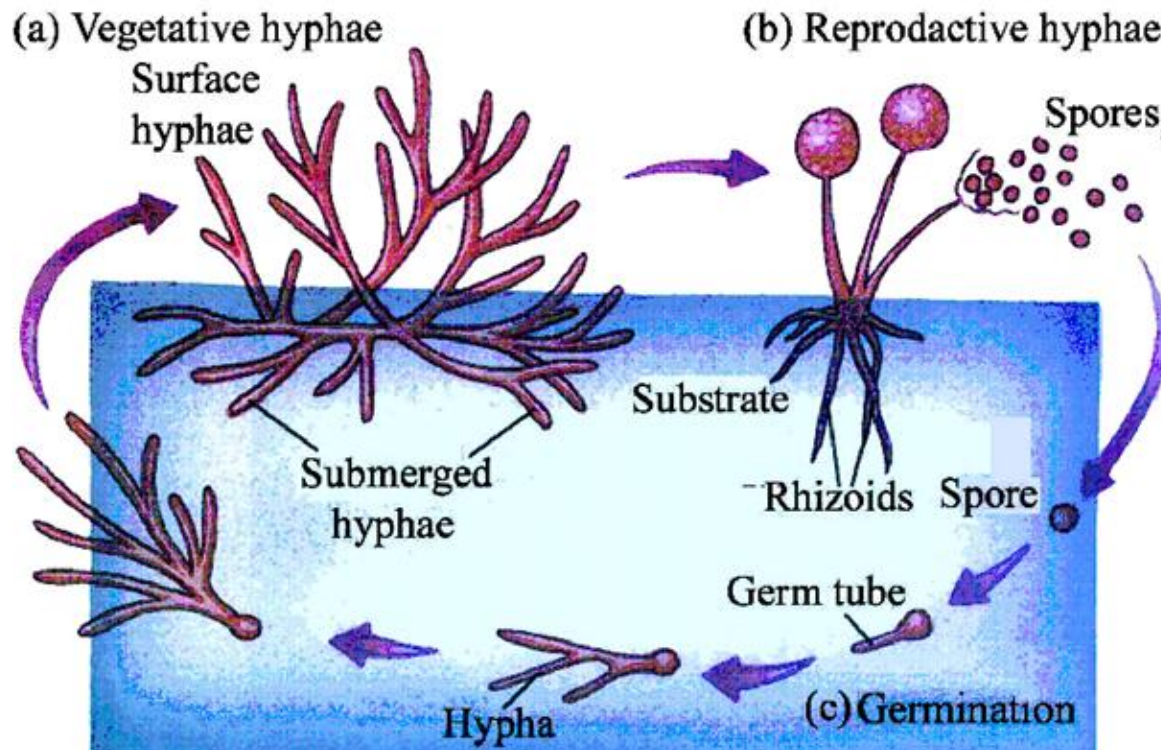
Moulds are aerobic, tolerate low a_w and low pH, and therefore spoil foods in which bacteria cannot grow.

Properties of molds

- Moulds are aerobic microorganisms. Although they can grow over a wide temperature range (0–60 °C), most are mesophilic, with an optimum between 22 and 32 °C. Their optimum pH is usually between 5 and 6, but some species grow in strongly acidic media down to about pH 2.5. They possess a rich extracellular enzyme system and take up nutrients by diffusion.
- Moulds are industrially important because of the positive and negative changes they cause in foods.
- Their benefits can be summarised as follows:
 - cultivated mushrooms (macrofungi) are consumed as food;
 - they increase soil fertility by degrading organic matter;
 - they develop the flavour, aroma and texture of cheeses such as Roquefort and Camembert;
 - they are used in the production of antibiotics (penicillin);
 - they are used to produce certain vitamins, enzymes, pigments, organic acids, ethanol and glycerol.

Reproduction of molds

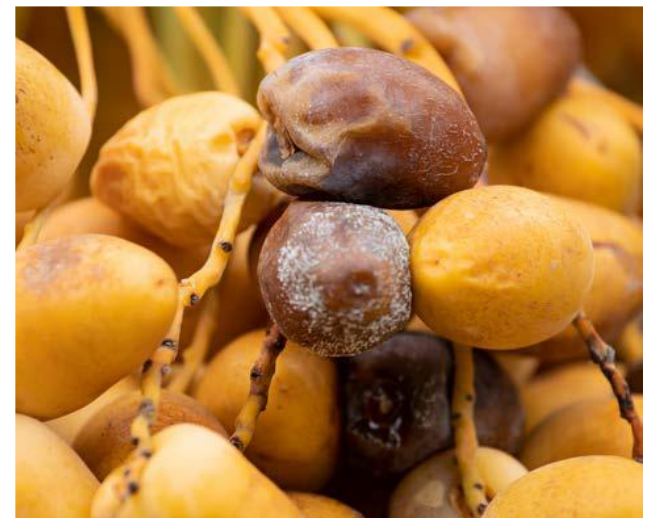
- Moulds that reproduce both asexually and sexually
 - are called perfect moulds and are classified as
 - » Zygomycetes (Mucoromycota), Ascomycetes and Basidiomycetes.
 - The Oomycetes, once included here, are no longer regarded as true fungi.
- Moulds that reproduce only by asexual spores
 - are called imperfect moulds (anamorphic fungi); their asexual spores are
 - » conidia, arthrospores (oidia), sporangiospores and chlamydospores.



Harms of Molds

Some mould species are pathogenic to humans and animals.

- Some species produce toxic secondary metabolites called mycotoxins.
- They cause nutrient losses and produce undesirable colour, odour and bitterness in foods.
- In humid environments they damage organic materials such as wood, textiles and leather.
- Thus, besides their many benefits, moulds also have harmful effects.



Importance of molds in Food Industry

Moulds are industrially important because of the positive and negative changes they cause in foods. The mould genera of importance in the food industry are:

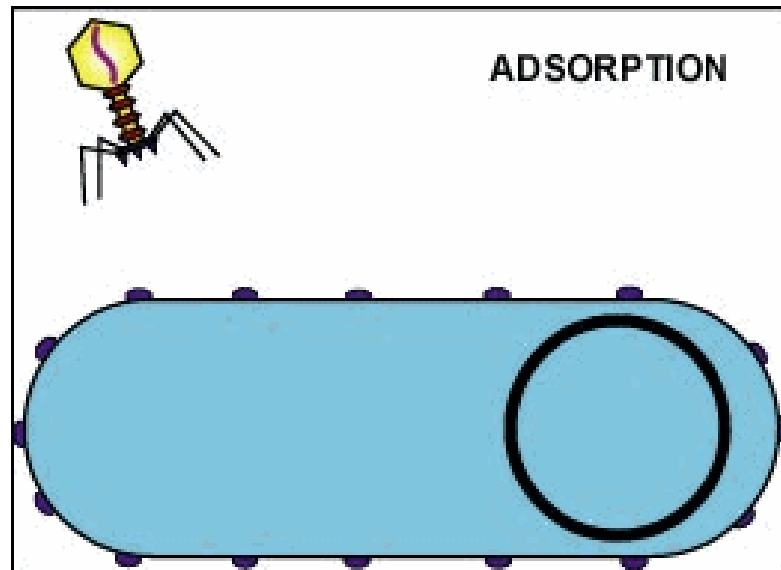
- ***Alternaria***: the genus contains nearly fifty species that are saprophytes or plant pathogens. They cause rots in plants before and after harvest, and significant losses are observed in produce such as apples, potatoes, tomatoes, courgettes and carrots. Some species produce mycotoxins (alternariol, tenuazonic acid).
- ***Aspergillus***: a genus with a very wide distribution in nature. Its species range in colour from yellow and green to black and have septate hyphae. They cause spoilage of fruits and vegetables, meat, cereal products and many other foods, and are responsible for black rot of plums, figs and citrus fruits. *A. flavus* and *A. parasiticus* produce aflatoxins.
- ***Fusarium***: forms cotton-like mycelium, usually pale pink, white or yellow. The genus contains many soil saprophytes and plant pathogens; it causes wilt, blight and rot in cereals, sugar beet, chickpea and maize (seedlings, roots, crown, stem and cob) and Fusarium rots of figs, citrus fruits, bananas, pineapples, pomegranates and potatoes. *F. verticillioides* (formerly *F. moniliforme*) and *F. graminearum* produce fumonisins, deoxynivalenol and zearalenone.
- ***Penicillium***: forms colonies ranging from blue to blue-green on foods such as citrus fruits; some species are used in cheese production and others produce mycotoxins.

Virus

- Viruses are obligate intracellular parasites that pass through filters which retain the smallest bacteria and that cannot be seen with a simple light microscope. They replicate only inside living cells and have no complete cellular organisation. Each virus infects only particular animal, plant or bacterial cells.
 - For example, the hepatitis A virus infects the human liver.
 - The rabies virus infects the nerve cells of both humans and animals.
- Viruses cannot grow in foods, but water and foods act as vehicles for their transmission.
- Viruses that cause infection in humans when ingested through the digestive tract are called enteric viruses.
 - Enteric viruses multiply in the intestine and are excreted into the environment with the faeces. Today the most important foodborne viruses are norovirus, which is the leading cause of foodborne outbreaks worldwide, and hepatitis A virus (the agent of jaundice, sarılık); hepatitis E virus, rotavirus and poliovirus (the agent of polio, çocuk felci) are also transmitted by food and water.

Reproduction of viruses

- A bacteriophage
 - attaches itself to the surface of a host bacterial cell and
 - injects its nucleic acid into the host cell;
 - many new phage particles are then formed inside the host and
 - are released when the cell lyses.
- Viruses cause diseases of humans, animals and plants; bacteriophages are a serious problem in dairy and other fermentations because they lyse the starter culture.



Parasites

- The parasites of importance in foods are roundworms, flatworms, tapeworms and protozoa.
- They cause human illness and are associated with foods.
- Trichinellosis is caused by *Trichinella spiralis* present in pork and game meat.
- Anisakiasis is caused by *Anisakis simplex* present in many marine fish.
- Taeniasis is caused by *Taenia saginata* from beef and *Taenia solium* from pork.
- Toxoplasmosis is caused by *Toxoplasma gondii* present in undercooked meat and unpasteurised milk.
- Giardiasis is caused by *Giardia duodenalis* (*G. lamblia*) present in water and raw vegetables.
- Ascariasis is caused by *Ascaris lumbricoides* in raw vegetables; cryptosporidiosis and cyclosporiasis are also important protozoan infections.

Important Bacterial Groups

1) Lactic acid bacteria (LAB)

- ferment carbohydrates and produce relatively large amounts of lactic acid.
- Major genera: *Lactococcus*, *Lactobacillus* and the genera split from it in 2020, *Leuconostoc*, *Pediococcus*, *Enterococcus*.

Table 2.2. Principal genera of the lactic acid bacteria			
Genus	Cell morphology	Fermentation	Lactate isomer
<i>Lactococcus</i>	cocci in chains	homo	L
<i>Pediococcus</i>	cocci	homo	DL
<i>Streptococcus</i>	cocci in chains	homo	L
<i>Lactobacillus</i>	rods	homo/hetero	DL, D, L
<i>Leuconostoc</i>	cocci	hetero	D

The 2020 Reclassification of Lactobacillus

Genus *Lactobacillus* before 2020
more than 260 species in one genus



Zheng et al. (2020) split it into 25 genera. The species names you meet in food microbiology became:

<i>Lactobacillus delbrueckii</i>	→	unchanged - yoghurt starter
<i>Lactobacillus acidophilus</i>	→	unchanged - probiotic
<i>Lactobacillus helveticus</i>	→	unchanged - cheese
<i>L. plantarum</i>	→	<i>Lactiplantibacillus plantarum</i>
<i>L. casei</i> , <i>L. rhamnosus</i>	→	<i>Lacticaseibacillus</i>
<i>L. brevis</i>	→	<i>Levilactobacillus brevis</i>
<i>L. sakei</i> , <i>L. curvatus</i>	→	<i>Latilactobacillus</i>
<i>L. fermentum</i> , <i>L. reuteri</i>	→	<i>Limosilactobacillus</i>
<i>L. viridescens</i>	→	<i>Weissella viridescens</i>

Older textbooks and many product labels still use the pre-2020 names, so both should be recognised.

i) Characteristics of LAB

- LAB are Gram-positive, non-motile, non-spore-forming rods or cocci. They are catalase- and oxidase-negative and produce lactic acid as the major end product of carbohydrate metabolism without requiring oxygen.
- LAB are microaerophilic; most are aerotolerant anaerobes.
 - They tolerate small amounts of oxygen.
 - Some LAB use oxygen to produce hydrogen peroxide.
 - They require amino acids, B vitamins and nucleic acid bases and are nutritionally fastidious.
 - Some grow at temperatures as high as 45 °C.
 - They grow over a wide pH range but mostly grow best at pH 4.0–6.5.
- The generation time of LAB ranges from 30 to 90 minutes.
- The lactic acid they produce inhibits spoilage bacteria; many LAB also produce bacteriocins such as nisin.
- They are divided into groups according to their glucose metabolism, growth temperature and other characteristics.

i) Groups of LAB depending on glucose metabolism

- LAB use one of two pathways, which differ in the end products of glucose metabolism.
- This gives two groups: homofermentative and heterofermentative LAB.
- The pathway of lactic acid production differs between the two groups.

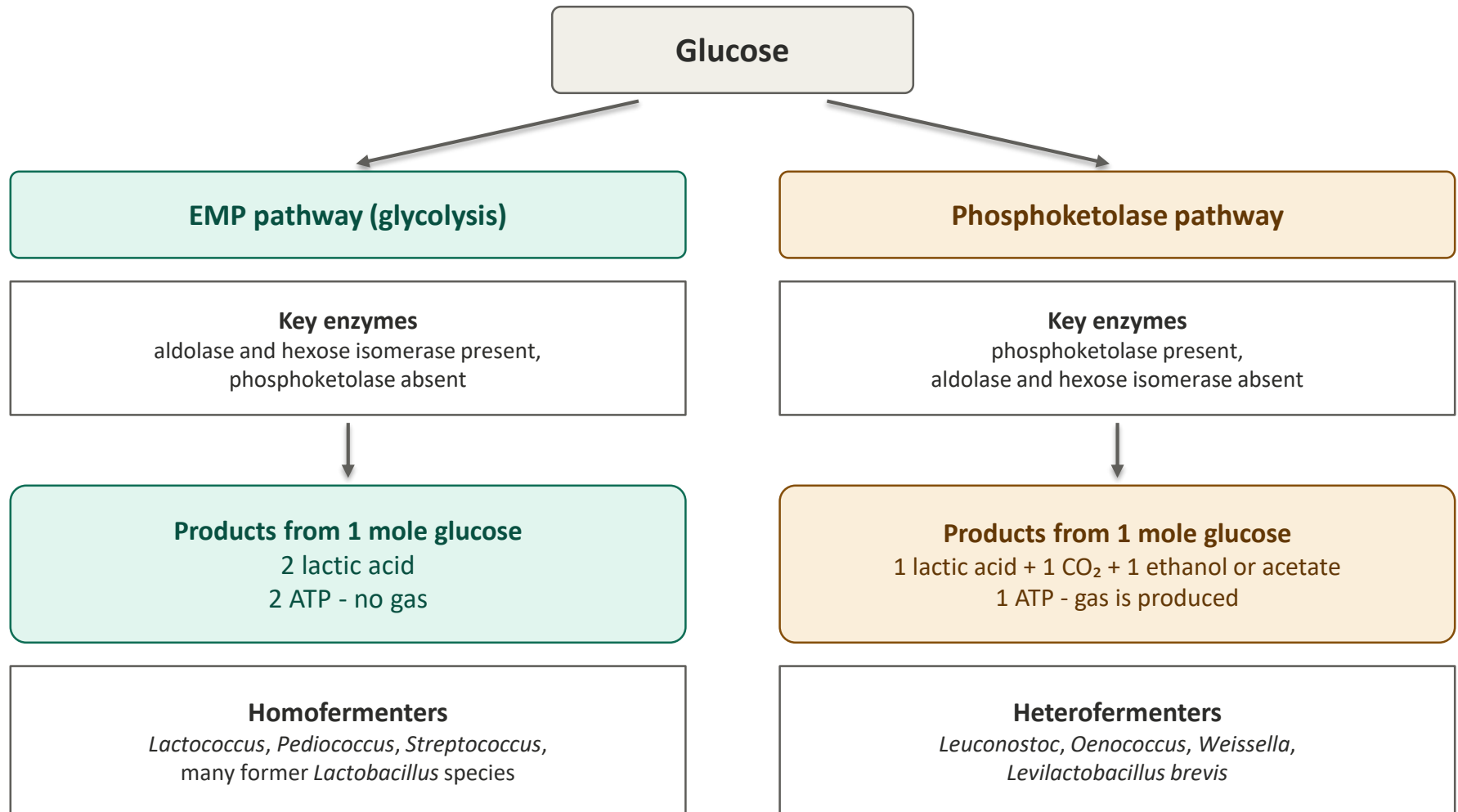
Homolactic fermentation

- Homofermentative LAB produce lactic acid as the major product (about 90%) of glucose fermentation.
 - They use the Embden–Meyerhof–Parnas (EMP) pathway, that is, glycolysis, to ferment sugars.
 - They possess the key enzymes aldolase and hexose isomerase
 - but lack phosphoketolase.
 - In the EMP pathway the six-carbon glucose molecule is split into two molecules of glyceraldehyde-3-phosphate.
 - These are then converted to pyruvate, with the production of two molecules of ATP by substrate-level phosphorylation.
 - The fermentation of 1 mole of glucose yields 2 moles of lactic acid.
- All members of the genera *Lactococcus*, *Pediococcus* and *Streptococcus*, and many species of the *Lactobacillus* group, are homofermenters,
 - whereas all members of *Leuconostoc*, *Oenococcus* and *Weissella* and some species of the *Lactobacillus* group are heterofermenters.

Heterolactic fermentation

- Heterofermentative LAB produce approximately equal amounts of lactate (about 50%) and of ethanol, acetic acid and CO₂ from glucose.
- They possess phosphoketolase
 - but lack aldolase and hexose isomerase.
- They use the phosphoketolase (6-phosphogluconate/pentose phosphate) pathway to metabolise hexoses and pentoses.
- Heterofermenters and homofermenters are easily distinguished in the laboratory
 - by the ability of the heterofermenters to produce CO₂ from glucose.
- The fermentation of 1 mole of glucose yields 1 mole each of lactic acid, CO₂ and ethanol or acetate through the phosphoketolase pathway.
- Heterofermenters are more important than homofermenters in the production of flavour and aroma compounds such as acetaldehyde and diacetyl.

Homolactic and Heterolactic Fermentation



Gas production from glucose is the simplest laboratory test that separates the two groups; heterofermenters also give the aroma compounds.

- The glycolytic (EMP) pathway is used by all LAB except *Leuconostoc*, the obligately heterofermentative lactobacilli (group III), *Oenococcus* and *Weissella*.
 - The glycolytic pathway operates with excess sugar and limited oxygen.
- Homofermenters obtain twice as much energy from glucose as heterofermenters.

ii) Groups of LAB depending on growth temperature

Mesophilic LAB

- Mesophilic LAB are used in the production of fermented milk products (fresh cheese, semi-hard cheese, butter) and of fermented sausage (sucuk).
- The optimum growth temperature of mesophilic LAB is between 20 and 30 °C.
- *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* are mesophilic LAB.
- *Lc. lactis* subsp. *cremoris* is more sensitive to salt (< 4%) and to temperature (does not grow at 40 °C) than *Lc. lactis* subsp. *lactis*.
- Mesophilic aroma cultures are *Lc. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*.
 - Their growth temperature range is 5–38 °C;
 - they produce diacetyl flavour at 18–25 °C and
 - they produce CO₂ from citrate.

Thermophilic LAB

- Their optimum growth temperature is 40–45 °C, with an overall range of 20–52 °C.
- They are used mainly in the production of yoghurt and of hard and soft cheeses.
- They include *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and subsp. *lactis*, *Lb. helveticus*, *Lb. acidophilus* and *Lacticaseibacillus casei* (formerly *Lactobacillus casei*).
- They may be single-strain or multiple-strain cultures.

Miscellaneous LAB and related bacteria

- *Bifidobacterium longum* (incl. subsp. *infantis*) and *Propionibacterium freudenreichii* subsp. *shermanii* grow optimally at 37–41 °C.
- *Bifidobacterium* species are widely used as probiotic cultures.
- Propionibacteria are used in the production of Swiss-type (eyed) cheeses.

2) Acetic acid bacteria

- The acetic acid-forming bacteria are *Acetobacter*, *Gluconobacter* and *Komagataeibacter*.
- The characteristics of the acetic acid bacteria are:
 1. they oxidise ethanol to acetic acid;
 - they are useful in vinegar production and
 - harmful in alcoholic beverages;
 2. *Acetobacter* further oxidises acetic acid to CO₂ and water (over-oxidation), whereas *Gluconobacter* does not;
 3. they oxidise D-sorbitol to L-sorbose in the manufacture of ascorbic acid;
 4. some strains cause excessive sliminess, for example *Gluconobacter oxydans* (formerly *Acetobacter suboxydans*).

3) Butyric acid bacteria

- These bacteria produce large amounts of butyric acid from carbohydrates under anaerobic conditions.
- For example *Clostridium butyricum* and *C. tyrobutyricum*, which causes late blowing in hard cheeses.

4) Propionic acid-forming bacteria

- Most of the bacteria in this group belong to the genus *Propionibacterium*:
 - *P. freudenreichii* (including subsp. *shermanii*) and *P. jensenii*.
- They
 - are pleomorphic, non-motile Gram-positive rods,
 - produce propionic acid, acetic acid and CO₂ (which forms the eyes in cheese), and
 - are used in dairy fermentations, particularly for Swiss-type cheeses such as Emmental and Gruyère.

5) Proteolytic bacteria

- They hydrolyse proteins by producing extracellular and intracellular proteinases,
 - yielding amino acids and small peptides.
- Proteolytic species are found in the genera *Kocuria*/*Micrococcus*, *Bacillus*, *Clostridium* (*C. sporogenes*, *C. putrefaciens*), *Pseudomonas* (*P. fluorescens*), *Shewanella*, *Flavobacterium*, *Alcaligenes*, *Brevibacterium*, *Serratia* and in the Enterobacteriaceae.
- Some bacteria are termed “acid-proteolytic”:
 - they carry out acid fermentation and proteolysis at the same time.
 - *Enterococcus faecalis* var. *liquefaciens* (formerly *Streptococcus faecalis*) and *Macrococcus caseolyticus* (formerly *Micrococcus caseolyticus*) are acid-proteolytic bacteria.
- Putrefactive bacteria degrade proteins and produce foul-smelling compounds such as hydrogen sulphide, mercaptans, amines and indole.

6) Lipolytic bacteria

- These bacteria produce extracellular lipases that
- hydrolyse fats to free fatty acids and glycerol.
- *Pseudomonas* species (*P. fluorescens*, *P. aeruginosa*) and *Alcaligenes* species are strongly lipolytic.
- *Serratia*, *Staphylococcus*, *Flavobacterium* and *Kocuria/Micrococcus* also contain lipolytic species.
- Many microbial lipases are heat-resistant and survive food processing, including the UHT treatment of milk, so that they cause rancidity during storage.

7) Saccharolytic bacteria

- These bacteria hydrolyse disaccharides and polysaccharides to simple sugars.
- They produce extracellular amylases that hydrolyse starch.
- Examples are *Bacillus subtilis*, *Clostridium butyricum*, *Aeromonas*, *Pseudomonas* and *Enterobacter*.

8) Pectolytic bacteria

- Pectins are complex carbohydrates present in fruits and vegetables.
- Pectolytic bacteria produce a mixture of pectolytic enzymes known as pectinases.
 - They hydrolyse pectin and cause softening of plant tissues (soft rot).
- This group includes species of *Bacillus*, *Clostridium*, *Pectobacterium* and *Dickeya* (both formerly *Erwinia*), *Achromobacter*, *Aeromonas*, *Arthrobacter*, *Flavobacterium* and *Xanthomonas*.

9) Thermophilic bacteria (or thermophiles)

- These bacteria grow at 55 °C and above.
- They are important in foods held at high temperatures,
 - where they cause spoilage.
- *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) causes flat-sour spoilage in low-acid canned foods.
- *Thermoanaerobacterium thermosaccharolyticum* causes gaseous spoilage, and *Desulfotomaculum nigrificans* sulphide stinker spoilage, in canned foods.

10) Thermotolerant bacteria

- These bacteria survive heat treatments such as pasteurisation and
 - then cause spoilage.
- Their optimum growth temperature is about 45 °C.
- This group includes *Bacillus* and *Clostridium* spores, *Kocuria*, *Lactobacillus*, *Microbacterium* and *Enterococcus*.

11) Psychrophilic bacteria (or psychrophile)

- These bacteria grow optimally at about 12–15 °C
- and have a growth temperature range of about –5 to 22 °C.
- They are able to grow in refrigerated and chilled foods.

12) Psychrotrophic bacteria (or psychrotrophs)

- These bacteria grow at refrigeration temperature (≤ 7 °C)
- and are therefore important in refrigerated foods.
- Their optimum growth temperature is between 25 and 30 °C;
 - most of them do not grow above 35 °C.
- This group is represented by the genera *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Shewanella*, *Psychrobacter*, *Brochothrix thermosphacta* and *Arthrobacter*,
- and less frequently by *Listeria*, *Yersinia*, *Aeromonas*, *Escherichia*, *Serratia marcescens*, *Proteus*, *Vibrio*, *Bacillus*, *Leuconostoc*, *Clostridium*, *Citrobacter*, *Kocuria*, *Lactobacillus* and *Enterobacter*.

13) Osmophilic bacteria

- These bacteria grow at higher osmotic pressures than other bacteria.
- They grow at high sugar concentrations,
 - for example species of *Leuconostoc*.
- Some species of *Staphylococcus*, *Leuconostoc* and *Lactobacillus* also belong to this group, although true osmophiles are mainly yeasts.

14) Halophilic bacteria (halophiles)

- These bacteria are important in heavily salted foods and in brines.
- They include species of *Halobacterium*, *Halococcus*, *Kocuria/Micrococcus*, *Staphylococcus*, *Pediococcus*, *Vibrio*, *Pseudomonas*, *Bacillus*, *Alcaligenes* and *Corynebacterium*.
- They require a certain concentration of dissolved NaCl for growth.
- Bacteria that grow both with and without salt are called salt-tolerant bacteria (for example *Kocuria*).
- Bacteria that survive but do not grow at high salt concentrations are called halotolerant.
 - Halophiles usually grow in foods containing 5% NaCl or more.
 - Halotolerant species occur in the genera *Bacillus*, *Kocuria*, *Staphylococcus*, *Corynebacterium*, *Streptococcus* and *Clostridium*.

- **Groups of halophilic** bacteria depending on salt requirements for growth:
 - Bacteria that grow best at 0.5–3% NaCl are called slightly halophilic;
 - they occur in the genera *Pseudomonas*, *Moraxella*, *Flavobacterium*, *Acinetobacter* and *Vibrio*.
 - They are found on fish and shellfish.
 - Bacteria growing at 3–15% NaCl are called moderate halophiles;
 - they occur in the genera *Bacillus*, *Kocuria*, *Vibrio*, *Acinetobacter*, *Moraxella* and *Staphylococcus*.
 - Bacteria growing at 15–30% NaCl are called extreme halophiles;
 - these are the archaea *Halobacterium salinarum* (formerly *H. halobium*) and *Halococcus* species.
 - They survive in heavily brined and salted foods, which they discolour pink or red.

15) Pigmented bacteria

- These bacteria produce pigments ranging from black to white on or in foods.
- All species of *Flavobacterium* (yellow to orange) and *Serratia* (red), and many species of *Kocuria/Micrococcus*, are pigmented bacteria.
- *Halobacterium* and *Halococcus* produce red to orange carotenoid pigments.
- *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) causes greening and discoloration of cheese and cured meats.

16) Slime or rope-forming bacteria

- These bacteria produce slime by synthesising exopolysaccharides.
- Some species of the genera *Klebsiella*, *Xanthomonas*, *Leuconostoc*, *Alcaligenes*, *Enterobacter*, *Lactococcus* and *Lactobacillus* are slime formers.
- *Leuconostoc* species produce dextran slime in sucrose solutions and on foods.
- Some *Bacillus* species (*B. subtilis*, *B. licheniformis*) are responsible for ropiness in bread.
- *Alcaligenes* species (the organism formerly named *A. viscolactis*) and *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*) cause ropiness in milk.
- Some species of *Streptococcus* and *Lactobacillus* make milk slimy or ropy; this property is used deliberately in some fermented milks.
- *Kocuria/Micrococcus* species cause sliminess on meat.
- Strains of *Lactiplantibacillus plantarum* can cause ropiness in various fruit, vegetable and cereal products.

17) Gas-forming bacteria

- These bacteria produce gas (CO_2 , H_2 , H_2S) from carbohydrates.
- Species of *Leuconostoc* and the heterofermentative lactobacilli produce only one gas, CO_2 .
- *Propionibacterium*, *Escherichia*, *Enterobacter*, *Desulfotomaculum*, *Proteus*, *Bacillus* and *Clostridium* produce both CO_2 and H_2 .

18) Sporeformers

- The rod-shaped genera *Bacillus*, *Clostridium*, *Sporolactobacillus*, *Desulfotomaculum*, *Alicyclobacillus* and *Sporosarcina* form endospores.
- Of these, *Bacillus*, *Clostridium* and *Alicyclobacillus* (the cause of taint in fruit juices) are the most important in the food industry.

19) Indicator bacteria

- Indicator organisms:
 - coliforms,
 - faecal coliforms and *Escherichia coli*,
 - Enterobacteriaceae,
 - enterococci, and
 - aerobic mesophilic (total viable) counts
 - are used as indicators of the hygienic quality of foods and of process efficiency. *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Vibrio* and *Listeria* are the enteric pathogens sought directly.

Important Yeast Genera

Brettanomyces (teleomorph *Dekkera*)

- produce high amounts of acetic acid and
- are used in the late fermentation of Belgian lambic and English ales;
- they may also occur in spoiled pickles and cause off-flavours (“Brett” character) in wine.
- *B. bruxellensis* is the typical species (*B. lambicus* is a synonym of it).

Debaryomyces

- form pellicles on meat brines;
- *D. hansenii* (formerly *D. kloeckeri*) grows on cheese and fermented sausage and is very salt-tolerant.

Kluyveromyces

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

Mycoderma (obsolete genus; now placed in *Candida* and *Pichia*)

- usually grow on the surface of beer, pickles, wine, fruit juices and vinegar;
- they form a heavy film or pellicle;
- the organism formerly called *M. vini* occurs in wine and vinegar.

Saccharomyces

- produce round, oval or elongated cells;
- may form a pseudomycelium;
- reproduce by budding and by ascus formation;
- are used in baking for the leavening of bread and in alcoholic fermentation;
- are also used in other bioprocesses (brewing, baking and distilling);
- and cause spoilage of foods by producing alcohol and CO₂.

Saccharomyces cerevisiae is used in many areas of the food industry:

- special strains are used for the leavening of bread;
- alcoholic beverages:
 - as top-fermenting yeasts,
 - which grow rapidly at about 20 °C;
 - the cells clump and are carried to the surface by the CO₂ produced, hence the name top yeast;
 - the strains formerly called *S. cerevisiae* var. *ellipsoideus* give high alcohol yields and are
used for industrial alcohol, wines and distilled spirits;
 - as bottom-fermenting yeasts,
 - which do not clump and grow more slowly, producing less CO₂,
 - so that the yeast settles to the bottom of the vessel;
 - they ferment best at low temperatures (10–15 °C);
 - *Saccharomyces pastorianus* (formerly *S. carlsbergensis*) is used in lager beer production;
- *S. cerevisiae* is also used to produce alcohol, glycerol and invertase.
- *Kluyveromyces marxianus* (formerly *S. fragilis*) and *K. lactis* (formerly *S. lactis*) ferment lactose.

Pichia

- form a pellicle on beer, wine and brines and cause spoilage;
- some species are also used in oriental food fermentations;
- *P. membranifaciens* grows as a pellicle on beers and wines.

Torulopsis (an obsolete genus; its species are now placed mainly in *Candida* and *Zygosaccharomyces*)

- cause spoilage of milk because they ferment lactose; the organism formerly called *T. sphaerica* is now *Kluyveromyces marxianus*, and *T. versatilis* is now *Candida versatilis*.
- They also spoil fruit juice concentrates and acid foods,
- as well as sweetened condensed milk.

Candida

- A few species are of both industrial and medical importance.
- They spoil foods with high acid, salt and sugar contents and
- form films and pellicles on the surface of liquids.
- They cause rancidity in butter and dairy products; *Candida lipolytica* is now *Yarrowia lipolytica*, *C. krusei* is *Pichia kudriavzevii* and *C. utilis* is *Cyberlindnera jadinii*.

Zygosaccharomyces

- are able to grow at high sugar concentrations and are therefore called osmophilic;
- they spoil honey, syrups and molasses;
- they are used in the fermentation of soy sauce and of some wines;
- *Z. mellis* and *Z. rouxii* grow in honey; *Z. bailii* spoils acid, preserved foods.

Groups of yeasts

- Film yeasts: *Pichia*, *Wickerhamomyces* (formerly *Hansenula*), *Debaryomyces*, *Candida* and *Trichosporon*,
 - grow on the surface of acid products such as sauerkraut and pickles;
 - they oxidise the organic acids and thus allow less acid-tolerant spoilage organisms to grow;
 - *Wickerhamomyces* and *Pichia* tolerate high levels of alcohol and may oxidise it in alcoholic beverages;
 - *Debaryomyces* is very salt-tolerant and grows on cheese brines containing as much as 24% salt;
 - film yeasts produce little or no alcohol from sugars.
- **Osmophilic yeasts**
 - grow well in
 - environments of high osmotic pressure, that is, at
 - high concentrations of sugar and salt (for example *Zygosaccharomyces rouxii* and *Z. mellis*);
 - they cause spoilage of dried fruits, concentrated juices, honey and other high-sugar foods;
 - *Debaryomyces* species are the most salt-tolerant of the osmophilic film yeasts.

Important Mold Genera

Aspergillus

- are xerophilic (able to grow at low water activity);
- appear yellow to black on a large number of foods;
- grow on grains, jams, cured ham, nuts, dried fruits and vegetables;
- some species are useful in the preparation of certain foods;
- species causing spoilage include *A. glaucus* and *A. repens* (the *Aspergillus* section *Aspergillus*, teleomorph *Eurotium*);
- some species produce mycotoxins (*A. flavus* and *A. parasiticus* produce aflatoxins, *A. ochraceus* and *A. carbonarius* produce ochratoxin A);
- others are used in food and food-additive processing;
 - *Aspergillus oryzae* is used to hydrolyse starch with α -amylase in the production of sake, soy sauce and miso;
 - *Aspergillus niger* is used in the commercial production of
 - citric and gluconic acids from sucrose and starch, and of
 - enzymes such as amylases, pectinases and β -galactosidase.

Alternaria

- cause food spoilage;
- colonies are dirty grey-green;
- the defects they cause in foods are:
 - rot of tomatoes and citrus fruits;
 - rancid off-flavours in dairy products;
- some species produce mycotoxins (alternariol, tenuazonic acid).
- *Alternaria citri* causes rot of citrus fruits;
- *A. alternata* (formerly *A. tenuis*) and *A. brassicae* are other common species.

Byssochlamys

- produces heat-resistant ascospores and is therefore important in heat-treated foods;
- it causes spoilage of high-acid canned foods and fruit products;
- it occurs in soils and on ripening fruits, especially grapes;
- *B. fulva* and *B. nivea* are the important species; they also produce the mycotoxin patulin.

Geotrichum

- produces cottony, cream-coloured colonies;
- it is associated with processing equipment and is therefore called the “machinery mould”;
- it often grows on dairy products (the “dairy mould”);
- *Geotrichum candidum* (teleomorph *Galactomyces candidus*) gives white to cream-coloured growth and is also used in the ripening of surface-mould cheeses.

Mucor

- produce cottony colonies;
- the colonies are white at first and later become grey to brown as the sporangia mature;
- they are used in food fermentations and as a source of enzymes;
- *Amylomyces rouxii* (formerly *Mucor rouxii*) is used for the saccharification of starch;
- *Mucor* species help to ripen some cheeses and are used in the production of certain oriental foods (for example tempeh and sufu);
- they also cause spoilage of foods such as vegetables.

Penicillium

- are used in food production:
 - *Penicillium roqueforti* (with blue-green conidia) and *Penicillium camemberti* (with greyish-white conidia) are used in cheese;
- they also cause spoilage of grains, bread and meat;
 - *P. expansum*, with blue-green spores, causes soft rot of fruits, and
 - *P. digitatum*, with yellow-green conidia, causes soft rot of citrus fruits;
- some species produce mycotoxins:
 - for example *P. verrucosum* produces ochratoxin A and *P. expansum* produces patulin in apples and apple juice;
- others produce antibiotics, such as penicillin from *P. chrysogenum*.

Rhizopus

- form colonies that become black as the sporangia mature;
- are widespread in nature and occur on foods such as fruits, cakes, preserves and bread;
- are involved in the spoilage of many fruits and vegetables;
- *Rhizopus stolonifer* is the common black bread mould;
- *R. oligosporus* is used in the production of tempeh, and some species ferment starch to produce alcohol.