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Food Microbiology

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Preface

This textbook is primarily intended for third-year undergraduate students in Microbiology. It has been developed in accordance with the official curriculum for the Food Microbiology module and is designed to serve as a comprehensive teaching and learning resource. It may also be beneficial to students in related disciplines, including biotechnology, food science, nutrition and quality control.

The textbook introduces the fundamental principles of food microbiology, with particular emphasis on the interactions between microorganisms and food. It covers the major foodborne microorganisms of public health importance, including pathogens responsible for food intoxications, foodborne infections, and foodborne toxicoinfections. It also examines the molecular mechanisms underlying microbial pathogenicity and virulence. In addition, the textbook addresses the principal sources of food contamination, the intrinsic and extrinsic factors influencing microbial growth and survival in food, and the fundamental principles of food hygiene, prevention, and microbiological control required to ensure food quality and safety.

Prepared in accordance with the official curriculum established by the Ministry of Higher Education and Scientific Research, this textbook has been designed to provide students with a clear, well-structured, and scientifically rigorous learning resource. Its primary objective is to facilitate the acquisition of fundamental knowledge in food microbiology while fostering a comprehensive understanding of the microbiological hazards associated with food, as well as the strategies used for their prevention, detection, and control. Ultimately, this textbook is intended to serve as a valuable academic reference, providing students with the essential scientific foundation required for advanced studies and future professional practice in the fields of food microbiology, food safety, and quality control.

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Chapter I : Concise Introduction to the Major Food Groups:

(Classification of foods according to their principal constituents, including proteins, lipids, carbohydrates, water, mineral elements and vitamins etc.)

I.1. Food Groups

Food groups may be classified according to their principal physiological role in the human body **(figure 1):**

I.1.1. Physiological roles of food

I.1.1.1. Functional (protective) foods

These foods provide dietary fibre, minerals and vitamins that are essential for the proper functioning of the body. This role is primarily fulfilled by the fruit and vegetable group.

I.1.1.2. Body-building foods

These are essential for bone formation and muscle development. They include starchy foods, meat, fish and eggs.

I.1.1.3. Energy-providing foods

These foods supply energy to the body'scells and also contribute to energy storage. This group includes cereal products, sugary products and fats.

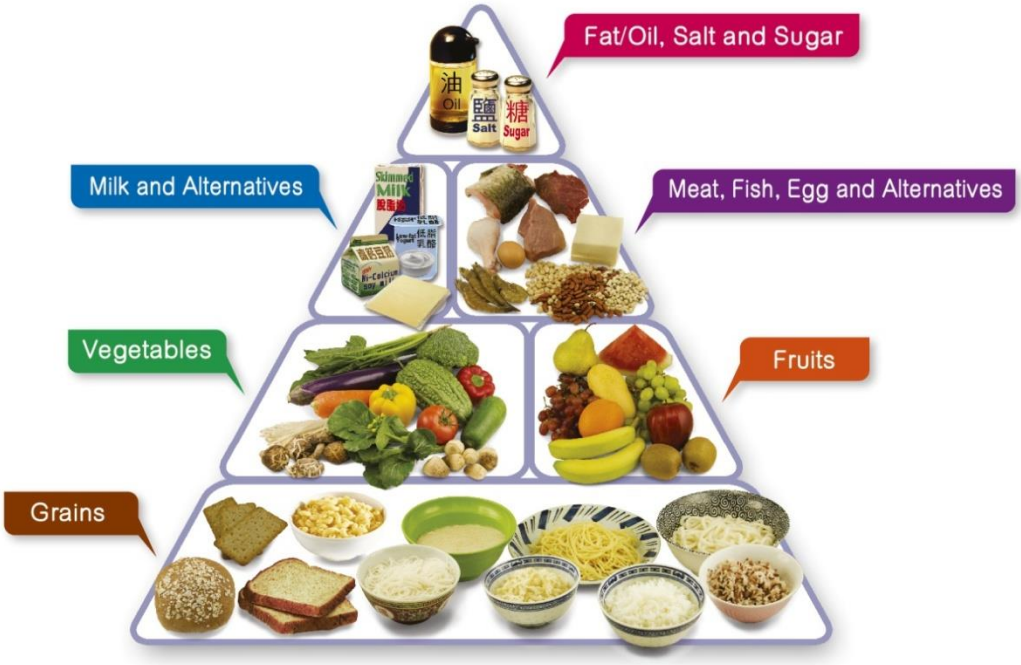


Figure 1: Food groups

I.2. Chemical composition

The seven food groups constitute a simplified classification based on their content of essential nutrients, including proteins, lipids and carbohydrates, as well as minerals such as iron, calcium and magnesium, and vitamins, whether fat-soluble (A, D, E and K) or water-soluble (B-complex and C).

The seven food categories considered are as follows: meat, fish and eggs; dairy products; fats and oils; vegetables and fruits; cereals and legumes; sugary foods; and finally, water and beverages (boissons) **(figure 2)**.

Figure 2: Foods considered to be rich sources of micronutrients

I.2.1. Fruit and Vegetables

I.2.1.1. Nutritional composition

Fruit and vegetables **(figure 3)** are primarily characterised by their high water content, which may represent between 80 and 95% of their fresh weight, as well as by their supply of essential nutrients that cannot be synthesised by the human body. Although they are low in energy due to their reduced caloric density, their nutritional value is considerable owing to their richness in dietary fibre, minerals and vitamins.

Fruit generally contains between 10 and 25% carbohydrates, predominantly in the form of fructose, together with sucrose and glucose. They are low in protein (less than 1%) and lipids (less than 0.5%). The carbohydrate fraction also includes dietary fibre components such as cellulose, hemicellulose, lignin and pectic substances. Many fruits are acidic, containing organic acids such as malic acid and citric acid, which may account for up to 2–3% of their total weight. Lemons, for example, are particularly rich in citric acid.

Figure 3 : Sources of micronutrients (vitamins and minerals)

Vegetables, by contrast, have an average water content of approximately 88%, and typically contain about 8.6% carbohydrates, 1.9% protein, 0.3% lipids and 0.84% ash (representing inorganic mineral matter). They constitute an important source of minerals such as potassium, calcium, magnesium, iron and copper, as well as water-soluble vitamins including vitamin C, B-group vitamins and provitamin A (β-carotene). Potatoes, for instance, are notable for their relatively high starch content (around 20%) and for a reduced vitamin C content, particularly after prolonged storage.

I.2.1.2. Dietary fiber: plays a crucial role in digestive physiology. It accelerates intestinal transit, promotes satiety and supports the development of the intestinal microbiota, thereby contributing to protection against various diseases. It also reduces lipid absorption and moderates postprandial glycaemic response. As fibre is not digested by human enzymes, it exerts significant beneficial effects on gastrointestinal health.

I.2.1.3. Minerals: present in fruit and vegetables are essential for normal physiological functioning. Potassium and sodium regulate fluid balance, while magnesium contributes to neuromuscular function and cardiac rhythm regulation. Calcium, although less abundant than in dairy products, is also present and contributes to bone formation and maintenance.

I.2.1.4. Vitamins: are abundant in this food group. Vitamin C supports immune function, enhances iron absorption and acts as an antioxidant. Vitamin B9 (folic acid) plays a fundamental role in protein metabolism and nucleic acid synthesis. β-carotene, found in the

coloured tissues of plants, exerts antioxidant activity and is converted in the body into vitamin A, which is essential for growth and vision.

I.2.2. Meat, Fish and Eggs

Meat, fish and eggs are highly recommended owing to their high protein content, which is essential for the growth, maintenance and renewal of body tissues, particularly skeletal muscle. These foods provide high-biological-value proteins containing all essential amino acids that cannot be synthesised by the human body. However, their lipid, vitamin and mineral composition varies considerably depending on the animal species and the cut or portion consumed, highlighting the importance of dietary diversification to ensure a balanced nutritional intake.

I.2.2.1. Proteins and Lipids

a. Proteins present in these foods are fundamental for growth, tissue repair and the maintenance of metabolic functions. Meat contains on average approximately 20% protein, mainly composed of myosin, myoalbumin and collagen. Fish provides comparable protein levels, with some variation according to species. Eggs also constitute an excellent protein source, with whole eggs containing approximately 14% protein.

b. Lipids serve as a major source of energy, although their quantity and quality vary significantly. Meat contains both saturated and monounsaturated fatty acids, with total fat content ranging from 2% to 30%, depending on species and cut. Fish exhibit greater variability, with lipid contents ranging from 0.5% to 15%, and eggs contain approximately 12–16% lipids, primarily in the yolk.

I.2.2.2. Minerals and Vitamins

The minerals present in meat, fish and eggs play essential physiological roles:

a. Iron: Essential for the synthesis of haemoglobin and myoglobin. Heme iron derived from animal sources exhibits a bioavailability approximately 2.5 times greater than that of non-heme iron from plant sources.

b. Zinc: Contributes to immune function and exhibits antioxidant activity.

c. Selenium: Plays a protective role against oxidative stress and cellular ageing.

These foods are also rich in B-group vitamins, which are crucial for energy metabolism and nervous system function. Offal, particularly liver, is especially rich in vitamins A and D, in addition to B vitamins and minerals but also contains substantial amounts of cholesterol.

Processed meat products, although protein-rich, often contain elevated levels of fat and cholesterol and should therefore be consumed in moderation.

I.2.2.3. Fish

Fish is an excellent source of high-quality protein and essential micronutrients, particularly vitamins B12 and E. Oily fish, such as salmon and mackerel, are especially rich in long-chain omega-3 fatty acids, which are associated with cardiovascular health benefits. Fish also provides significant amounts of phosphorus and selenium.

I.2.2.4. Eggs

Egg proteins, notably ovalbumin in the albumen and ovovitellin in the yolk, possess high biological value. Whole eggs contain approximately 14% protein (around 8 g per egg) and 12–16% lipids. These lipids are located exclusively in the yolk, which is also rich in phospholipids.

Egg yolk contains approximately 300 mg of cholesterol per yolk, in addition to phosphorus and iron. Eggs are a good source of B-group vitamins and also contain vitamins A and D. They contain negligible amounts of carbohydrates and consist of approximately 75% water.

I.2.3. Milk and Dairy Products

They play a crucial role in nutrition, particularly during adolescence, a period in which the development of bone mass is critical. During puberty, bone mass can increase substantially, and dairy products, due to their high calcium and nutrient content, contribute significantly to this process. These foods are nutritionally complete and provide a broad range of elements essential for health.

I.2.3.1. Proteins and lipids

a. Proteins: Dairy proteins are composed of essential amino acids necessary for the synthesis and renewal of body tissues, including muscles. The principal proteins in milk are caseins and whey proteins, such as lactalbumins.

b. Lipids: Dairy fats provide energy and comprise saturated, monounsaturated and polyunsaturated fatty acids in varying proportions. For example, cow's milk contains predominantly triglycerides, with saturated and monounsaturated fatty acids predominating and a small proportion of essential fatty acids.

I.2.3.2. Minerals and vitamins

a. Calcium: One of the most significant minerals in dairy products, calcium is essential for the formation of bones and teeth and plays a key role in the development of bone mass, which increases by 7–8% per year during adolescence. Milk is a particularly rich source (approximately 1200 mg/L).

b. Phosphorus: Closely associated with calcium, phosphorus contributes to skeletal development and energy metabolism.

c.Other minerals present include sodium, potassium, magnesium, sulphur and copper, although milk contains negligible iron. Milk's near-neutral pH (6.5–6.8) and high water activity facilitate the growth of microorganisms, rendering it highly perishable.

d. Vitamins: Whole milk is a valuable source of vitamins A and D. Most B vitamins are present, including vitamin B12. Fat-soluble vitamins are largely absent from skimmed milk.

I.2.4. Fats

Fats are essential dietary components, playing a fundamental role in physiological functions. They include foods such as butter, cream, oils and margarines, each providing a unique composition of fatty acids and vitamins.

I.2.4.1. Composition and types of fats

I.2.4.1.1. Creams and butter: Concentrated lipid sources, with cream containing approximately 30–35% fat and butter 82–84%. Saturated fatty acids predominate (>60% of total fatty acids). Butter contains short- and medium-chain saturated fatty acids (~13%) but low polyunsaturated fatty acids (~2%) and approximately 250 mg cholesterol per 100 g. These products are excellent sources of vitamin A, with some vitamin D, but contain negligible calcium.

I.2.4.1.2. Animal fats: Such as goose or duck fat, contain 90–100% lipids. Saturated fatty acids are lower (~30%) and polyunsaturated (11–15%), while monounsaturated fatty acids are higher (50–60%). Cholesterol content is ~100 mg/100 g.

I.2.4.1.3. Oils: Generally liquid at room temperature and cholesterol-free. Their health effects depend on fatty acid composition:

a. **Olive oil:** Rich in monounsaturated fatty acids (70–75%), beneficial for cardiovascular health.

b. **Rapeseed oil:** High in monounsaturated fatty acids (60–65%) and essential fatty acids (~30%, mainly linolenic acid).

c. **Peanut oil:** Contains 45–50% monounsaturated, ~20% saturated and 30–35% polyunsaturated fatty acids.

d. **Corn, soybean, sunflower, grape seed, nut oils:** Excellent sources of polyunsaturated fatty acids (60–70%) and rich in vitamin E.

I.2.4.2. Nutritional Importance

- **Energy source:** Fats provide concentrated energy reserves.
- **Cellular components:** Fatty acids are essential constituents of cell membranes.
- **Vitamin absorption:** Facilitate the uptake of fat-soluble vitamins (A, D, E, K).
- **Cardiovascular health:** Monounsaturated and polyunsaturated fatty acids, particularly omega-3 and omega-6, support heart health.

I.2.5. Cereals and Derivatives – Legumes: An Essential Source of Nutrients

I.2.5.1. Cereals and legumes : are fundamental components of the global diet, providing a substantial proportion of the energy and nutrients required by the world population **(figure 4)**. Cereals, such as wheat, rice, maize, barley, oats, and rye, are primarily composed of carbohydrates, mainly in the form of starch (70–76%), and contain 8–12% protein, with variations depending on the type (up to 14% in durum wheat). Their lipid content is low (2–4%), largely concentrated in the germ. Whole grains and their derivatives are also an important source of dietary fiber. Although cereals are low in calcium, they are rich in phosphorus, particularly in the form of phytic acid in whole flour products. They do not contain vitamins A, C, or D, but are rich in B-group vitamins, especially vitamin B1 (thiamine) and vitamin B2 (riboflavin).

Figure 4: Major Food Cereals

I.2.5.2. Oilseed legumes, soybeans, and peanuts, are rich in proteins (approximately 24 %), minerals (phosphorus, iron at 85–90 %), and B-group vitamins. They also contain a high amount of dietary fiber (12 to 25 % of dry weight), which can sometimes make them difficult to digest. Common dried pulses have a low lipid content (1–5 %), whereas soybeans and peanuts provide approximately 18 % lipids.

I.2.5.3. Starchy foods

They encompassing potatoes, cereals, bread, and pulses, constitute the principal source of dietary energy and should account for approximately half of daily food intake. This food group provides several essential components:

- **Dietary fibre:** Predominantly present in whole grains and unrefined products, fibre contributes to proper digestive function, particularly by regulating intestinal transit and providing a sense of satiety.
- **Complex carbohydrates:** Mainly in the form of starch, the glucose released from their breakdown serves as the primary energy source for the body. This glucose is liberated gradually, ensuring prolonged satiety and supporting both physical activity and cognitive function over time.
- **Proteins:** Although plant proteins contain limiting amino acids, i.e., those present in low quantities, they remain an important source of nutrients.

- **Minerals and vitamins:** Starchy foods are a source of magnesium, which is involved in maintaining nervous system balance and regulating heart rhythm. They also contain vitamin E, an antioxidant, and B-group vitamins, which participate in nutrient metabolism. The content of vitamins and minerals varies considerably according to the type of cereal and the degree of refinement.

I.2.6. Sugars and Sugary Products (sweetened products)

Sugars and sugary products comprise a variety of foods with a pronounced sweet taste, such as chocolate, honey, jam, pastries, and spreads. While these foods are valued for their sweet flavour, they often contain so-called "hidden" fats, necessitating moderate consumption to avoid dietary imbalances. The primary nutritional contribution of this group lies in carbohydrates, particularly sucrose, glucose, and fructose, although they generally provide little additional nutrients, except in the case of chocolate.

I.2.6.1. Composition and Types of Sugary Products

a. Confectionery: These are food preparations in which sugar is the main ingredient, excluding jams, jellies, and marmalades. They are predominantly composed of sucrose and provide "empty calories" without significant additional nutrients.

b. Honey: Composed of 3–6% sucrose, 35% glucose, and 35% fructose, honey also contains trace amounts of vitamins and minerals. Although often considered a natural sweetener, it remains a concentrated source of simple sugars.

c. Chocolate: Produced by blending sugar with cocoa mass, chocolate typically contains 50–65% sucrose, 20–30% lipids (mainly cocoa butter), 6% protein, minerals such as magnesium and iron, and small amounts of vitamins. Dark chocolate, in particular, is often regarded as beneficial to health due to its antioxidant content.

I.2.6.2. Nutritional Importance and Consumption

Simple carbohydrates present in sugary products provide a rapid source of energy by releasing glucose, which is the essential fuel for all cells in the body. The brain, in particular, relies exclusively on glucose as an energy source, as it cannot synthesise it on demand. However, these carbohydrates are rapidly digested, resulting in a swift rise in blood glucose levels, which may be problematic for individuals with diabetes or those aiming to manage their body weight.

Consumption of sugary products should be moderate, as excessive intake can lead to dietary imbalance, promote weight gain, and increase the risk of chronic diseases such as type 2 diabetes and cardiovascular conditions. Conversely, athletes may benefit from these foods during prolonged physical exertion, as they provide a quick source of energy to support activity.

I.2.7. Water and Beverages: The Importance of Hydration

Water is a fundamental component of the human diet, essential for life and the proper functioning of the body. Comprising approximately 60–70% of body weight, the human body relies on a regular intake of water to maintain vital functions. Daily water losses, through urine, faeces, perspiration, and respiration, must be continuously replenished to prevent dehydration, which can lead to severe physiological dysfunctions.

I.2.7.1. Types of water and their characteristics

Drinking water is classified into several categories according to legislation:

a. Tap water (distribution water): Sourced from surface water or groundwater, tap water undergoes various treatments to ensure purification before distribution. It is regularly monitored to guarantee its safety for human consumption.

b. Bottled water: Derived from well-protected underground sources, bottled water is not subjected to disinfecting treatments and is bottled under strict hygienic conditions. It offers a convenient alternative to tap water.

c. Spring water: Must originate from a specific source and be marketed without any treatment. Its mineral composition may vary depending on the source.

d. Mineral water: Exclusively of underground origin, mineral water is obtained from a source or via borehole. Its mineral composition is stable, and it undergoes no chemical treatment or disinfection before bottling or therapeutic use. Mineral waters are often selected for their specific properties, such as beneficial mineral content.

I.2.7.2. Sugary and Other Beverages

Sugary beverages, including soft drinks, syrups, cola, and fruit drinks, are popular but should be consumed in moderation. They contain high amounts of added sugars, with fruit drinks containing between 90 and 120 g of sugar per litre. Regular consumption may contribute to excessive caloric intake and health problems such as obesity and diabetes.

a. Fruit drinks: Unlike fruit juices, these contain little actual fruit extract and a high sugar content. They should not be confused with fruit juices, which are more nutrient-dense.

b. Tea and coffee: These beverages are valued for their stimulating properties due to caffeine and theine. Although they provide negligible assimilable nutrients, they can be part of a balanced diet if consumed without excessive sugar or milk.

c. Fruit juices: Contain nutrients from the fruits from which they are derived, including minerals, vitamins, and natural sugars. Sugar content varies by fruit: grape juice contains approximately 200 g per litre, while orange juice contains 90–100 g per litre.

I.2.7.3. Importance of Hydration

Regular fluid intake is crucial to compensate for daily water losses. Daily requirements range from 1.2 L to 2.5 L, depending on physical activity and environmental conditions. It is recommended to vary water sources to benefit from the different minerals they provide. In summary, adequate hydration is essential for maintaining health and well-being, and beverage choices should consider both their composition and their impact on overall health.

I.1/ Microorganisms and food

(pathogens linked to intoxications, intoxication, toxico-infection, and virulent infection…).

I.1.1. Microorganisms associated with food

Food microbiology focuses on the study of microorganisms whether saprophytic, pathogenic, or beneficial—that play a role in the food industry. This discipline mainly covers three aspects:

I.1.1.1. Food safety

It involves the analysis of microbial characteristics, their effects, and preventive measures against food intoxications and foodborne infections. For example, *Clostridium botulinum* is responsible for food intoxication, while *Salmonella Typhimurium* causes foodborne infections.

I.1.1.2. Food preservation

This aspect aims to:

- Identify and report possible routes of food contamination by microorganisms.
- Control factors (such as medium composition, pH, water activity, temperature, redox potential, etc.) that influence their survival.
- Study and control methods that allow their complete destruction (such as sterilization), the total inhibition of their growth and activity, as well as techniques ensuring their stabilization.

I.1.1.3. Beneficial effects and industrial applications

It explores the positive effects of microorganisms and their use in industry. For example, molds used in cheese production and lactic acid bacteria in the dairy industry.

Foods can act as vectors or as actual culture media for microorganisms, potentially causing various disorders in consumers. The severity of these conditions depends on the nature and number of microorganisms, as well as the toxicity of their metabolic by-products.

I.1.2. Microbial Contamination Routes of Food

Microbial contamination of food depends on several factors **(figure 5):**

a) Unpreserved plant-based foods mainly act as carriers for microorganisms, which generally remain on their surface.

b) Animal-derived foods can be contaminated in two main ways:

- The animal was healthy at the time of slaughter, but the product was later contaminated through contact with an infected or carrier person or animal, or by microorganisms from the environment (soil, air, dust, work surfaces).
- The animal was already affected by a microbial disease, whether apparent or not, and the microorganisms were transmitted to the consumer through meat or milk.

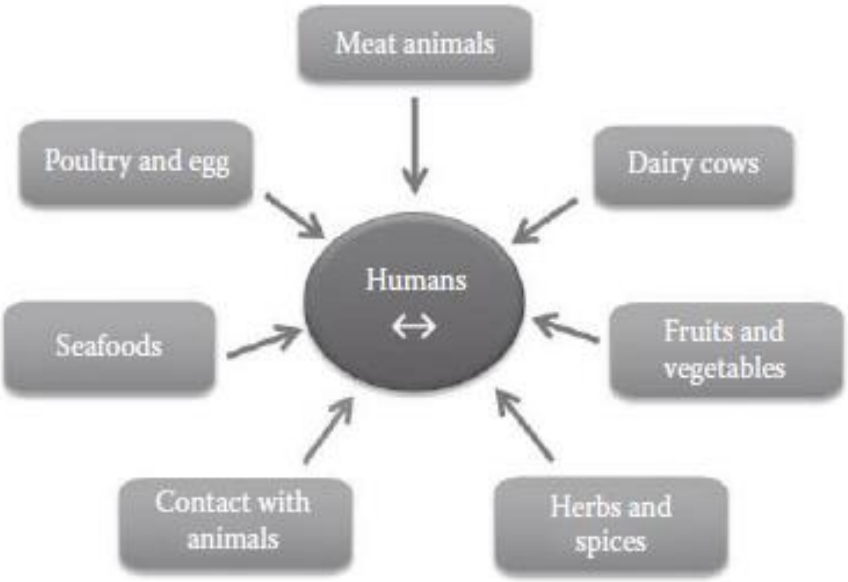


Figure 5 : Diagram showing various sources for foodborne pathogens, indicates person to person transmission.

I.1.3. Foodborne Outbreaks (FBO)

A foodborne outbreak (FBO) is characterized by the occurrence of at least two cases presenting similar symptoms that can be attributed to the same food source. Three main types are distinguished:

I.1.3.1. Toxi-infection

Toxi-infection results from the ingestion of a sufficient number of viable microorganisms, which multiply in the host (generally in the intestine) and produce toxins *in vivo* responsible for the symptoms.

Example: *Clostridium perfringens* food poisoning — the spore-forming bacterium is ingested, multiplies in the intestine, and produces its enterotoxin.

I.1.3.2. Intoxination

Intoxination refers to the pathological effect of a toxin on the organism, regardless of its origin or mode of acquisition.

It therefore corresponds to the toxic mechanism rather than the mode of contamination.

I.1.3.3. Infection

Infection results from the ingestion (or entry) of viable microorganisms (bacteria, viruses, or parasites) that multiply in the host and cause an inflammatory or tissue response.

Example: salmonellosis — invasion of the intestinal mucosa and bacterial multiplication.

The main mechanism is microbial multiplication, not necessarily toxin production.

I.1.3.4. Intoxication

Food intoxication results from the ingestion of toxins already formed in the food, even if the producing microorganism is absent or has been destroyed.

Example: staphylococcal food poisoning — ingestion of preformed heat-stable enterotoxins.

The formation of biogenic amines, particularly histamine, results from the bacterial decarboxylation of histidine during the spoilage of protein-rich foods. Histamine intoxication is characterized by digestive disorders and vasomotor symptoms **(figure 6)**. It is caused by the ingestion of preformed histamine in food produced by bacteria such as *Morganella morganii*, *Klebsiella pneumoniae*, certain strains of *Escherichia coli*, and species of *Pseudomonas*.

The formation of toxic metabolites from amino acids derived from proteins is common during microbial spoilage of foods, particularly fish rich in histidine.

For example, histamine intoxication manifests as nausea, vomiting, diarrhea, flushing, headaches, palpitations, and sometimes edema. It results from the consumption of foods containing high levels of histamine formed by bacterial decarboxylation of histidine.

The responsible bacteria possess histidine decarboxylase, including:

- *Morganella morganii* (formerly *Proteus morganii*)
- *Klebsiella pneumoniae*
- certain strains of *Escherichia coli*
- certain species of *Pseudomonas*

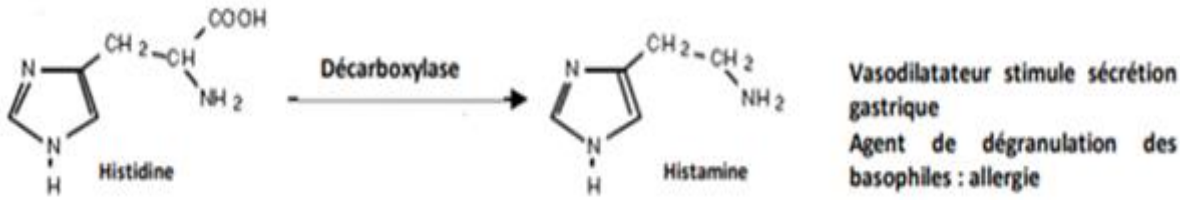


Figure 6: Production of toxic metabolites from proteins

I.2. Microbial Foodborne Diseases

Among foodborne infectious diseases, the most common are caused by the ingestion of microorganisms belonging to the genera *Salmonella*, *Shigella*, *Listeria*, *Brucella*, *Escherichia*, *Campylobacter*, *Clostridium*, *Yersinia*, and *Vibrio*, as well as certain viruses **(figure 7).**

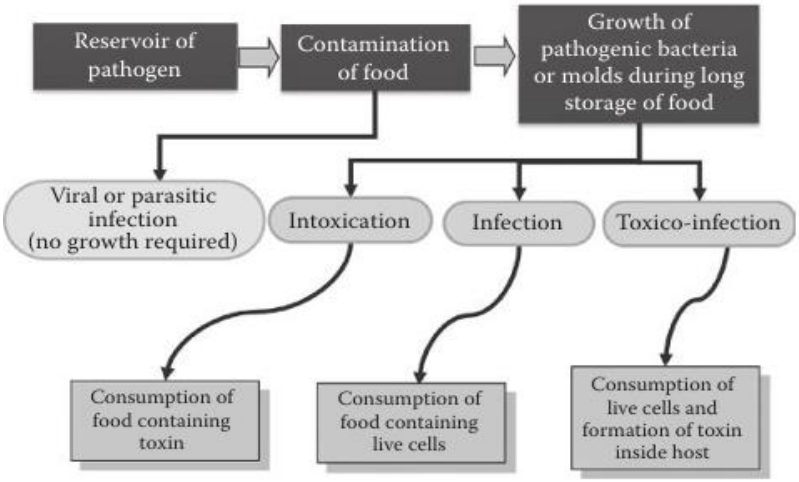


Figure 7: Sequence of events that lead to foodborne disease by bacteria and viruses.

These microorganisms may act as intracellular or extracellular pathogens, multiplying by using host components as a source of nutrients. They can be invasive and/or produce toxins, leading to localized lesions in the digestive tract as well as in other tissues, potentially progressing to septicemia.

The presence of these microorganisms in food represents a major public health risk. For certain highly pathogenic organisms (e.g., *Salmonella*, *Listeria monocytogenes*), regulations require their complete absence in a defined sample volume or weight.

For others, such as *Escherichia coli*, which may be naturally present at low levels, monitoring is based on acceptable counts according to regulatory thresholds.

Therefore, microbiological control methods are adapted to the specific risk, using either qualitative detection (presence/absence) or quantitative enumeration.

The diagram illustrating the transmission pathways of foodborne pathogens shows that these microorganisms can be transmitted either vertically or horizontally **(figure 8)**

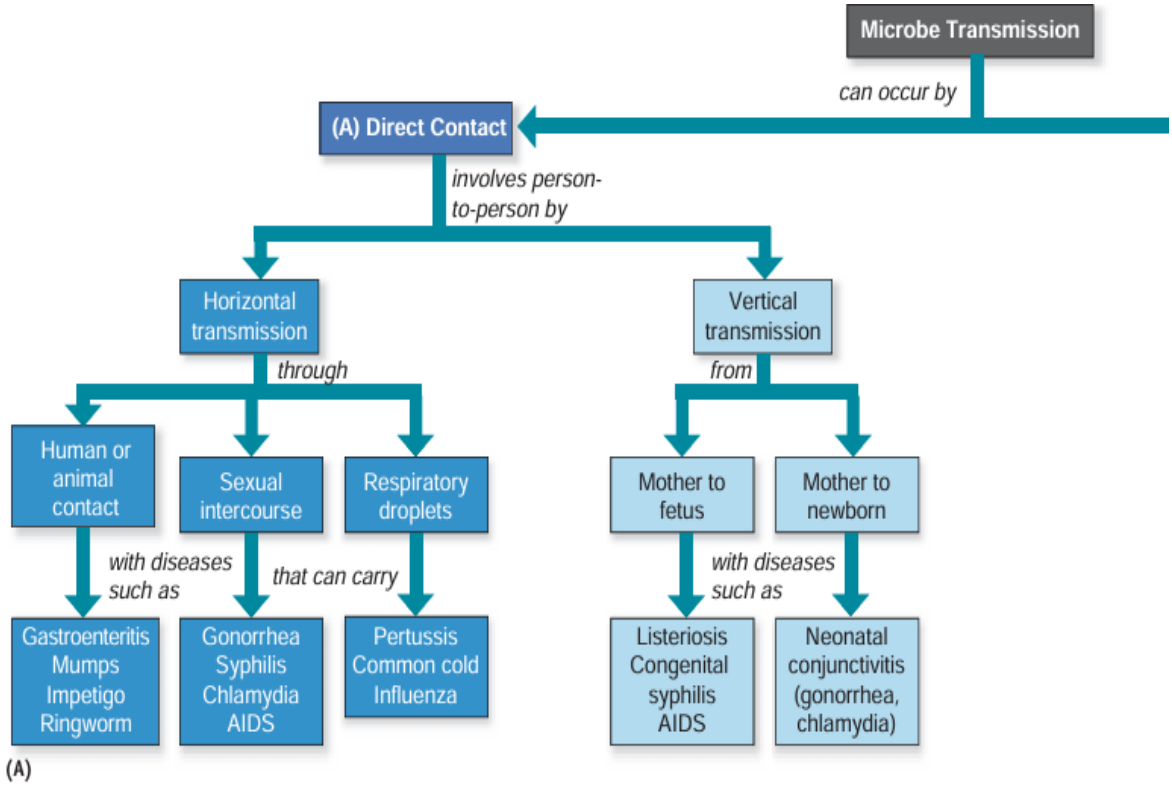


Figure 8 : Transmission of microorganisms and viruses. (A) Pathogens can be transmitted by direct contact involving horizontal or vertical transmission. (B) Pathogens also can be transmitted by fomites, contaminated food and water, and vectors.

I.2.1. Enterobacteriaceae

I.2.1.1. General characteristics of the group

Enterobacteriaceae constitute a highly diverse family, both ecologically and in terms of pathogenicity. Species in this family can be:

- **Parasites:** Some species are strictly pathogenic for humans or animals, such as *Shigella* or *Yersinia pestis*.
- **Commensals:** Naturally present in humans and animals, without causing disease under normal conditions, such as *Escherichia coli*, *Proteus mirabilis*, or *Klebsiella spp.*
- **Saprophytes:** Living in the environment (soil, water, decomposing plants) and feeding on dead organic matter, such as *Serratia* spp. or *Enterobacter* spp.

Enterobacteriaceae are widespread in nature, often due to contamination from animal feces and wastewater. They are frequently involved in food contamination, and some species can cause disease or foodborne intoxications.

I.2.1.2. Key characteristics of the Enterobacteriaceae family:

- Gram-negative bacilli or coccobacilli
- Non-spore-forming
- Motility: motile via peritrichous flagella or non-motile
- Growth: grow on standard culture media
- Respiration: aerobic or facultatively anaerobic
- Glucose fermentation: with or without gas production
- Nitrate reductase positive: reduce nitrates to nitrites (except *Erwinia*)
- Oxidase negative and catalase positive, with a few exceptions
- Mesophilic
- Neutrophilic
- Non-pigmented (except *Erwinia* and *Serratia*)

The presence of Enterobacteriaceae, fecal coliforms, or thermotolerant coliforms is often indicative of poor hygienic quality of a product, whether during production or in the finished product. It may indicate fecal or environmental contamination, failures in processing procedures, poor hygiene of equipment and tools, or cross-contamination from different sources (direct/indirect).

I.2.1.3. Coliforms and *Escherichia coli*

Among Enterobacteriaceae, which are mainly found in the intestines of humans and animals, coliform bacteria are distinguished by their ability to ferment lactose at varying rates. Fecal coliforms have two additional characteristics related to their habitat: they can grow at 44 °C and in the presence of bile salts. Moreover, *E. coli* **(figure 9)** *i*s generally characterized by its ability to produce indole from indole-free peptone water.

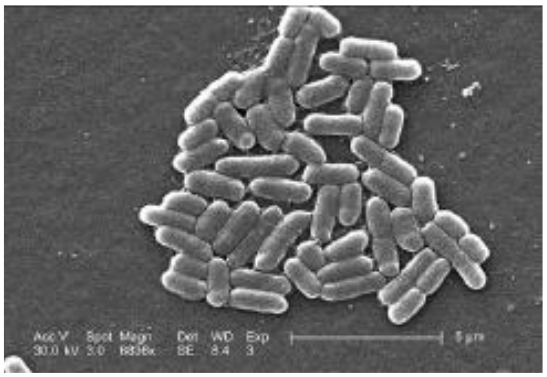


Figure 9 : *Escherichia coli* O157:H7. This group of bacterial cells was obtained from a pure bacterial culture and examined by scanning electron microscopy

Infantile gastroenteritis (IGE) caused by *Escherichia coli* (*E. coli*) is an infection primarily affecting the gastrointestinal tract of young children, although adults can also be affected. Specific strains of E. coli are responsible for this disease and are distinguished by their ability to produce toxins or adhere to the intestinal wall **(figure 10).**

I.2.1.3.1. Types of *E. coli* associated with IGE

a. Enterotoxigenic *E. coli* (ETEC):

- **Characteristics:** Produces toxins that stimulate the secretion of fluids and electrolytes in the intestine, causing watery diarrhea.
- **Symptoms:** Profuse watery diarrhea, abdominal cramps, sometimes vomiting and fever.

b. Enteropathogenic *E. coli* (EPEC):

- **Characteristics:** Adheres to the intestinal wall and causes lesions, disrupting nutrient and water absorption.
- **Symptoms:** Persistent diarrhea, often accompanied by fever and vomiting.

c. Enterohemorrhagic *E. coli* (EHEC), including E. coli O157:H7:

- **Characteristics:** Produces a toxin called verotoxin or Shiga toxin, which can damage blood vessels, leading to bloody diarrhea.
- **Symptoms:** Bloody diarrhea, severe abdominal pain, and sometimes serious complications such as hemolytic uremic syndrome (HUS).

d. Enteroinvasive *E. coli* (EIEC):

- **Characteristics:** Invades intestinal mucosal cells, causing inflammation and ulceration.
- **Symptoms:** Watery or bloody diarrhea, fever, and abdominal pain.

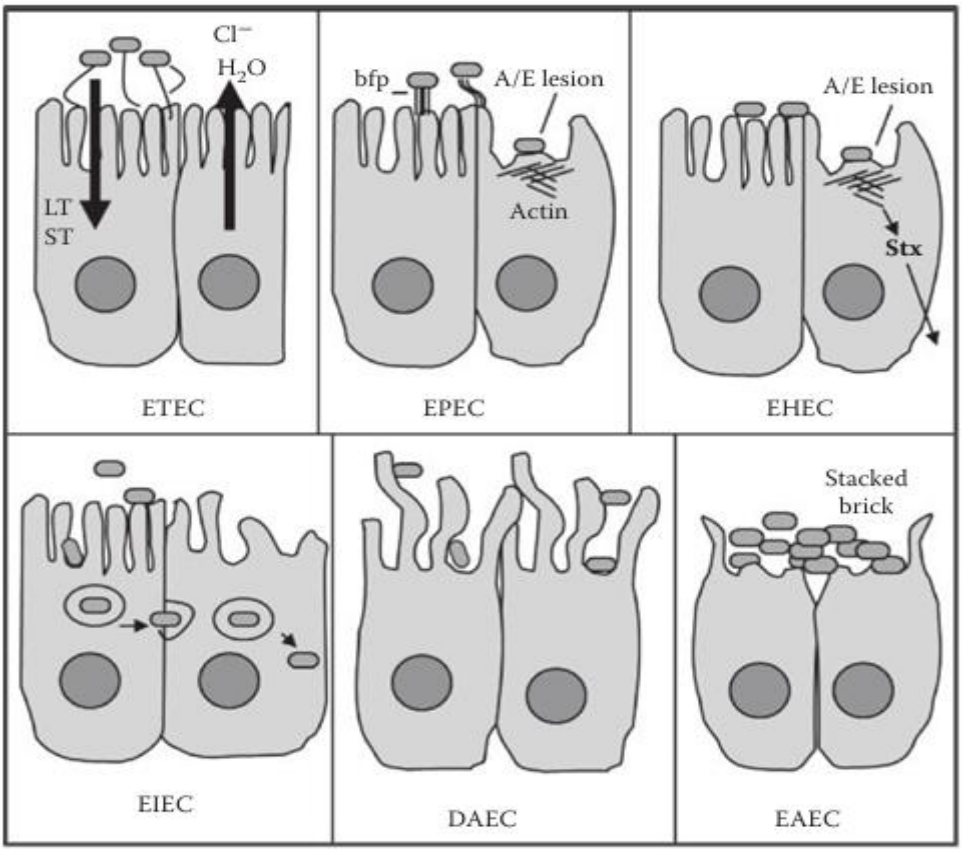


Figure 10 : Mechanism of *Escherichia coli*–induced cell damage in intestinal villous epithelial cells by enterotoxigenic *E. coli* (eteC), enteropathogenic *E. coli* (ePeC), enterohemorrhagic *E. coli* (eHeC), enteroinvasive Esc. coli (eieC), diffusely adhering Esc. coli (DAeC), and entero aggregative *E. coli* (eAeC). Lt, heat-labile toxin; Ht, heat-stable toxin; bfp, bundle-forming pili; A/e lesion, attachment/effacement lesion showing actin accumulation; Stx, Shiga-like toxin.

I.2.1.3.2. Transmission

a. **Food sources:** Contamination occurs mainly through the consumption of contaminated foods, such as undercooked beef, unpasteurized dairy products, raw fruits and vegetables, and contaminated water.
b. **Human-to-human transmission:** It can also occur through direct contact with an infected person or contact with contaminated surfaces.

Biotypes of *E. coli* known as UPEC (Uropathogenic *E. coli*) are specifically associated with urinary tract infections (UTIs). These strains possess structures called common pili or fimbriae, allowing strong attachment to the cells of the urinary mucosa. This adhesion is a critical step in the infection process. Some UPEC strains can produce toxins that damage urothelial cells, contributing to inflammation and tissue injury.

I.2.1.3.3. Detection and isolation media

Isolation of Enterobacteriaceae, particularly coliforms, requires the use of specific culture media that promote their growth while inhibiting other microorganisms:

a. MacConkey Agar

A selective and differential medium. Bile salts and crystal violet inhibit the growth of Gram-positive bacteria, while lactose and neutral red allow differentiation of lactose-fermenting bacteria (pink/red colonies) from non-fermenters (colorless colonies).

b. Eosin Methylene Blue (EMB) Agar

A selective and differential medium that inhibits Gram-positive bacteria and allows differentiation of lactose-fermenting bacteria. Used for the isolation and differentiation of coliforms and *E. coli*.

-*E. coli* colonies typically appear metallic green or dark blue-green with a metallic sheen.

-Enterobacter and Klebsiella may form pink to red colonies.

-Salmonella and Shigella colonies usually appear colorless to slightly opaque and do not show the pink to red coloration typical of lactose-positive bacteria.

c. Violet Red Bile Glucose (VRBG) Agar

A selective and differential medium used for the detection and isolation of Enterobacteriaceae, particularly glucose-fermenting bacteria. Its selectivity is provided by bile salts, which inhibit the growth of Gram-positive bacteria.

d. Violet Red Bile Lactose (VRBL) Agar

Contains peptone, lactose, bile salts, sodium chloride, neutral red, and crystal violet. A selective and differential medium that promotes the growth of Gram-negative bacteria and differentiates lactose fermenters. Colonies appear red/purple.

I.2.2. Genus *Salmonella*

Salmonella are Enterobacteriaceae characterized by their inability to ferment lactose and produce urease. They grow easily on standard culture media, ferment glucose, reduce nitrates to nitrites, and are oxidase-negative. Their identification relies on several criteria,

including the ability to produce hydrogen sulfide (H_2S), although exceptions exist, as well as the presence of lysine decarboxylase and the absence of tryptophan deaminase.
They are mainly found in the intestines of humans and animals.

The genus *Salmonella* **(figure 11)** currently includes over 2,500 serotypes, all considered potentially pathogenic to humans. Among these, four serotypes — S. typhi, S. paratyphi A, B, and C — are responsible for infectious diseases known as typhoid and paratyphoid fevers. These diseases have become less frequent, and their treatment with antibiotics (tifomycin or chloramphenicol) is well controlled. Moreover, a vaccine (TAB-Typhim) provides effective protection.

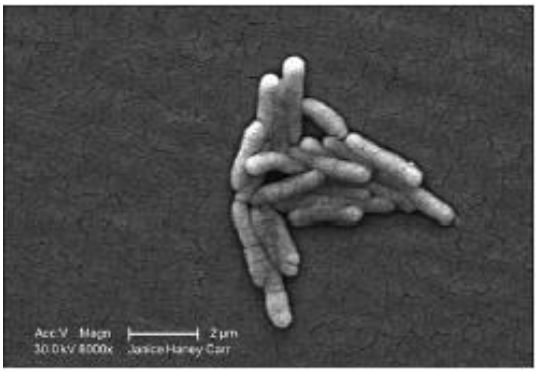


Figure 11 : *Salmonella enterica*. This scanning electronmicrograph shows a clump of bacterial cells obtained from a pure culture.

Salmonella foodborne infections are very common, with these bacteria being the pathogens most frequently involved in foodborne illnesses (FBI).
The infectious dose varies by serotype: it can be very low — a few cells — for *Salmonella enterica subsp. enterica* serovar *Typhi*, *Salmonella paratyphi* A, B, and C; it is higher for certain serotypes responsible for foodborne infections, reaching approximately 10^9 cells for *Salmonella Typhimurium* and 10^6 cells for *Salmonella Anatum*. Among foodborne infections, *Salmonella Enteritidis* is the most frequently implicated serotype.

Food contamination by *Salmonella* bacteria can result from several factors **(figure 12)**: it may be primary, when animals are infected or asymptomatic carriers; it can occur through contact with a contaminated environment (water, soil, surfaces, slaughtering or processing equipment); or it may involve food handlers who are ill or asymptomatic carriers, facilitating the transmission of the bacteria during food preparation or distribution.

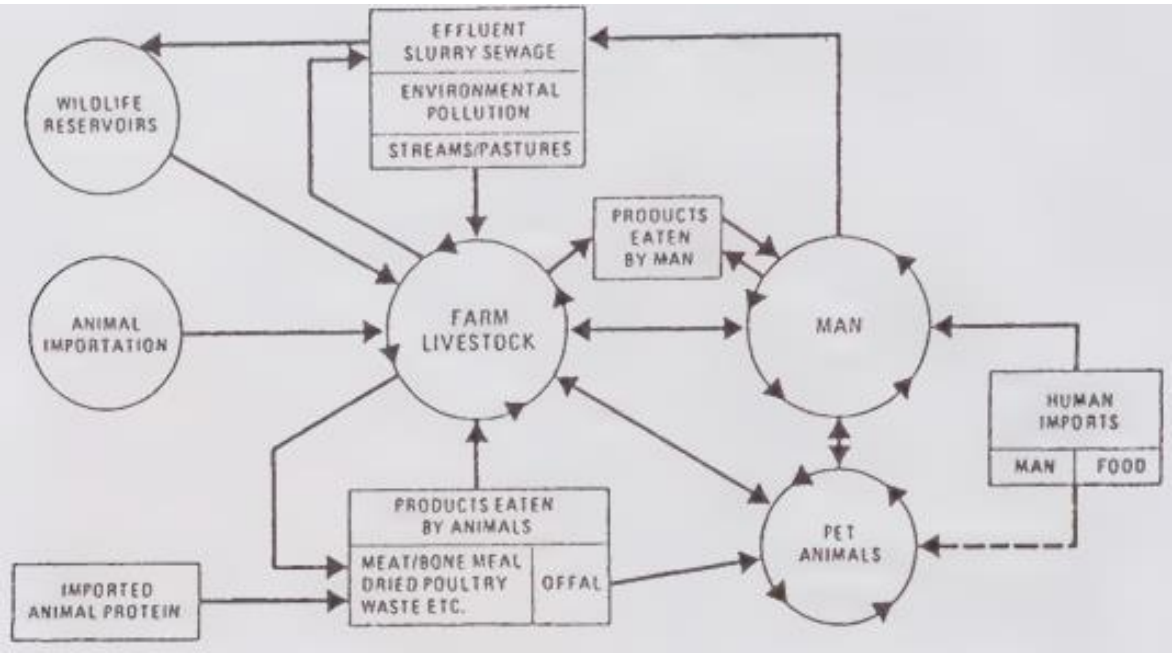


Figure 12 : The *Salmonella* cycle of infection

The diagram of food contamination pathways by these bacteria shows that these processes can occur either vertically (direct) and/or horizontally (indirect).

All types of foods can potentially be contaminated by these microorganisms. If temperature, water activity, and pH conditions are favorable, Salmonella can multiply. Foods most frequently associated with salmonellosis cases include poultry (40 %), meats, particularly ground meat (10 %), milk and dairy products (15 %), eggs (5 %, with higher risk for duck or quail eggs), ice cream and pastries (5 %), as well as shellfish, among others.

Salmonelloses are divided into two main categories: on one hand, typhoid fever (caused by *S. enterica* serovar *Typhi*) and paratyphoid fevers (caused by *S. paratyphi* A, B, and C), which involve two invasion mechanisms: an entero-invasive process and the action of endotoxin (LPS). On the other hand, there are non-typhoidal salmonelloses, often responsible for foodborne infections (such as those caused by *S. Typhimurium* and *S. Enteritidis*). These gastroenteritis cases are associated either with an entero-invasive process or with an endotoxin-mediated mechanism.

I.2.2.1. Typhoid Fever: Specifically caused by *Salmonella typhi*. Typhoid fever is a systemic disease (**Figure 13)**, with symptoms such as high fever, fatigue, headache,

abdominal pain, and sometimes a rash. It can lead to serious complications if untreated. Like *Salmonella* foodborne infections, it is primarily transmitted through the consumption of contaminated food or water, often linked to poor hygiene and sanitation conditions.

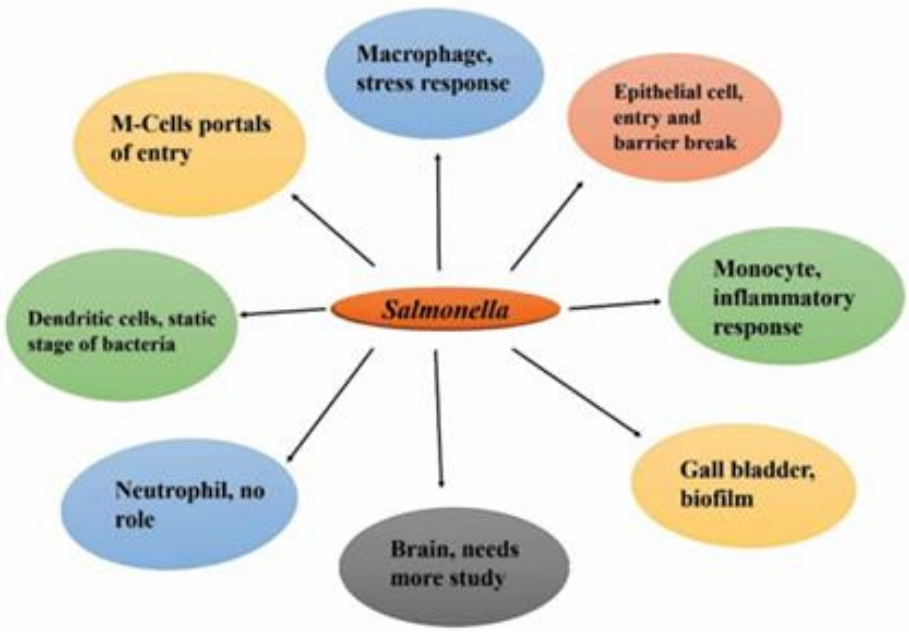


Figure 13 : *Salmonella* infection in different cells and organs.

I.2.2.2. Paratyphoid Fever: Caused by *Salmonella paratyphi*, which includes three serovars: A, B, and C. Symptoms are similar to those of typhoid fever but generally less severe. It is also a systemic disease, but complications are less frequent.

I.2.2.3. *Salmonella* Foodborne Infection: The most common serotypes include *Salmonella Enteritidis* and *Salmonella Typhimurium*. Clinical signs vary depending on the species, age, and physiological condition of the consumer, appearing 5 to 72 hours after ingestion. They are characterized by diarrhea, abdominal pain, chills, fever, vomiting, prostration, anorexia, headache, and malaise. Occasionally, enteritis or localized infection may occur. These symptoms typically last a few days, with children and the elderly being particularly susceptible to this foodborne infection.

An enterotoxin (LPS) produced by *Salmonella enteritidis* in the intestine has been identified. This toxin disrupts water and electrolyte metabolism, which can lead to symptoms such as diarrhea and dehydration, typical of gastrointestinal infections caused by this bacterium **(figure 14)**. Diagnosis is based on the microbiological analysis of the patient's

stool, who may become an asymptomatic carrier. The proportion of such carriers ranges from a few percent in the general population to over 20 % among people living in groups under poor hygiene conditions, such as workers in a meat processing plant. Currently, one of the major challenges in food bacteriology is the increasing contamination levels of many raw materials. It is important to note that these bacteria are easily eliminated by pasteurization.

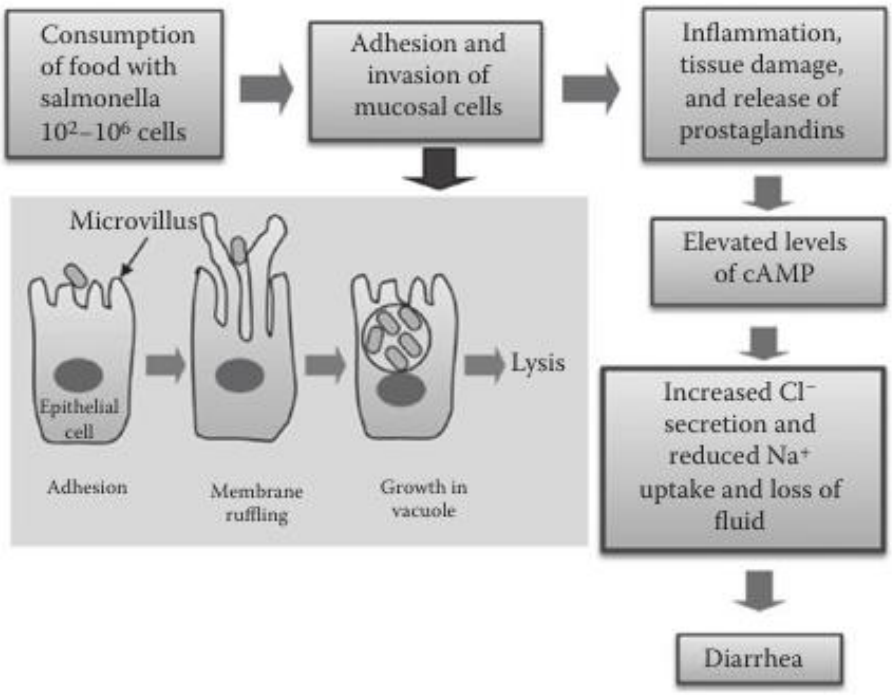


Figure 14: Schematic drawing showing steps involved in *Salmonella* pathogenesis.

I.2.2.4. Detection of *Salmonella*

I.2.2.4.1. Pre-enrichment medium

Tryptone Salt Water (TSW) is a non-selective pre-enrichment medium designed to promote the growth of *Salmonella* when present in low numbers in a sample. The usual inoculation ratio is 1:10.

I.2.2.4.2. Enrichment media

Selenite broth (SFB) and tetrathionate broth (SFM) are commonly used for the selective isolation of *Salmonella* from clinical, food, or environmental samples.

-The pre-enriched sample is inoculated into the SFB or SFM medium, usually at a 1:10 ratio.

-The medium is incubated at 35–37°C for 18–24 hours.

I.2.2.4.3. Isolation

***a. Salmonella-Shigella* (SS) :** agar is a selective isolation medium that inhibits Gram-positive bacteria and is used for the detection of *Salmonella*.

Bile salts combined with brilliant green inhibit the growth of Gram-positive bacteria and partially inhibit some Gram-negative bacteria.

On this medium:

-*Salmonella* form colorless colonies with or without a black center **(figure 15)**: Lactose$^-$ and H_2S^+ or H_2S^-

-*Shigella* form colorless colonies without a black center: Lactose$^-$ and H_2S^-

-Coliforms, including *Escherichia coli*, form red/pink colonies: Lactose$^+$ and H_2S^-

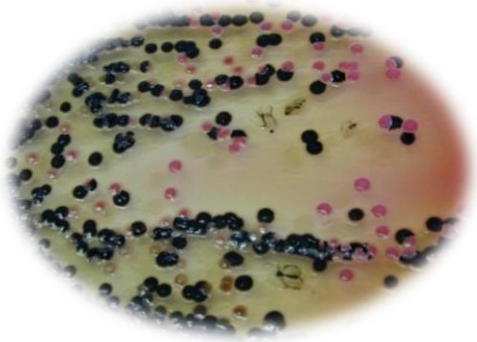


Figure 15 : Mixed culture of *Salmonella* (Lactose$^-$, H_2S^+) and *Escherichia coli* (Lactose$^+$, H_2S^-) on *Salmonella-Shigella* (SS) agar, 24 h at 37 °C.

b. Hektoen agar : is a selective isolation medium for non-fastidious Gram-negative bacilli and is used for the detection of *Salmonella* and *Shigella* in stool samples. Sodium deoxycholate inhibits the growth of Gram-positive bacteria.

This medium allows simultaneous detection of lactose, sucrose, and salicin utilization. Carbohydrate fermentation results in acidification, which turns the bromothymol blue indicator to yellow:

-Yellow or orange colonies: acidification of the medium due to fermentation of at least one of the sugars present.

-Green or blue colonies: no acidification = none of the sugars are utilized: lactose$^-$, sucrose$^-$, salicin$^-$.

Sodium thiosulfate serves as a sulfur source, from which some bacteria produce hydrogen sulfide (H_2S). H_2S reacts with ferric ions to form a black iron sulfide (FeS) precipitate **(figure 16)**.

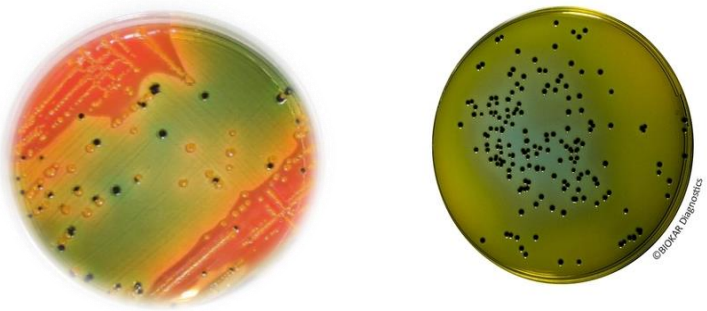


Figure 16 : *Salmonella* (green colonies with black centers) and *Escherichia coli* (orange colonies) on Hektoen agar, 24 h at 37 °C.

c. Wilson-Blair Agar (modified) : is a selective medium used for the isolation of *Salmonella typhi* and other *Salmonella* species from pathological animal products, water, dairy products, and other food items.

-The concentrations of brilliant green and bismuth sulfite inhibit the growth of Gram-positive secondary flora and most Enterobacteriaceae, except *Salmonella* and a few *Shigella* species.

-Using the sulfur compounds in the medium, *Salmonella* produces hydrogen sulfide, which gives the colonies a black or sometimes green coloration.

-Characteristic *Salmonella typhi* colonies appear black, flat, dry, and are usually surrounded by a dark-brown metallic sheen **(figure 17)**. Green or brownish colonies without the dark-brown zone are typical of *Salmonella Enteritidis*, *Salmonella Gallinarum*, *Salmonella Choleraesuis*, and *Salmonella Paratyphi*. Coliforms, *Proteus*, and *Shigella* are strongly inhibited but may occasionally produce greenish or brownish colonies.

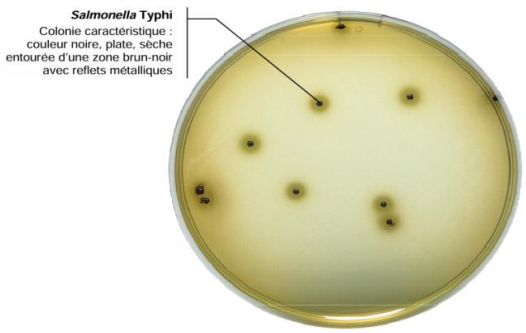


Figure 17 : *Salmonella typhi* on Wilson-Blair agar at 37 °C / 24 h incubation (surface inoculation).

To confirm the presence of *Salmonella Typhi*, immunological methods may be employed, such as the slide agglutination test.

I.2.3. Micrococci

I.2.3.1. Genus *Staphylococcus*

Members of this genus are Gram-positive cocci, often grouped in clusters. They are non-motile, facultatively anaerobic, and chemo-organo-heterotrophic. Their carbon sources include various sugars, and they are generally halotolerant and catalase-positive.

Staphylococcus species are classified as either coagulase-positive or coagulase-negative. Among the former are *Staphylococcus aureus* **(figure 18)** and *Staphylococcus intermedius*; among the latter is *Staphylococcus epidermidis*.

These bacteria can be both commensal and pathogenic in humans and other animals. Their G+C content is approximately 30 to 39%. Common culture media used for their isolation and growth include Chapman medium and Baird-Parker medium.

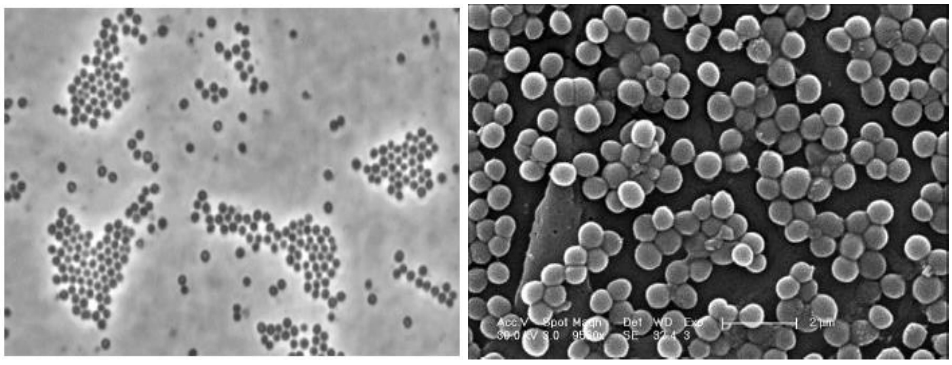


Figure 18 : a) *S. aureus* cells. Phase contrast microscopy (1000× magnification) ; b) *S. aureus.* Scanning electron micrograph of many MRSA cells at a magnification of 9,560 times.

I.2.3.1.1. Staphylococcal Enterotoxicosis

This is an extremely common microbial disease in many countries. It is caused by consuming foods contaminated with enterotoxin-producing strains of *Staphylococcus aureus*. Currently, six types of enterotoxins have been identified: A, B, C, D, E, and F.

I.2.3.1.2. Food Poisoning by *Staphylococcus aureus*

The intoxication is characterized by a short incubation period, generally 1 to 4 hours. The symptoms of this illness are typical: excessive salivation, nausea, vomiting, abdominal pain, profuse diarrhea, sweating, headache, prostration, and sometimes fever. These symptoms usually resolve within 24 to 48 hours, and the patient does not develop specific immunity.

The near-constant presence of this bacterium on human and animal skin and mucous membranes facilitates its widespread dispersion. For a contaminated food to cause intoxication, it must be stored long enough at a temperature that allows microbial growth. Since staphylococcal enterotoxin is a secondary metabolite, it is produced during the late exponential phase and the stationary phase of bacterial growth. The minimum number of bacteria required to produce enough toxin to cause poisoning is estimated by various authors to be between 5×10^5 and 5×10^6 cells per gram.

Enterotoxins A, B, C, D, E, and F are secreted by enterotoxigenic strains of *Staphylococcus aureus*, and a single strain can produce several of these toxins simultaneously. Toxin C exists in three variants (C1, C2, and C3).

These proteins resist hydrolysis by digestive enzymes such as pepsin and trypsin, and they are extremely heat-stable. Consequently, their toxic activity can persist even after sterilization at 121 °C for 15 minutes.

It is therefore clear that if a food is contaminated, a heat treatment such as pasteurization (60 °C for 30 minutes) will destroy the microorganisms, but the food may remain potentially hazardous due to the possible presence of enterotoxin.

Staphylococcal enterotoxins target peripheral gastrointestinal sensory receptors **(figure 19)**. After interacting with these receptors, they transmit nerve impulses via the vagus nerve to the intestinal motility center located in the hypothalamic region of the brain, causing vomiting and intestinal hypermotility.

The foods most frequently associated with this intoxication, in decreasing order of frequency, are: meats and cold cuts, pastries, poultry, cheeses, vegetables, and fish.

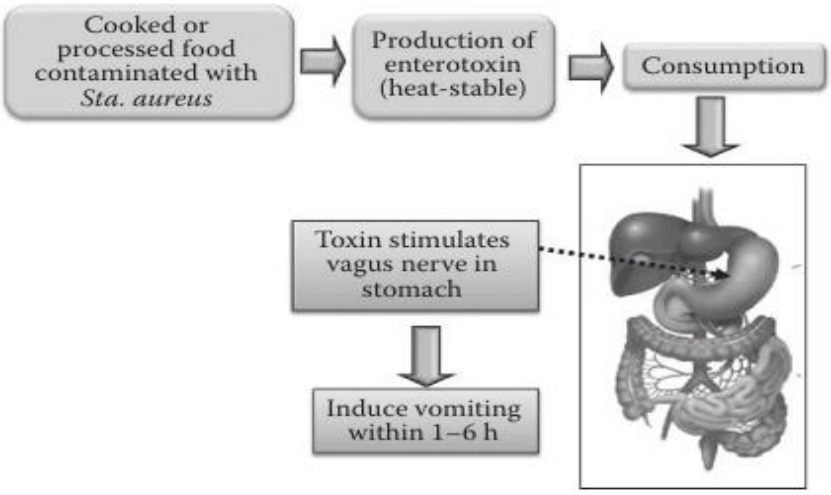


Figure 19 : Pathogenic mechanism of intoxication with enterotoxin from *Staphylococcus aureus.*

I.2.3.1.3. Detection of *S. aureus*

a. Chapman agar is a selective isolation medium used for the detection of *Staphylococcus* species. The selective power of this medium is based on its very high NaCl concentration (75 g/L), which selects for halotolerant bacteria such as *Staphylococcus* **(figure 20).**

The presence of mannitol and phenol red allows determination of the bacteria's ability to ferment mannitol.

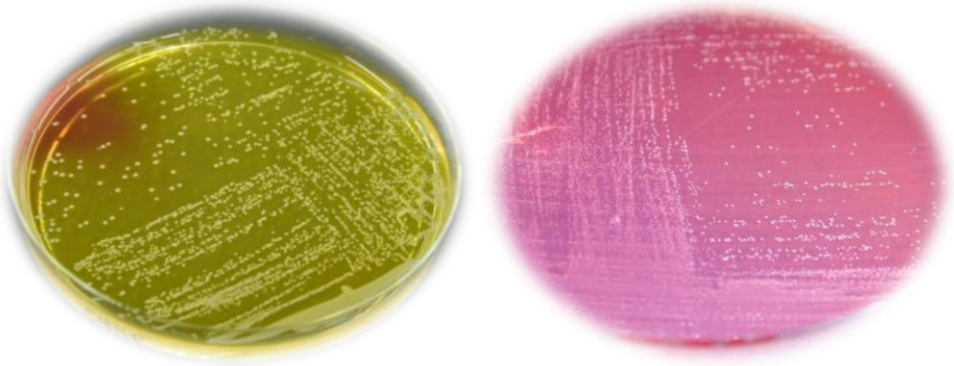


Figure 20 : a) *Staphylococcus aureus* on Chapman medium (mannitol+) ; b) *Staphylococcus epidermidis* (mannitol−) after 24 hours at 37°C.

b. Baird-Parker Agar is a selective and differential culture medium primarily used for the isolation and identification of *S. aureus* from food, clinical, or environmental samples.

- **Potassium tellurite:** Inhibits the growth of many Gram-negative bacteria and some Gram-positive bacteria while allowing the growth of *S. aureus*, which can reduce tellurite to metallic tellurium, forming characteristic black colonies **(figure 21)**. Nearly 100% of coagulase-positive staphylococci have this ability. Tellurite is toxic to other bacteria, and its interaction with the egg yolk in the medium contributes to the formation of well-defined black colonies.
- **Lithium chloride:** Helps inhibit the growth of certain Gram-negative bacteria and other staphylococci.
- **Egg yolk:** Acts as an enrichment; staphylococci that contain lecithinase break down the egg yolk, creating clear zones around the colonies.
- **Incubation:** Plates are incubated at 35–37°C for 24 to 48 hours.
- **Typical *Staphylococcus aureus* colonies:** Appear black, shiny, convex, and are surrounded by a clear zone of hydrolysis.
- **Additional tests:** Coagulase and catalase tests are often required to confirm the identification of *Staphylococcus aureus*.

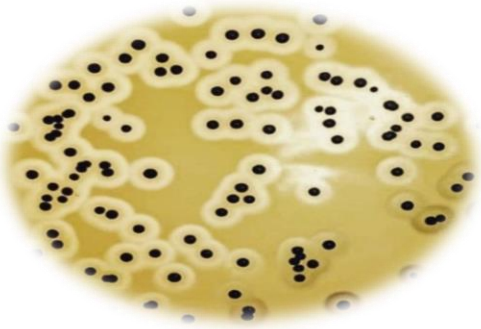


Figure 21: *Staphylococcus aureus* on BAIRD-PARKER medium after 24 hours of incubation at 37°C

I.2.4. Spore-Forming Bacteria

I.2.4.1. Genus *Clostridium* (Anaerobic)

These bacteria belong to the family *Bacillaceae*. They are frequently found in soil, wastewater, and the intestine. Under strictly anaerobic conditions, they can contaminate various products such as water, milk, fish, fermented or frozen foods, and especially canned foods. They degrade sugars and proteins, producing butyric acid and gases such as H_2S.

Clostridium are Gram-positive bacilli, often large, occurring either singly or in chains. The morphology and position of the spore are important taxonomic criteria, and these spores can resist temperatures of 100°C for several minutes. Generally non-motile, these bacteria are catalase-negative. They grow readily on standard culture media. Under anaerobic conditions, they can be either proteolytic or saccharolytic depending on the species and are often gas-producing.

Clostridium are classified according to their metabolism and substrate activity:

- **Saccharolytic *Clostridium*** are gas-producing (CO_2^+, H_2^+, H_2S^-), often produce butyric acid, acidify and coagulate milk, and generally do not liquefy gelatin. Mesophilic species include *Clostridium butyricum* and *Clostridium tyrobutyricum*.
- **Proteolytic or putrefactive *Clostridium*** are highly active in protein degradation. They are weakly fermentative and produce little gas (H_2S^+). They liquefy gelatin and digest milk casein, with or without coagulation, as seen in *Clostridium botulinum* and *Clostridium putrefaciens*.
- **Proteolytic and saccharolytic *Clostridium*** liquefy gelatin and have significant fermentative activity (H_2S^+). They are often called "sulfite-reducing" *Clostridium*, such as *Clostridium butyricum*.

Some species of the genus *Clostridium* are involved in foodborne intoxications and gastroenteritis, notably *C. perfringens* **(figure 22)**. Other species, such as *C. botulinum*, produce extremely potent neurotoxins responsible for botulism, a severe and potentially fatal intoxication.

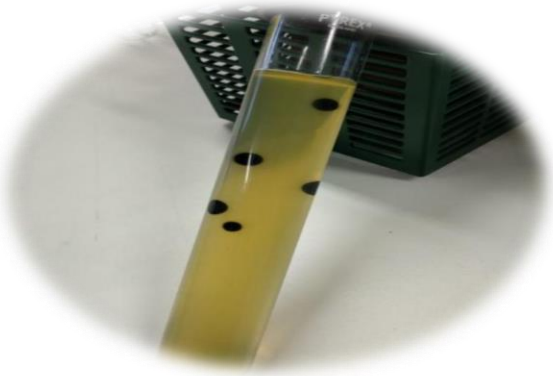


Figure 22: *Clostridium perfringens* colonies on Meat-Liver agar after 24 h at 44 °C

I.2.4.1.1. Foodborne illnesses Caused by *Clostridium perfringens*

This bacterium is probably the most widespread anaerobic microorganism in the natural environment. Found in soil and water, it occurs in a wide range of natural products. It is also a commensal of humans and animals, colonizing the skin as well as the digestive and respiratory tracts.

The ability of this bacterium to form spores allows it to survive under extremely unfavorable conditions. However, its strictly anaerobic nature limits its growth in our foods. Canned and cooked foods provide ideal conditions for the proliferation of *Clostridium perfringens*, as cooking reduces the oxygen content.

Strains of *C. perfringens* are classified into at least six types based on the toxins they produce and secrete, with more than a dozen toxins having been identified.

Foodborne illness often occurs when toxin-producing type A strains of *Clostridium perfringens* multiply in meat left to cool for several hours at temperatures near or above ambient temperature. This growth results from the germination of spores, which can survive simmering-type cooking for 3–4 hours or temperatures of 110 °C for 30 minutes.

To trigger foodborne illness, a microbial load of at least 10^8 cells per gram is required. Symptoms typically appear 8–24 hours after consuming the contaminated food and primarily

include severe abdominal pain and diarrhea. Nausea, vomiting, fever, chills, or prostration are less common.

The enterotoxins, with a molecular mass of approximately 35,000 daltons, are antigenic and heat-labile. This protein disrupts cellular energy production and directly affects the structure and function of cells, particularly enterocytes.

I.2.4.1.2. Botulinum Intoxication

Botulinum intoxication is caused by the ingestion of botulinum toxin, produced during the growth of *Clostridium botulinum* in food. This soil-dwelling, spore-forming, strictly anaerobic bacterium poses a major contamination risk for many foods, particularly canned goods (jars and bottles) that have undergone insufficient heat treatment.

Botulinum toxin is one of the most potent poisons known, with an estimated LD_{50} between 10^{-8} and 10^{-9} g/kg of body weight. Due to this extreme toxicity, mortality remains high despite treatments such as antitoxin sera or anatoxins.

Six types of *Clostridium botulinum* (A, B, C, D, E, and F) have been identified based on the serological specificity of their toxins. Types A, B, and E are most frequently involved in human botulism.

Botulinum toxins are large proteins that, when ingested, are absorbed by the lymphatic system of the digestive tract and enter the bloodstream. They act by binding to the neuromuscular junctions of cholinergic nerve fibers in the peripheral nervous system, thereby preventing the release of acetylcholine, an essential neurotransmitter (**figure 23)**.

This inhibition of acetylcholine release leads to a series of neurological disturbances, including **(figure 23)**:

- Headaches and nausea
- Vomiting and abdominal cramps
- Constipation
- Dryness of mucous membranes and skin
- Respiratory problems that can progress to paralysis

These symptoms result from the disruption of communication between nerves and muscles, highlighting the potency and danger of botulinum toxins.

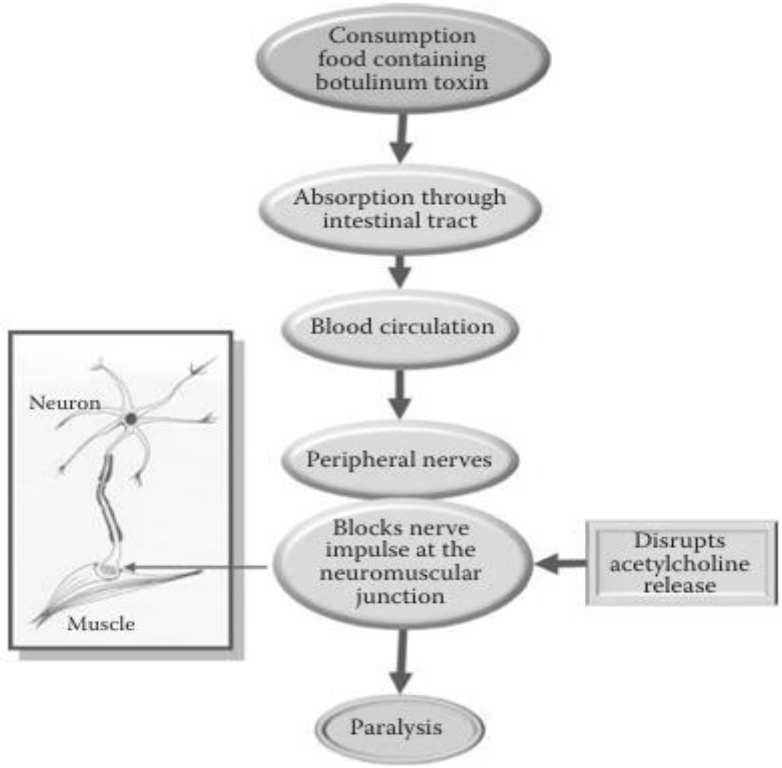


Figure 23 : Sequence of events in foodborne botulism (*C. botulinum*).

I.2.4.1.3. Prevention of Bacterial Growth and Toxin Production

- **Acidic foods (pH < 4.5):** Inhibit the growth of *C. botulinum*.
- **Water activity (Aw < 0.94):** Foods with low water activity, such as dried or salted products (often nitrite-cured), prevent bacterial growth and therefore toxin production.

I.2.4.1.4. Food Safety Measures

- Prevention relies on producing properly sterilized canned foods.
- Foods that are not true canned products (e.g., semi-preserved foods) should be stored under refrigeration.
- In non-acidic foods, the addition of nitrites at levels of 20 ppm or more inhibits bacterial germination and proliferation.

I.2.4.1.5. Heat Inactivation of Toxins

Botulinum toxins are denatured and inactivated by heat. According to studies, at 80°C, inactivation takes 8 to 90 minutes, while at 100°C, only a few seconds are required. Proper cooking of food can therefore render it safe by denaturing the toxins.

I.2.4.2. Genus *Bacillus* (aerobic)

These bacteria are Gram-positive rods, generally motile, and capable of forming endospores. Spore morphology is a key criterion for their classification. Depending on the species, they can be aerobic or facultatively anaerobic and are catalase positive. Most species are chemo-organo-heterotrophs, and many can grow on nutrient agar. They are commonly found as saprophytes in soil and water. Some species are pathogenic to humans or other animals, including insects. Their G+C content ranges from 30 to 70%.

These bacteria are responsible for contaminating numerous food products and often possess proteolytic properties. Thanks to their ability to form spores, they are resistant to unfavorable environmental conditions and can cause spoilage of canned foods.

Among the frequently encountered species are *Bacillus stearothermophilus*, *Bacillus coagulans*, and *Bacillus cereus* **(figure 24),** which are found in soil, on plants (especially cereals), and on animal skin. They are often involved in foodborne intoxications, with contamination levels ranging from 10^4 to 10^7 cells per gram.

I.2.4.2.1. *Bacillus cereus*

B. cereus is a Gram-positive, spore-forming **(figure 24b)**, facultatively aerobic-anaerobic, and thermoresistant bacterium. Its growth temperature ranges from 5 to 50 °C. The formation of endospores provides high resistance to unfavorable environmental conditions, including bactericidal agents, disinfectants, radiation, desiccation, as well as cooling and freezing cycles.

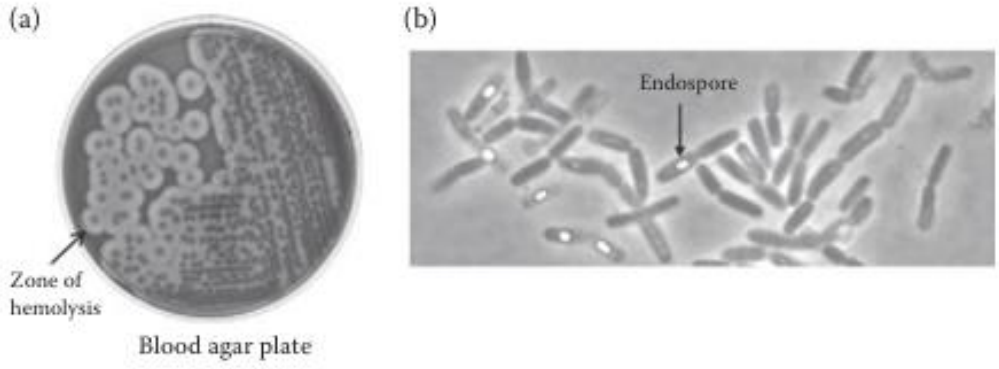


Figure 24 : (a) *Bacillus cereus* colonies on blood agar plate showing zone of hemolysis and (b) microscopic picture of cells showing endospores.

This bacterium can produce two types of toxins:

a. Emetic toxin – a polypeptide preformed in foods, resistant to a wide range of temperatures and pH levels.

b. Diarrheal toxins – protein enterotoxins such as hemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe), and cytotoxin K (CytK), produced during bacterial growth in the small intestine after ingestion of a sufficient number of vegetative cells or spores.

The incubation period after consuming contaminated food typically ranges from 6 to 15 hours, with recovery occurring within 24 to 48 hours.

Two types of clinical manifestations may occur:

-The first is characterized by intense vomiting, which appears rapidly, usually 1 to 5 hours after ingestion.

-The second involves severe diarrhea accompanied by abdominal pain, appearing approximately 10 hours after consuming the contaminated meal.

It is important to note that over 10% of individuals can be asymptomatic carriers of this bacterium.

I.2.5. Vibrios

Vibrio cholerae is a very thin, comma-shaped, Gram-negative bacillus, motile thanks to a single polar flagellum. It is neither encapsulated nor spore-forming.

Its growth temperature ranges between 20 and 40 °C, with an optimum between 30 and 37 °C. *V. cholerae* grows at neutral or alkaline pH (pH 9–10) but is highly sensitive to acidification and does not grow below pH 6.

V. cholerae exhibits a respiratory and fermentative metabolism, is facultatively anaerobic, and oxidase-positive. It ferments mannose, mannitol, and sucrose without gas production, and ferments lactose only slowly. It does not ferment arabinose or inositol. It does not catabolize arginine (ADH-) but possesses lysine decarboxylase (LDC) and ornithine decarboxylase (ODC). It produces lecithinase, lipase, and amylase. The bacterium is sensitive to novobiocin and vibriostatic agents and does not produce H_2S or urease.

Cholera Toxin (Exotoxin) is the primary virulence factor in the pathophysiology of cholera. Cholera toxin (CT) is secreted across the bacterial membrane in its active form. CT, also called cholera holotoxin, is a complex holoprotein of 84 kDa composed of two parts, A

and B, linked non-covalently. The A part (28 kDa) consists of two subunits, A1 (21 kDa) and A2 (7 kDa). The B part is made up of five subunits of 11.5 kDa each, also linked non-covalently.

The B part is responsible for binding CT to the microvilli of the enterocyte (duodenum and jejunum). The A2 subunit facilitates the translocation of the A1 subunit across the membrane, while A1 is the active component of the toxin. The A1 subunit has enzymatic activity that activates enterocyte adenylate cyclase. The resulting increase in intracellular cAMP levels leads to overactivation of protein kinase A, which in turn opens ion channels, especially chloride channels **(figure 25)**. This causes massive secretion of chloride ions and water into the intestinal lumen, leading to the characteristic diarrhea of cholera infection. This rapid loss of fluids and electrolytes can result in severe, potentially fatal dehydration if untreated.

Other virulence factors produced by *V. cholerae* include various enzymes such as mucinase, neuraminidase, and proteases, which facilitate the bacterium's penetration through the mucus layer and adherence to enterocytes in the small intestine.

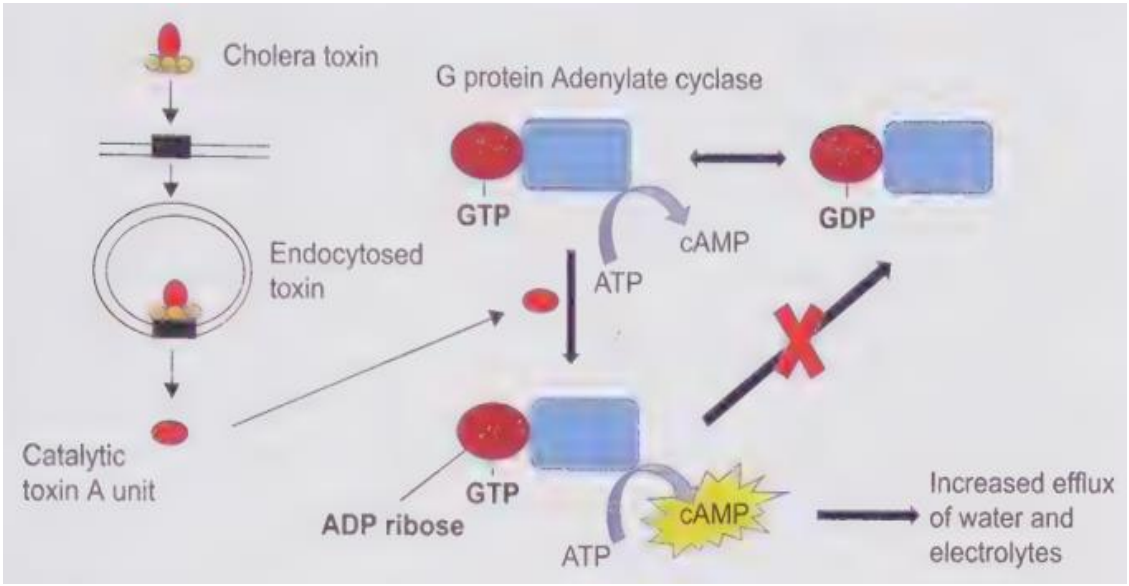


Figure 25 : Cholera toxin and its mode of action.

Although *V. cholerae* can grow in many common media, its isolation from stool samples is more effective using selective media. Alkaline peptone water (APW) is recommended as an enrichment broth, and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar is the selective medium of choice **(figure 26).**

After 18 to 24 hours of incubation at 35–37°C, colonies suspected to be *V. cholerae* appear on TCBS agar as small colonies 2–4 mm in diameter **(Figure 26)**. The yellow color results from sucrose fermentation in the medium. Organisms that do not ferment sucrose, such as *V. parahaemolyticus*, produce green or blue-green colonies.

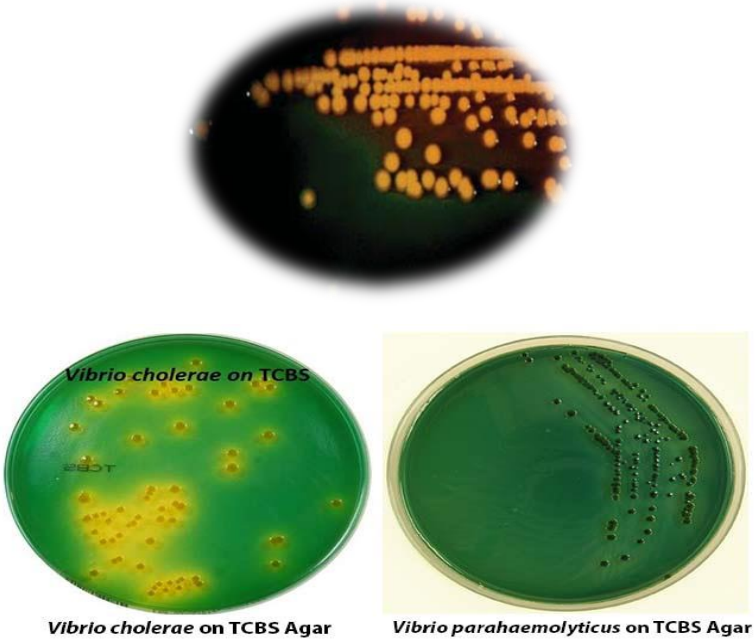


Figure 26 : *V. cholerae* cultured on a TCBS selective isolation medium

I.2.6. *Brucella*

Brucellosis, also known as Malta fever or Mediterranean fever, is an anthropozoonosis primarily affecting domestic animals such as cattle, sheep, and goats. Humans are infected accidentally through direct or indirect contact. Originally described in the Mediterranean basin, brucellosis is now recognized worldwide. It is a notifiable disease and is classified as an occupational disease, representing a frequently underappreciated public health issue.

Brucella are small Gram-negative coccobacilli (0.5–0.7 µm in diameter and 0.5–1.5 µm in length), facultative intracellular organisms, non-motile, non-encapsulated, and non-spore-forming. Their genome consists of two circular DNA molecules and is responsible for a zoonosis transmissible to humans.

The genus *Brucella* currently comprises 12 species, classified according to their pathogenicity and preferred hosts (reservoirs):

- Seven species can be isolated from terrestrial mammals: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, and *B. microti*, with the first three also subdivided into biovars.
- More recently discovered species include *B. ceti* and *B. pinnipedialis* (also found in marine mammals), *B. inopinata* (in humans), *B. vulpis*, and *B. papionis*.

Brucella bacteria are strictly aerobic and fastidious, characterized by slow growth in primary culture. Some strains, such as certain biovars of *B. abortus* and *B. ovis*, require a CO_2-enriched atmosphere (5–10%) for optimal growth. Certain biotypes also require thiamine, niacinamide, or biotin.

They grow at pH 6.6–7.4, with an optimal temperature of 37°C, although they can develop between 20 and 40°C on suitable media. They are capable of producing catalase (+), nitrite reductase (+), and cytochrome oxidase (+) (except *B. neotomae* and *B. ovis*). H_2S production and urease activity vary among species. Most human-pathogenic strains are urease-positive, citrate-negative, indole-negative, VP-negative, and RM-negative.

Their culture requires enriched media such as Columbia agar with fresh blood or chocolate agar **(figure 27)**, or trypticase soy agar supplemented with serum. Optimal growth occurs at 34–35°C. Isolation of *Brucella*, particularly in primary culture, requires incubation periods of at least 3–4 days up to 2–3 weeks. Colonies are translucent, round, and have regular edges. Liquid cultures show slight turbidity (BHI).
Brucella agar is used for culturing *Brucella* spp. and other fastidious microorganisms in the laboratory. It is also recommended for isolating *Brucella* species from food samples.

Figure 27: *Brucella* genus on blood-enriched medium

I.2.6.1. Transmission

a. Direct Cutaneous-Mucosal Contamination

- Represents the primary route of occupational exposure for farmers, shepherds, and veterinarians, accounting for 60–70% of cases.
- Handling lambs, aborted fetuses, fetal membranes, and placentas, as well as contact with vaginal discharges, poses a major risk.
- Contaminated soil and manure remain hazardous for approximately 2 months.
- Laboratory contamination is associated with handling live bacteria or vaccine suspensions.
- The cutaneous route is dominant, especially when working with bare hands, as even intact skin is a barrier easily breached.
- Mucosal entry appears rare; the conjunctiva can be contaminated by a soiled hand.

b. Indirect Contamination

- Milk and dairy products (raw milk, unpasteurized dairy products, artisanal cheese, curd, fresh or even aged cheese) can harbor viable *Brucella* for extended periods.
- Meat plays a much smaller role. Raw vegetables fertilized with animal manure and subsequently poorly washed cannot be completely ruled out as a source.

c. Other Routes

- Brucellosis can occur in tourists in contact with livestock.
- Human-to-human transmission via sexual contact is rare.

I.2.6.2. Reservoir

- The bacterium is ubiquitous, but ruminant mammals and swine are the main reservoirs: *B. melitensis* primarily infects sheep and goats, *B. abortus* infects cattle, and *B. suis* infects pigs.
- *Brucella* spp. can survive for several weeks, and in some cases, several months in dust, water, fetal tissues, meat, and dairy products.

I.2.6.3. Pathophysiology

Brucellosis is a lymphatic-origin septicemia caused by bacteria of the genus *Brucella*, which are facultative intracellular parasites of the reticuloendothelial system (mononuclear

phagocyte system). The pathogenesis of the disease is primarily associated with the endotoxin produced by these bacteria. *Brucella* species have the ability to multiply within macrophages by inhibiting phagosome-lysosome fusion, allowing them to evade host defense mechanisms. *B. melitensis* and *B. suis* are generally considered more virulent than *B. abortus*.

I.2.6.4. Clinical Signs

Brucellosis presents with a variety of symptoms that can be classified into several stages:

a. Incubation: The incubation period ranges from 1 to 4 weeks.

b. Acute Primary Infection (Acute Septicemic Brucellosis)

- Flu-like syndrome with undulant fever.
- Lymphadenopathy (swelling of lymph nodes).
- Splenomegaly (enlargement of the spleen).
- Hepatomegaly (enlargement of the liver).

c. Subacute Focal Brucellosis: This stage is characterized by secondary localization in various organs, including:

- Osteoarticular (joints and bones),
- Cardiac,
- Neurological,
- Genitourinary, and
- Hepatic systems.

d. Chronic Brucellosis: The disease course may last more than one year.

e. Mortality: Generally less than 5%.

f. Pregnancy Complications: In pregnant women, brucellosis may lead to spontaneous abortion, preterm delivery, or intrauterine fetal death.

The complexity of brucellosis pathophysiology and the diversity of clinical manifestations emphasize the importance of early diagnosis and management.

I.2.7. Psychrotrophic Bacteria

Psychrotrophic bacteria are defined by their ability to grow at temperatures below +7 °C. They are characterized by growth that allows colony formation on agar at 7 °C within 10 days.

These bacteria, whether as agents of foodborne illness or as contributors to the deterioration of the commercial quality of food products, represent a limiting factor for the shelf life of refrigerated goods. Control of this type of microbiota relies primarily on improving the performance of refrigeration equipment to ensure storage of food between 0 °C and +2 °C, as well as on validating the shelf life of food products through appropriately designed scientific studies.

I.2.7.1. Main Psychrotrophic Bacteria

Psychrotrophic bacteria can be classified into different groups based on their effects: foodborne pathogenic agents and food spoilage agents.

a. Foodborne pathogenic agents

Based on currently available data regarding the frequency of contamination in food products, as well as recent reports, *Listeria monocytogenes* occupies a predominant position as a psychrotrophic bacterium pathogenic to humans.

Other species can also be involved, such as *Y. enterocolitica*, *B. cereus*, and *C. botulinum* type E. Additionally, *C. perfringens*, certain strains of *Salmonella* and *E. coli*, as well as, more rarely, bacteria of the genus *Pseudomonas*, may also be implicated.

b. Food spoilage agents

Psychrotrophic bacteria responsible for food spoilage are far more numerous and diverse. The family *Pseudomonadaceae* is often the most represented. Other genera include *Alteromonas*, *Flavobacterium*, *Acinetobacter*, and certain *Staphylococcus* species.

These bacteria, along with numerous psychrotrophic molds, are responsible for the deterioration of foods, particularly during cold storage.

I.2.7.2. Genus *Pseudomonas*

Pseudomonas aeruginosa is a bacterium belonging to the family *Pseudomonadaceae*. It is a Gram-negative bacillus, strictly aerobic, motile via a polar flagellum, and does not have particular nutritional requirements.

P. aeruginosa cells measure approximately 0.5–1 µm in width and 1.5–4 µm in length. This bacterium can metabolize a wide variety of organic compounds and exhibits notable resistance to several antibiotics and disinfectants.

Pseudomonas species possess an oxidative metabolism, meaning they use enzymatic redox reactions to exploit nutrients as sources of carbon and energy. Key biochemical characteristics of *P. aeruginosa* include the production of cytochrome c oxidase, nitrate reductase, and nitrite reductase, enabling the reduction of nitrates (NO_3^-) to nitric oxide (NO) and subsequently to nitrogen gas (N_2). This bacterium also possesses arginine deiminase (ADH positive).

Pseudomonas are ubiquitous bacteria, found in water, soil, and vegetation. Although generally of low virulence, several strains are opportunistic pathogens in humans and spoilage agents in foods, particularly meat, fish, and dairy products. The species most frequently encountered in humans include *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, and *P. stutzeri*.

Their presence in slaughterhouses, especially in cold storage rooms, constitutes a major source of meat contamination. Consequently, *Pseudomonas* species are often used as indicators of spoilage in fresh meat and milk.
Confirmation of their identification can be based, at minimum, on the following characteristics: Gram-negative bacilli, motile, strictly aerobic, oxidase positive, capable of growth at 41 °C, and resistant to kanamycin.

I.2.7.2.1. Pigments Produced by Pseudomonas

The identification of *P. aeruginosa* strains is facilitated by the frequent production of an aromatic molecule (o-aminoacetophenone), which is responsible for a characteristic odor, often described as reminiscent of acacia or grapes.
This species also produces two main pigments **(figure 28)**:

- **Pyocyanin**, specific to *P. aeruginosa*;
- **Pyoverdine**, a fluorescent pigment common to so-called fluorescent *Pseudomonas* species, such as *P. putida*, *P. fluorescens*, and *P. aeruginosa*.

Some strains may be non-pigmented, while others produce different pigments, such as pyorubin (red) or pyomelanin (black).

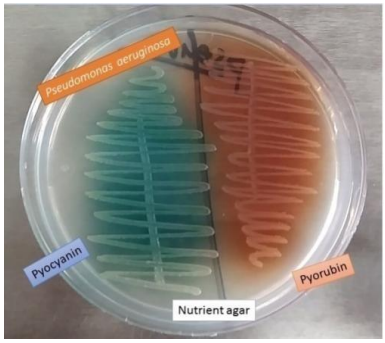


Figure 28 : Pigments produced by the genus *Pseudomonas*

I.2.7.2.2. Isolation

Cetrimide agar is a selective medium designed for the isolation and presumptive identification of *Pseudomonas aeruginosa* in animal-derived biological products, as well as in pharmaceutical and cosmetic products. Its formulation is derived from King A medium, which promotes pyocyanin production by this species.

Cetrimide (cetyltrimethylammonium bromide), a quaternary ammonium compound, acts as an inhibitor of a wide range of microorganisms, including *Pseudomonas* species other than *P. aeruginosa*. Pyocyanin production (a blue, non-fluorescent pigment) is stimulated by the presence of magnesium chloride and potassium sulfate. The medium also supports the production of fluorescent pigments (pyoverdines) by certain *P. aeruginosa* strains.

P. aeruginosa may exhibit the following characteristics **(figure 29)**:

-A distinctive yellow-green pigmentation with fluorescence under ultraviolet light (254 nm);

-Mucoid colonies, pigmented or non-pigmented.

This agar becomes highly selective for *Pseudomonas aeruginosa* when incubated at 42 °C. It is available with or without nalidixic acid.

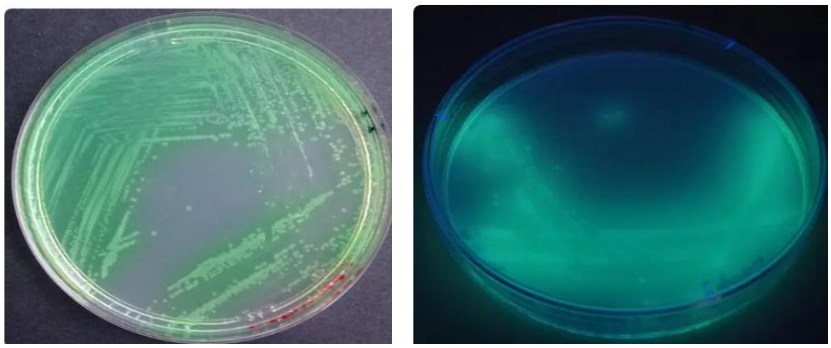


Figure 29: a) *P. aeruginosa* on cetrimide agar; b) *P. aeruginosa* on cetrimide agar under UV light

Colonies of *P. aeruginosa* growing on agar are typically yellow-green, and their fluorescence can be observed under ultraviolet light. They are collected for further strain identification. It is recommended to determine their serotype by slide agglutination using specific antisera, particularly for epidemiological studies.

The virulence mechanisms of *Pseudomonas aeruginosa* are multiple, interconnected, and rely on a combination of factors. The Type III secretion system (T3SS) enables the direct injection of exotoxins, such as ExoU and ExoS, into host cells. Rhamnolipids, produced at a late stage of growth, induce lysis of immune cells and contribute notably to pulmonary infections.

The LasB protease degrades antimicrobial peptides as well as components of the extracellular matrix, thereby promoting infection progression. Quorum sensing regulates the expression of virulence factors according to bacterial density. Finally, biofilm formation provides bacteria with protection against host defenses and increases their resistance to antibiotics, making infections more difficult to treat.

Together, these mechanisms allow the bacterium to colonize, invade, and persist within the host.

I.2.8. *Listeria monocytogenes*

Listeria monocytogenes (family *Listeriaceae*) is responsible for a disease affecting humans and animals known as listeriosis. The genus *Listeria* currently comprises 21 species. Among them, only two are pathogenic for humans and animals: *Listeria monocytogenes*, which is pathogenic for both humans and animals, and *Listeria ivanovii*, which is primarily pathogenic for animals and only rarely for humans.

L. monocytogenes is a small Gram-positive bacillus, occurring singly or in short chains, motile at 20–25 °C and non-motile at 37 °C (due to intracellular actin polymerization), and non-spore-forming. It is a facultative anaerobe that can also grow under microaerophilic conditions. It is catalase-positive (except for rare strains), oxidase-negative, ferments a wide range of carbohydrates without gas production, and produces lecithinases. The strains are generally β-hemolytic.

I.2.8.1. Modes of transmission

a. Foodborne transmission: by far the most important route (≈ 99% of cases). It occurs through the consumption of contaminated foods (milk and dairy products, raw meat and meat

products, vegetables, fish and shellfish, ready-to-eat meals) **(figure 30)**. The incubation period can be long, ranging from 3 to 70 days.

b. Cutaneous and mucosal transmission: cases have been reported in veterinarians and farmers, particularly during the delivery of infected animals or in the context of abortions associated with animal listeriosis.

c. Maternal–fetal transmission: as mentioned, transmission from mother to child can occur via the placenta or during delivery.

Listeriosis presents in invasive forms (maternal–neonatal and non-maternal–neonatal) and non-invasive forms.

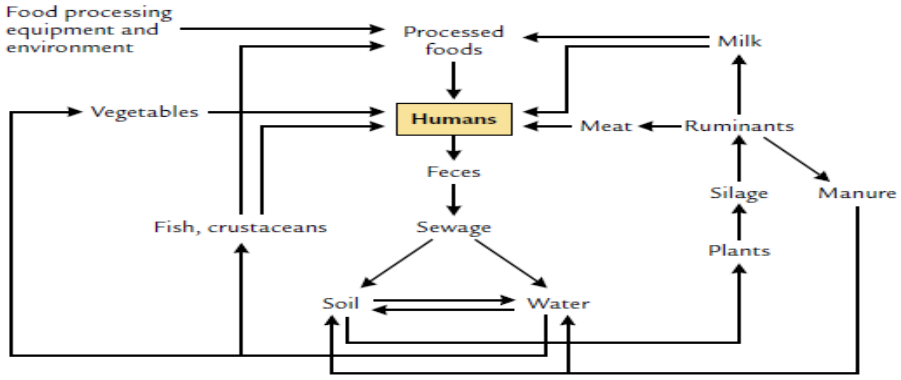


Figure 30 : Ruminants Potential routes of transmission of *L. monocytogenes.*

I.2.8.2.1. Invasive forms

a. Maternal–neonatal forms

Pregnant women infected through the foodborne route can transmit the bacterium to the fetus via transplacental passage or during delivery through contaminated genital tract secretions. This transmission may result in spontaneous abortions, preterm birth, stillbirth, or severe neonatal infections such as meningitis or septicemia.

b. Non-maternal–neonatal forms

At-risk populations include immunocompromised individuals, the elderly, and patients with chronic diseases, who are more likely to develop invasive forms of listeriosis. These forms can lead to severe infections, such as meningitis, septicemia, or localized infections affecting various organs.

I.2.8.2.2. Non-invasive forms

These forms are generally less severe and mainly present as febrile gastroenteritis. They are rarely diagnosed, as they can be confused with other types of gastroenteritis.

Symptoms of listeriosis include fever, headache, nausea, vomiting, pharyngitis, myalgia, and monocytosis; in severe cases, meningitis, cutaneous lesions, septicemia, or abortion may occur. Clinical manifestations often resemble an influenza-like syndrome.

I.2.8.3. Invasion and dissemination of *Listeria monocytogenes*

L. monocytogenes is a bacterium capable of invading the human host by crossing the intestinal barrier and using macrophages as vehicles for dissemination **(figure 31)**:

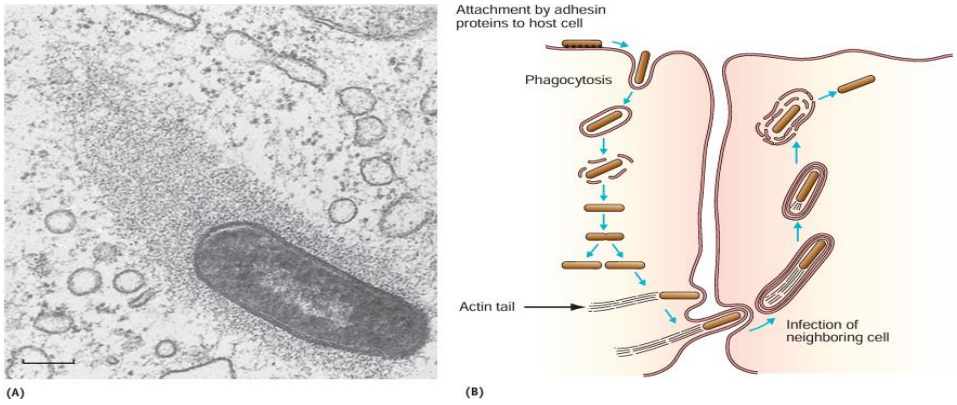


Figure 31 : Invasion by *L. monocytogenes*. (A) A transmission electron micrograph of a *Listeria* cell with its actin tail. (Bar = 0.3 μm.) (B) After entering a host cell by phagocytosis, the bacterial cell loses the surrounding membrane and divides to form a larger population. Invasion of neighboring cells uses the actin tail to "drive" the cells into the adjacent cell. In that cell, the cell again loses the surrounding cell membranes and initiates another infection.

a. Intestinal translocation: the bacterium crosses the intestinal epithelium and penetrates the underlying tissues.

b. Phagocytosis: it is internalized by macrophages into a vacuole known as a phagosome.

c. Phagosome–lysosome fusion: lysosomal enzymes attempt to destroy the bacterium.

d. Production of listeriolysin O (LLO): the bacterium produces LLO, a toxin that disrupts the phagosomal membrane.

e. Cytoplasmic escape: the bacterium escapes into the macrophage cytoplasm.

f. Intracellular multiplication: it replicates actively, leading to host cell destruction.

g. Systemic dissemination: released bacteria spread via the bloodstream to the liver, spleen, and brain, causing severe infections **(figure 32)**.

This process highlights the ability of *L. monocytogenes* to evade host immune defenses and ensure intracellular survival and dissemination.

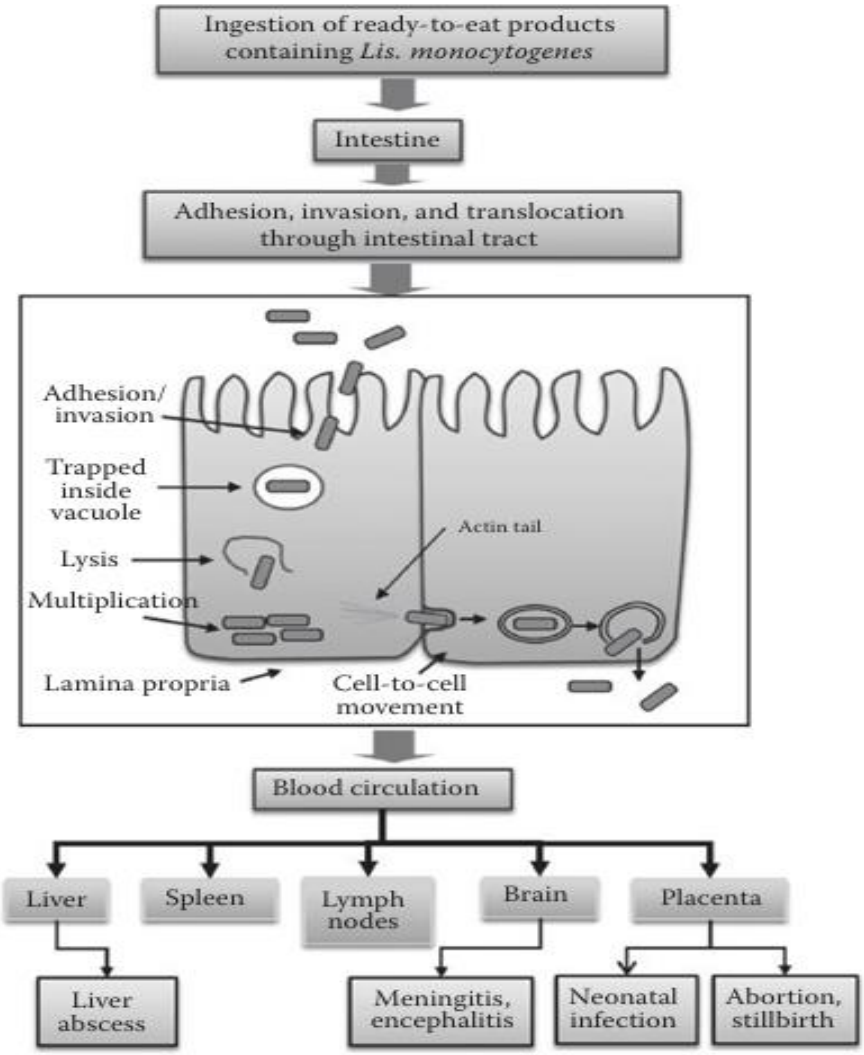


Figure 32 : Mechanism of *L. monocytogenes* pathogenesis and tissue distribution

I.2.8.4. Isolation

a. Fraser broth is used for the secondary selective enrichment of *Listeria monocytogenes* and other *Listeria* species in food products.

b. PALCAM agar is a selective medium used for the differentiation and isolation of *L. monocytogenes* from other *Listeria* species in foods such as milk and cheeses, as well as in other food products, even those heavily contaminated.

After 24 to 48 hours of incubation, *Listeria* forms olive-green colonies with a characteristic concave center, surrounded by a black halo. When colonies become confluent, the medium turns brown-black **(figure 33)**. PALCAM agar is highly selective, although occasional growth of staphylococci or enterococci may be observed.

Selectivity is achieved through the inclusion of lithium chloride, ceftazidime, polymyxin B, and acriflavine hydrochloride. The medium employs a dual-indicator system, containing both esculin with ferric ions and mannitol with phenol red. *Listeria* hydrolyzes esculin, producing colonies surrounded by a black zone. In contrast, enterococci and staphylococci ferment mannitol, forming colonies with a yellow zone, whereas *Listeria* is mannitol-negative.

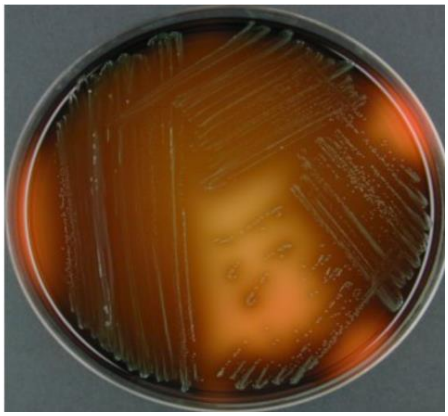


Figure 33: *L. monocytogenes* on PALCAM agar.
The colonies are green and surrounded by a black zone.

c. Oxford formulation for *Listeria* for the selective isolation and detection of *Listeria monocytogenes* in clinical and food samples.

The Oxford selective supplement for Oxford agar is used for the detection and enumeration of *Listeria*. It is an inhibitory mixture composed of three antibiotics (colistin, cefotetan, and fosfomycin), an antifungal agent (cycloheximide), and an antiseptic dye (acriflavine). Provided in lyophilized form, this supplement inhibits nearly all contaminating microflora in tested samples, thereby promoting the growth of *Listeria monocytogenes*. *Listeria monocytogenes* forms brown colonies with black zones due to esculin hydrolysis **(figure 34).**

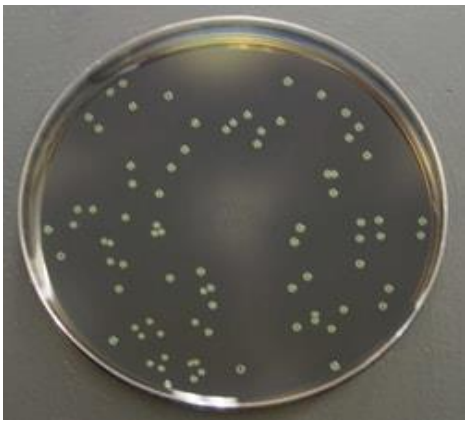


Figure 34: Selective agar for *L. monocytogenes* (Oxford formulation)

I.2.9. Yeasts and Molds

Yeasts and molds are important agents of food spoilage. They degrade fresh foods (fruits and vegetables), refrigerated products (meat products, cheeses), dry products (processed meats), and even acidic foods (fruit juices).

I.2.9.1. Yeasts

Yeasts are heterotrophic eukaryotic organisms belonging to the fungal kingdom. They are primarily characterized by their unicellular nature, although some species can form pseudohyphae or a pseudomycelium. Their size varies depending on the species, generally ranging from 1 to 10 µm in width and 2 to 50 µm in length. Morphologically, they exhibit diverse shapes, including spherical, globose, ovoid, elongated, or cylindrical forms **(figure 36a, b)**.

Each cell is surrounded by a rigid cell wall representing approximately 20% of its dry weight and contains a cytoplasmic membrane as well as a small nucleus. Yeasts may be haploid or diploid, with an optimal growth temperature ranging from 20 to 28 °C **(figure 35)** and a maximum tolerated temperature between 35 and 47 °C. They grow well in acidic environments, with an optimal pH of 4.5 to 6.5, but can also adapt to more extreme or alkaline conditions. They are widely distributed in nature, occurring in water, on plant leaves, in fruit musts, and in many other environments.

In general, yeasts present in food products are not pathogenic and are not considered major agents of foodborne illness. However, their proliferation can impair food quality by causing organoleptic changes such as visual defects, off-odors, and off-flavors, as well as gas production leading to swelling of products and their packaging.

Nevertheless, some yeasts can be pathogenic to humans, although such cases remain relatively rare and mainly affect immunocompromised individuals. For example, candidiasis is caused by *Candida albicans*, which is responsible for oral thrush in infants and vaginitis. Similarly, cryptococcosis is caused by *Cryptococcus neoformans*, an environmental yeast associated with pigeon droppings, which can lead to potentially fatal meningitis.

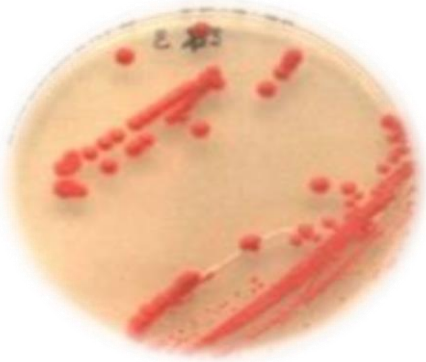


Figure 35: Macroscopic appearance of *Rhodotorula rubra*

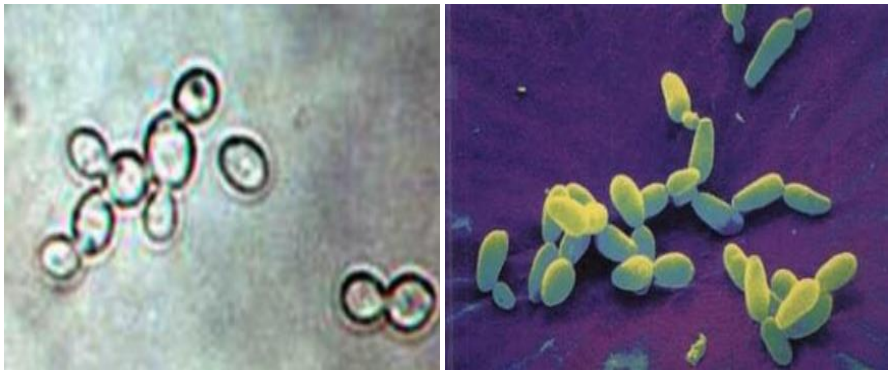


Figure 36: (a) Light micrograph; (b) Scanning electron micrograph of *Saccharomyces cerevisiae*

I.2.9.1.1. Role of yeasts in the agri-food and biotechnological industries

- Yeasts are widely used in brewing for beer production, in winemaking for wine production, and in cheesemaking for the ripening of certain cheeses. Their role in fermentation contributes to improving the texture, flavor, and preservation of foods.
- They also play a role in the valorization of agricultural and industrial waste into useful resources. For example, some yeasts can convert agricultural by-products into biofuels, thereby contributing to environmental sustainability and reducing the ecological impact of human activities.
- Yeasts are used to produce a wide range of compounds, including proteins, enzymes, lipids, and vitamins. These products have applications in human and animal nutrition as well as in various industrial processes.
- Yeasts are extensively exploited in biomedical research and biotechnology due to their eukaryotic nature. Unlike bacteria, they are capable of synthesizing glycosylated proteins, a key feature for the production of certain pharmaceuticals and vaccines.

- Genetically engineered yeasts are used to produce the hepatitis B virus surface antigen, an essential component in vaccine production.
- Yeasts are also used to produce human serum albumin, hormones, growth factors, and other therapeutic proteins, thereby playing a crucial role in the treatment of various diseases.

I.2.9.2. Molds

Molds are filamentous **(figure 37, 38, 39)** heterotrophic, and non-motile microorganisms with a typical eukaryotic cell structure. Some live in symbiosis with plants, others are parasites of plants or animals, while others are saprophytes, growing on inert or decaying substrates. Their vegetative structure consists of a filamentous thallus called the mycelium, composed of a network of filaments known as hyphae. The mycelium can form various specialized structures depending on the fungal group, involved in reproduction and dispersal through spore formation.

Molds are generally aerobic, acidophilic (pH range 3–7), and mesophilic (optimal temperature 20–30 °C). Some species are psychrophilic and can grow at low temperatures (T < 0 °C), such as *Cladosporium herbarum* and *Thamnidium elegans*. They typically have low water requirements compared to other microorganisms (aw ≈ 0.65).

Molds often possess significant lytic activities (cellulolytic, pectinolytic, amylolytic, proteolytic, lipolytic, etc.), making them powerful agents of degradation, but also useful in certain applications, such as cheese ripening and enzyme production.

Molds are generally saprophytic, feeding on inert or decomposing organic matter. Thanks to their sophisticated enzymatic systems, they can degrade food, leading to sometimes irreversible spoilage. However, their biochemical activities also enable them to act as agents of biodegradation, biosynthesis, or bioconversion, transforming low-nutrient raw materials into nutrient-rich foods with desirable flavors or into valuable condiments.

I.2.9.2.1. Role in food

The mold *Penicillium camemberti* is used in the production of soft cheeses with a bloomy rind, such as Camembert and Brie. Its crucial role during ripening consists in consuming lactic acid, thereby deacidifying the curd. In addition, it releases enzymes that influence the development of cheese flavors and aromas.

The green mold *Penicillium roqueforti* has long been used in the production of Roquefort and other blue-veined cheeses.

Molds are also used in the production of food additives. For example, *Aspergillus niger* **(figure 37a,c)** is employed for citric acid production. They are also involved in the production of enzymes (α-amylase, catalase, cellulase, lactase, pectinase, etc.), pigments, vitamins (β-carotene, riboflavin), as well as flavors and fragrances.

However, filamentous fungi can have detrimental effects in the food industry. Certain phytopathogenic fungi, such as *Penicillium expansum*, infect fruits and vegetables, causing blue mold rot in apples. Citrus molds are generally caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). Saprophytic molds, although not necessarily pathogenic, contaminate food and deteriorate its quality.

Some molds produce mycotoxins, representing a major health risk:

- *Aspergillus flavus* produces aflatoxins, including aflatoxin B1, a potent carcinogen.
- *Aspergillus clavatus* produces patulin, a toxin causing pulmonary and cerebral hemorrhages.
- *Fusarium sporotrichioides* and related species produce various toxins such as trichothecenes, zearalenone, and fumonisins. Some of these toxins are highly thermostable, withstanding 200 °C for 30 minutes.

I.2.9.3. Enumeration of yeasts and molds

a. Yeast Extract Glucose Agar supplemented with oxytetracycline (Oxytetracycline-Glucose Agar) is recommended for the analysis of yeasts and molds in milk, dairy products, and foods.

b. Sabouraud agar is a general-purpose medium that supports the growth and isolation of a wide variety of yeasts and molds. The addition of chloramphenicol inhibits the growth of Gram-positive and Gram-negative bacteria.

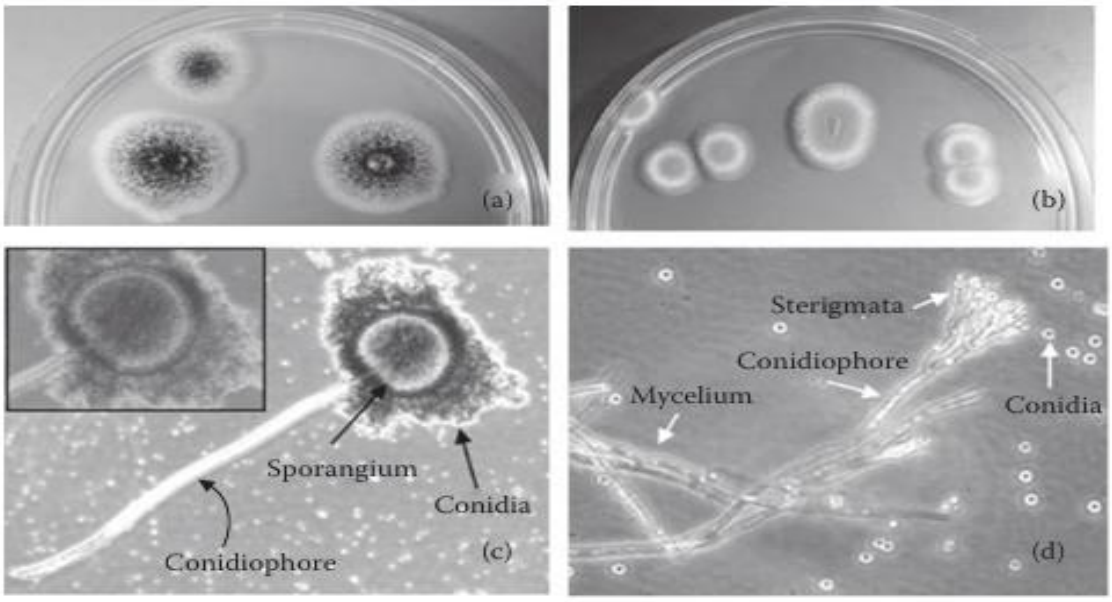


Figure 37 : Photographs of molds. Panel (a) and (b) showing growth of *Aspergillus niger* and *Penicillium citrinum* on agar plates. Panel (c) and (d) are light microscopic photograph of *Aspergillus niger* (c) and *Penicillium citrinum* (d) (magnification 400×)

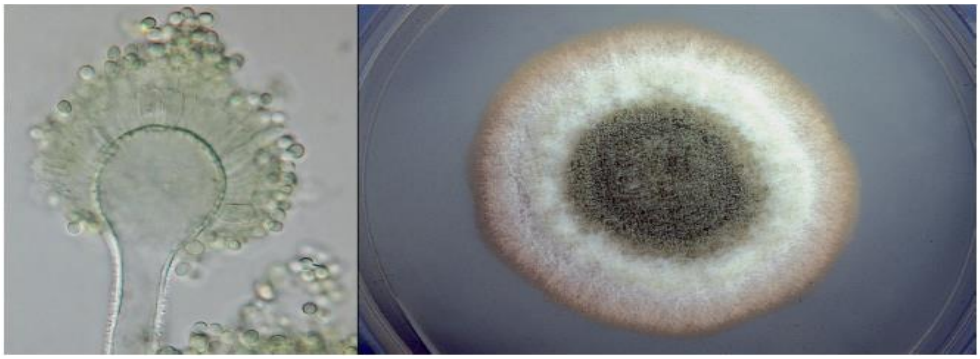


Figure 38 : *Aspergillus* fungi. (Left) Conidiophores of *A. flavus* are shown attached to phialides over most of the vesicle. Courtesy of MedMyco, under license CC BY-SA 4.0. (Right) Colony of *A. flavus* on potato dextrose agar showing typical yellow-green to olive surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores.

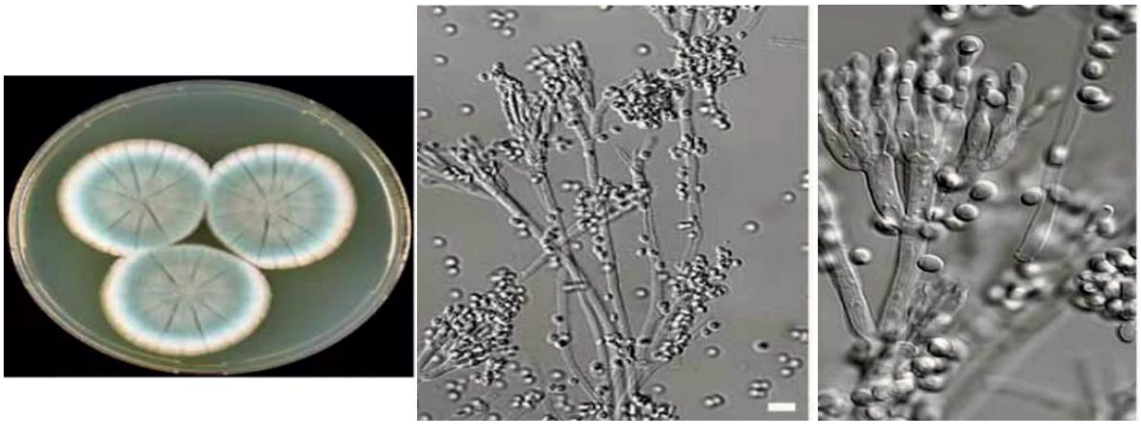


Figure 39 : *Penicillium* fungi. (Left) Growth of *Penicillium chrysogenum* colony on Czapek yeast extract agar. (Center) *P. chrysogenum* conidiophores. (Right) Close-up of *P. chrysogenum* conidia. Scalebars, 10 μm.

I.2.10. Foodborne parasitic

I.2.10.1. Major Foodborne Parasitic Diseases

Parasites **(figure 40)** are organisms that derive their nutrition and protection from other living organisms, known as hosts. When present in food, they can cause diseases in humans. Some parasites are transmitted directly or indirectly between animals and humans, particularly through the consumption of contaminated food or water. Diagnosis generally relies on parasitological examination of stool samples. Other parasites may be transmitted to humans or other animals through various routes, including via vectors.

The health effects of foodborne parasitic infections vary considerably depending on the type of parasite. Clinical manifestations may range from mild discomfort to severe disorders, primarily affecting the digestive system **(figure 40)** but potentially involving other organs as well.

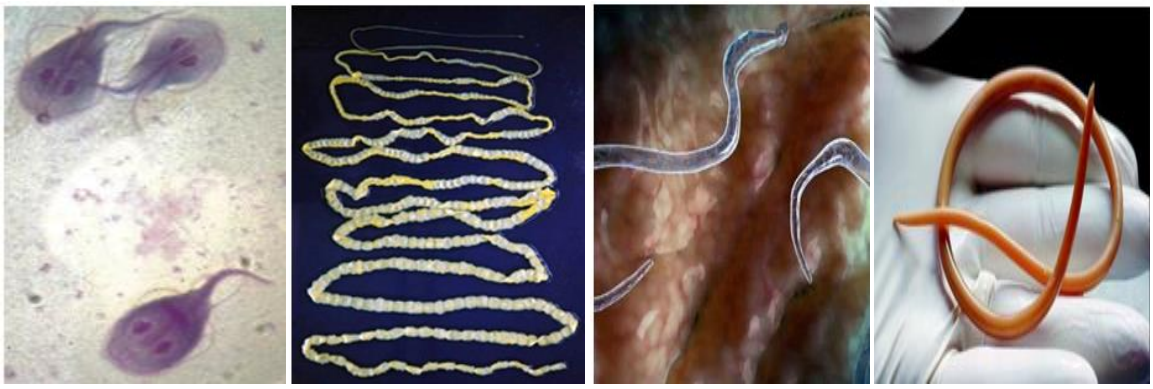


Figure 40: *Giardia intestinalis, Taenia saginata, Enterobius vermicularis, Ascaris lumbricoides*

I.2.10.2. Understanding the Life Cycle of Foodborne Parasites

Understanding the life cycle of foodborne parasites is essential for implementing effective prevention and control strategies. Each stage of the parasite life cycle represents a potential point of intervention. Most foodborne parasites have complex life cycles involving multiple stages and often more than one host. In general, these cycles include the following stages:

- **Egg/Larval stage:** The parasite produces eggs, often excreted in the feces of an infected host. In the case of helminths, these eggs can contaminate food or water. For some protozoa, eggs or cysts are part of the organism's survival strategy and can withstand unfavorable conditions.
- **Intermediate host stage:** Eggs or larvae usually need to develop within an intermediate host before becoming infectious to humans. This host is often a specific

animal. For example, the beef tapeworm, *Taenia saginata*, requires cattle as an intermediate host.

- **Human host stage:** Humans become infected through the consumption of contaminated food. Parasites establish themselves in the human body, typically in the intestines, but sometimes in other organs. At this stage, they mature, reproduce, and complete their life cycle.

I.2.10.3. Global impact of foodborne parasites

The presence of parasites in food is a global issue requiring international attention and cooperation. Understanding their life cycles helps develop prevention strategies, but controlling them requires a coordinated effort from all stakeholders.

- Foods may be contaminated with parasites from various sources, including soil, water, and the parasites themselves, through cross-contamination during food handling or the consumption of raw or undercooked foods.
- Prevention of foodborne parasites relies on the use of effective cooking methods to eliminate them, adherence to safe food-handling practices, and hygienic storage methods to avoid contamination.
- Some foods possess antiparasitic properties that may help eliminate parasites in the human body. Several foods, such as garlic, probiotic-rich foods, coconut oil, and spices like turmeric and clove, contain chemical compounds that may contribute to killing parasites.

I.2.10.4. Top ten most harmful foodborne parasites worldwide

1. *Taenia solium*: pork
2. *Echinococcus granulosus* (hydatid worm): fresh produce
3. *Echinococcus multilocularis* (tapeworm species): fresh produce
4. *Toxoplasma gondii* (protozoan): small ruminant meat, pork, beef, game
5. *Cryptosporidium* spp. (protozoa): fresh produce, fruit juices, milk
6. *Entamoeba histolytica* (protozoan): fresh produce
7. *Trichinella spiralis*: pork
8. Opisthorchiidae (family of flatworms): freshwater fish
9. *Ascaris* spp.: fresh produce
10. *Trypanosoma cruzi* (protozoan): fruit juices

These parasites pose a significant threat to global public health, highlighting the importance of strict food hygiene practices and effective sanitary controls.

I.2.11. Foodborne viruses

I.2.11.1. Viruses in food and the agri-food industry

Viruses are among the major agents responsible for foodborne diseases. They are transmitted to humans through food following direct or indirect contamination (poor hygiene) by fecal matter or other contaminated fluids.

I.2.11.2. Viral infections in the small intestine

Viral infections primarily occur in the small intestine. The infectious dose required for most viruses is relatively low, typically ranging from 100 to 1,000 viral particles.
These viruses, present as inert particles in food, measure between 25 and 100 nanometers, making their detection difficult. As obligate intracellular parasites, they can replicate only within living cells. They can be inactivated by various physical or chemical treatments, but thermal methods, such as pasteurization, are the simplest and most effective to apply.

I.2.11.3. Transmission and sources of contamination

The main route of transmission of foodborne viruses is the fecal–oral route. Foods are contaminated by human fecal matter containing viruses, often due to poor hygiene or the use of contaminated water **(figure 41)**.

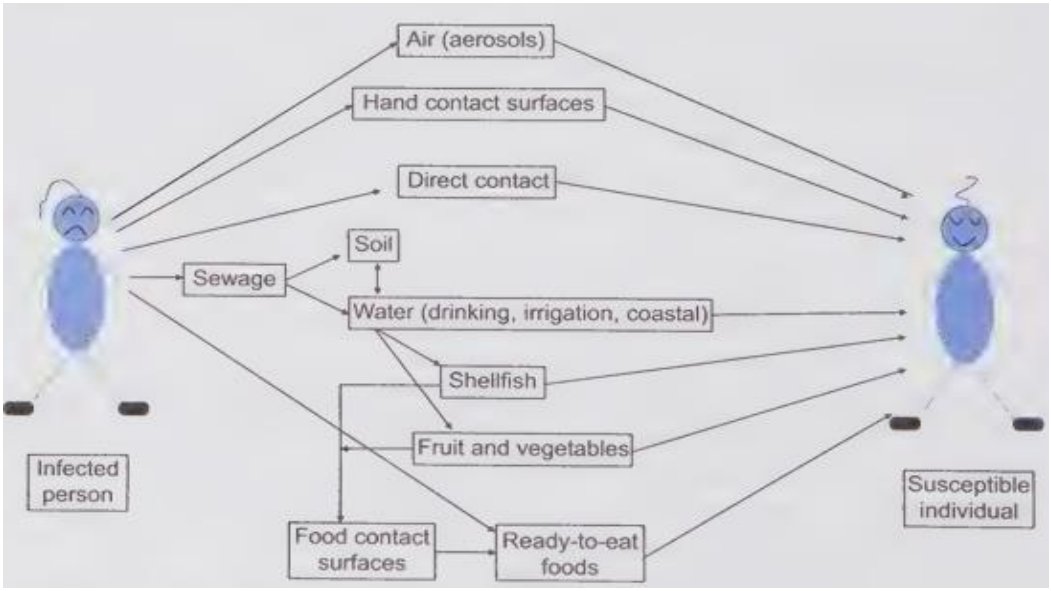


Figure 41 : Transmission of enteric viruses.

The foods most frequently contaminated include shellfish harvested from polluted waters, seafood, raw vegetables (salads, fresh herbs), and drinking water. Infected food handlers who do not follow hygiene rules can also introduce viruses into food **(figure 42)**.

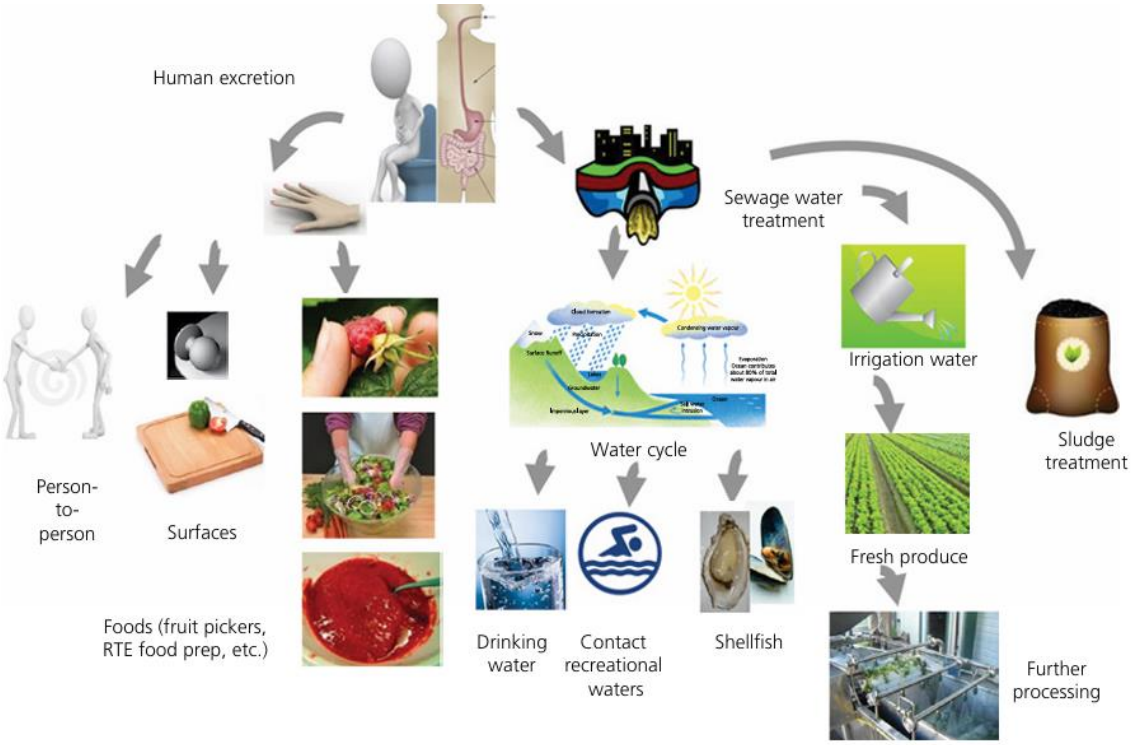


Figure 42 : Transmission routes of food-borne viruses.

The viruses most commonly associated with foodborne infections are hepatitis A virus (HAV) (approximately 10% of cases are linked to the consumption of contaminated food) and noroviruses (formerly known as Norwalk viruses). Other viruses, such as rotaviruses and certain adenoviruses, may also cause gastroenteritis.

I.2.11.4. Characteristics of Foodborne Viruses

Viruses do not grow in food, but they can survive and remain infectious. They can be inactivated by sufficient thermal treatments such as pasteurization, as well as by chemical agents such as sodium hypochlorite and other oxidants. Ultraviolet (UV) radiation is also effective in inactivating viruses.

I.2.11.5. Impact on Health and Industry

a. Associated diseases: Foodborne viral infections can cause serious illnesses such as hepatitis A and gastroenteritis. Some infections may be particularly dangerous for immunocompromised individuals.

b. Neglect in the industry: Although the agri-food industry focuses on controlling bacteria and molds, viruses are often overlooked due to the complexity of their detection and the perception that standard thermal treatments are sufficient to inactivate them.

Prevention of foodborne viral infections relies on improved hygiene practices, monitoring of water and food sources, and proper training of food handlers. Thermal treatments remain one of the most effective methods for virus inactivation in foods.

Foodborne viruses represent a major challenge for food safety. Their detection and control require increased attention from health authorities and the agri-food industry. By improving hygiene practices and adopting effective preventive measures, the risk of viral contamination can be reduced and public health protected.

I.2/ Lactic Acid Bacteria (*Lactococci, Lactobacilli, Leuconostoc, Bifidobacteria*): Beneficial and detrimental effects of lactic acid bacteria; lactic starter cultures: pure, mixed, and natural; use of lactic acid bacteria in milk processing (yogurt and cheese production).

I.2.1. Lactic Acid Bacteria (LAB)

LAB comprise a heterogeneous group of species whose common characteristic is the production of lactic acid following carbohydrate fermentation. They are ubiquitous microorganisms found in various ecological niches and colonize numerous food products such as dairy products, meat, vegetables, and cereals. They also constitute part of the intestinal and vaginal microbiota of humans and animals. LAB are involved in a large number of spontaneous food fermentations and are generally considered non-pathogenic, being classified as GRAS organisms (*Generally Recognized As Safe*). However, some members of the genera *Streptococcus* and *Enterococcus*, as well as certain other lactic acid bacteria, are regarded as opportunistic pathogens.

LAB are Gram-positive chemo-organotrophic bacteria, among which the most extensively studied genera are *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Enterococcus*, and *Pediococcus*. These bacteria may occur as rods, cocci, or coccobacilli, and are generally non-motile and non-spore-forming **(figure 43)**.

They exhibit facultative anaerobic or microaerophilic metabolism and are capable of growing at temperatures ranging from 10°C to 45°C and at pH values between 4.0 and 4.5. Their DNA G+C content is generally below 50%. They do not possess nitrate reductase, oxidase, or catalase enzymes, although some species may acquire pseudocatalase activity. For growth, they require organic carbon sources (fermentable carbohydrates), and many LAB display complex nutritional requirements.

Their growth requires rich and complex culture media containing yeast extract or meat extract and animal peptones, due to their high polyauxotrophic requirements for various amino acids, peptides, nucleotides, vitamins, and fatty acids, resulting from their limited biosynthetic capacity.

MRS medium (*de Man, Rogosa and Sharpe agar*) is commonly used for the cultivation of LAB. It is a highly enriched medium containing a mixture of various carbon sources (acetate, citrate, carbohydrates) and complex nitrogen sources.

Inoculation is performed by pour plating with a double-layer technique. Incubation is carried out for 2 days at 37°C under an atmosphere enriched with 5% CO_2.

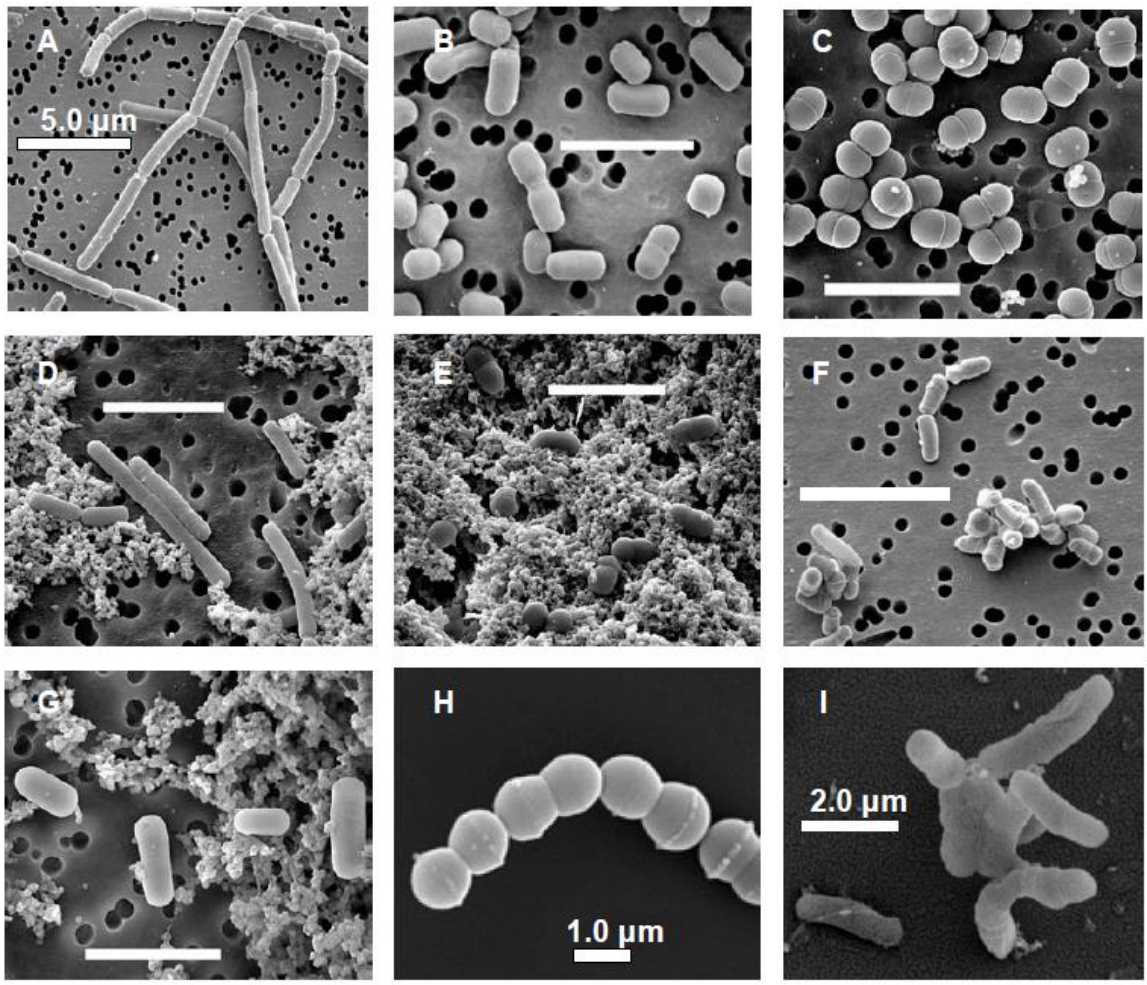


Figure 43 : Electron micrographs of lactic acid and related bacteria: A, *Lactobacillus delbrueckkii* subsp. *bulgaricus*; B, *Lactobacillus brevis*; C, *Pediococcus pentosaceus*; D, *Lactobacillus casei*; E, *Lactococcus lactis*; F, *Brevibacterium linens*; G, *Lactobacillus helveticus*; H, *Streptococcus thermophilus*; and I, *Bifidobacterium longum.* Scale bars are 3.0 µm, unless otherwise indicated. Not shown: *Lactobacillus gasseri* and *Oenococcus oeni.*

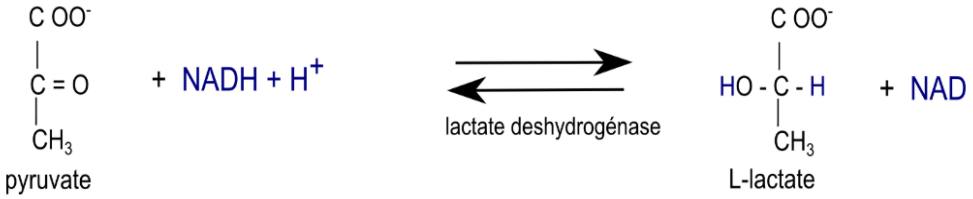
I.2.1.1. Metabolic pathways of LAB

LAB produce lactic acid as the principal end product of fermentative metabolism. According to the preferential fermentation pathway utilized, lactic acid bacteria are classified as follows:

I.2.1.1.1. Strictly homofermentative pathway

In the homofermentative (or homolactic) pathway, lactate is the major final product. This pathway proceeds through glycolysis, also known as the Embden–Meyerhof–Parnas (EMP) pathway. During this process, glucose is converted into two molecules of pyruvate, accompanied by the phosphorylation of two molecules of ADP into ATP. Pyruvate is subsequently reduced to lactate by lactate dehydrogenase, allowing the reoxidation of cofactors. The overall reaction is as follows:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{Pi} \longrightarrow 2\ \text{lactate} + 2\ \text{ATP}$$



Strictly homofermentative bacteria possess fructose-1,6-bisphosphate aldolase, but lack glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which are key enzymes of the pentose phosphate pathway **(Figure 44)**.

The genera *Lactobacillus, Lactococcus, Enterococcus, Streptococcus,* and *Pediococcus* include several homofermentative lactic acid bacteria. However, depending on the species, *Lactobacillus* may be either homofermentative or heterofermentative. For example, *Lactobacillus delbrueckii, Lactobacillus acidophilus,* and *Lactobacillus helveticus* are homofermentative species.

I.2.1.1.2. Strictly heterofermentative (heterolactic) pathway

In this pathway, in addition to lactic acid, carbon dioxide (CO_2), ethanol, and/or acetate may be produced. Glucose is metabolized mainly through the pentose phosphate pathway **(figure)**. The simplified overall fermentation balance is as follows:

$$\text{Glucose} + \text{ADP} + \text{Pi} \rightarrow \text{lactate} + \text{ethanol and/or acetate} + CO_2 + \text{ATP}$$

Strictly heterofermentative bacteria possess the enzymes of the pentose phosphate pathway, particularly the dehydrogenases, but lack fructose-1,6-bisphosphate aldolase **(Figure 45).**

These bacteria include the genera *Leuconostoc*, *Weissella*, and *Oenococcus*, as well as certain *Lactobacillus* species such as *Lactobacillus brevis* and *Lactobacillus hilgardii*.

I.2.1.1.3. Facultatively heterofermentative pathway

These bacteria possess the key enzymes of both metabolic pathways (homofermentative and heterofermentative pathways) and can therefore adapt their metabolism according to environmental conditions. A representative example is *Lactobacillus casei* **(figure 44, 45).**

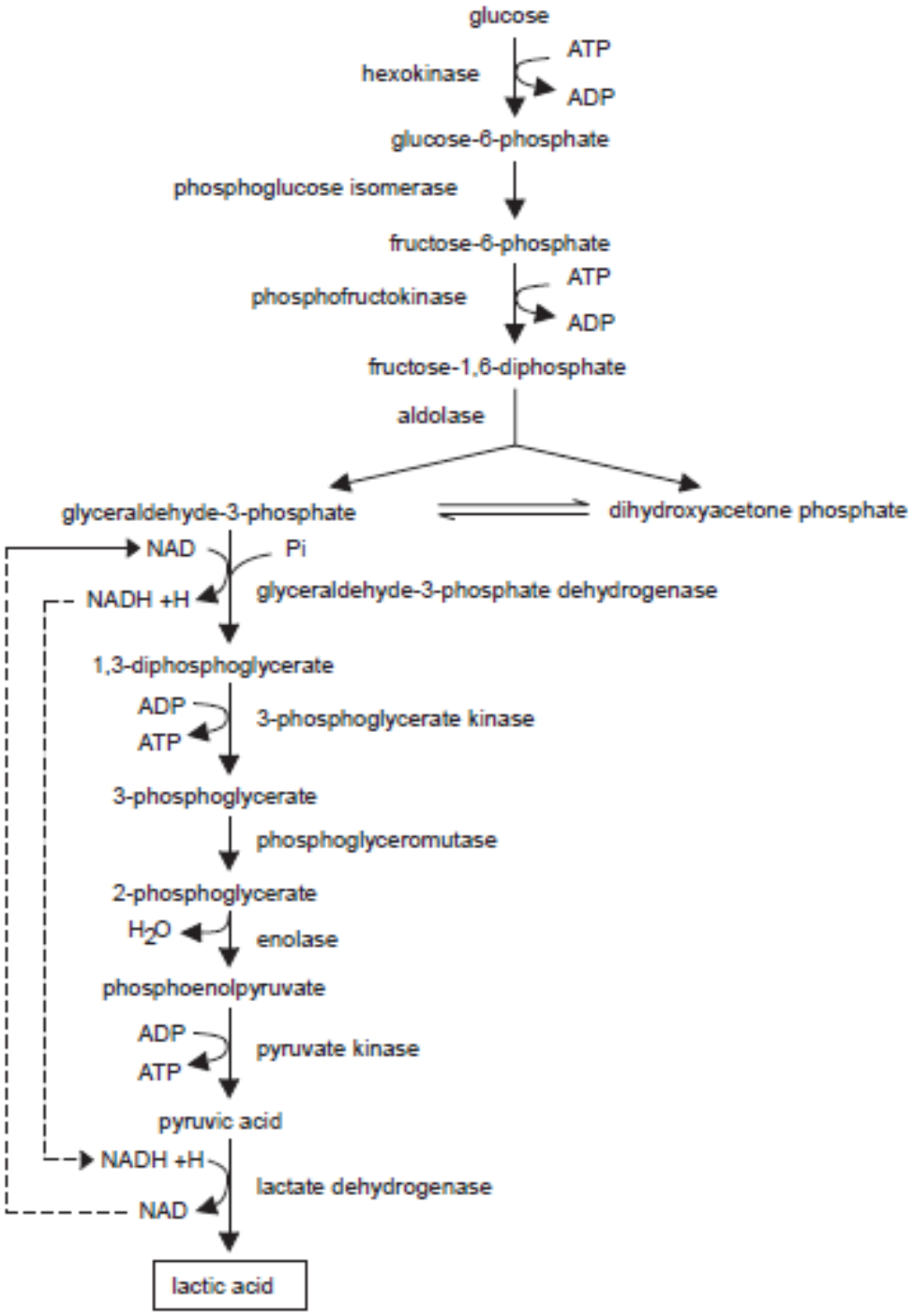


Figure 44 : The Embden-Meyerhoff pathway used by homofermentative lactic acid bacteria. The dashed line indicated the NAD/NADH oxidation-reduction part of the pathway.

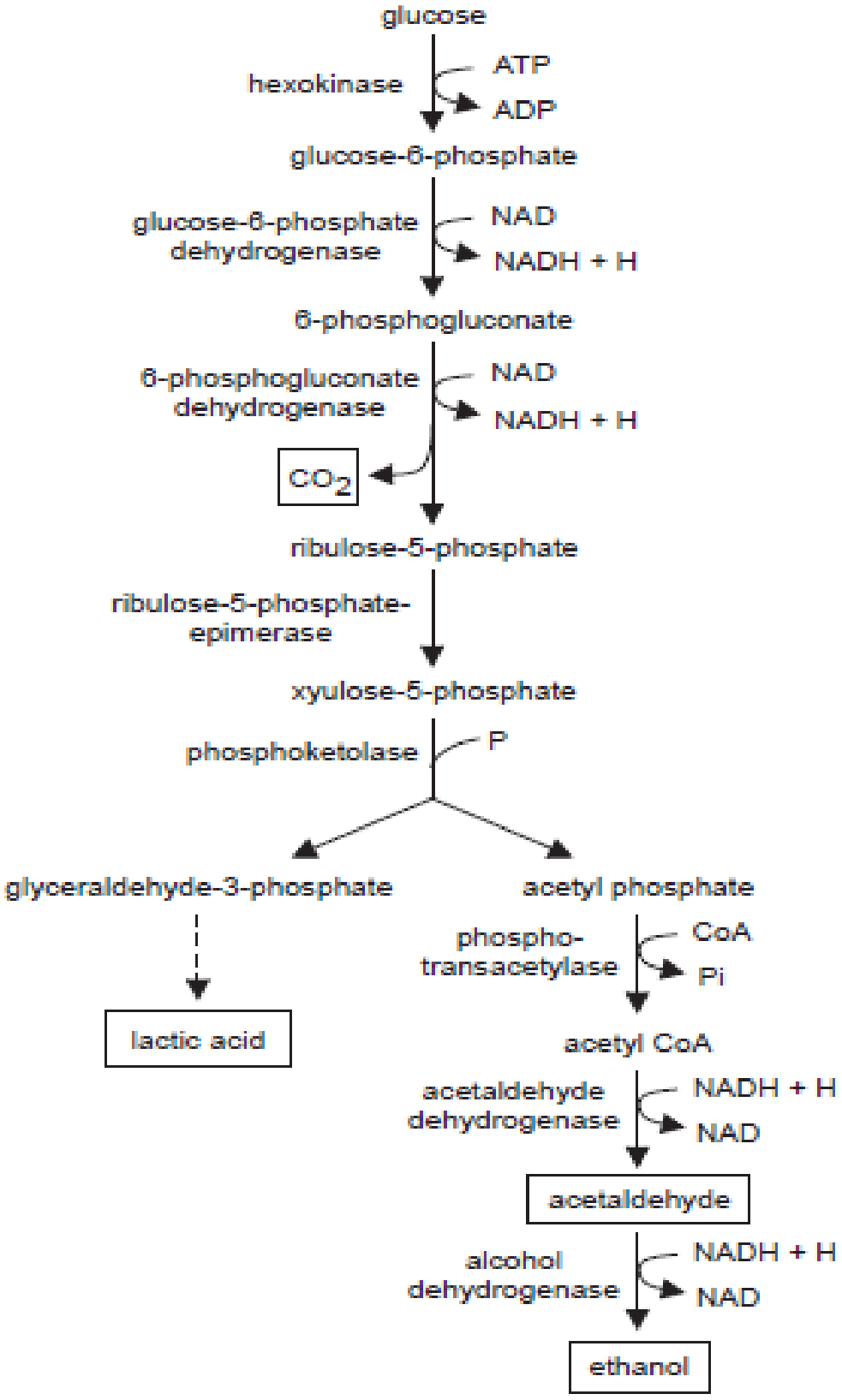


Figure 45 : The phosphoketolase pathway used by heterofermentative lactic acid bacteria.

Figure 46 illustrates the central role of pyruvate as a key metabolic intermediate in lactic acid bacteria and other fermentative microorganisms. Pyruvate serves as a metabolic branching point from which several fermentation pathways originate, depending on the species, enzymatic equipment, and environmental conditions. It may be reduced to lactate by lactate dehydrogenase**,** representing the major pathway in homofermentative lactic acid bacteria. Alternatively, pyruvate can be converted into α-acetolactate by α-acetolactate synthase**,** leading to the production of acetoin and subsequently 2,3-butanediol**,** compounds that contribute to the characteristic aroma and flavor of fermented dairy products. Through the action of pyruvate-formate lyase, pyruvate may also be metabolized into formate**,** acetate**,** acetaldehyde**,** and ethanol**,** whereas the pyruvate dehydrogenase pathway generates acetate**,** acetaldehyde**,** ethanol, and carbon dioxide. The distribution of carbon flux among these pathways determines the final composition of fermentation products and has a major influence on food acidification, preservation, texture, and sensory quality.

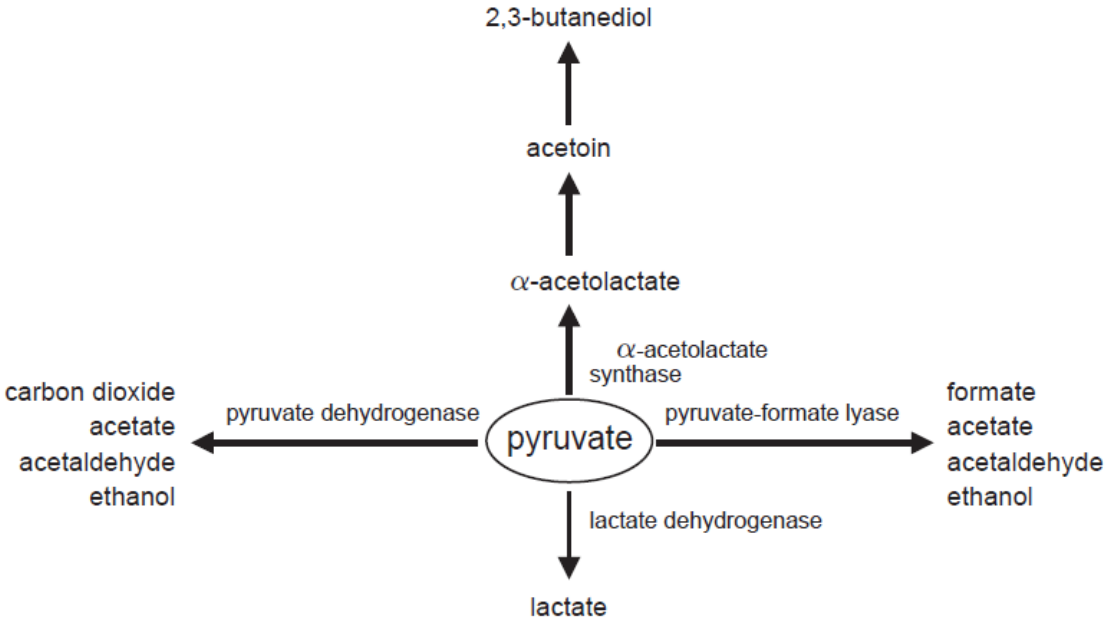


Figure 46 : Heterolactic end products from pyruvate metabolism.

I.2.1.2. Taxonomy and Classification of Lactic Acid Bacteria

In 1981, Tamime proposed a comprehensive classification of lactic acid bacteria (LAB), although their first classification had been established in 1919 by Orla-Jensen based on morphological and physiological criteria. This classification remains a major historical reference and strongly influenced LAB systematics, although it has been regularly revised and expanded.

The classical approach to bacterial taxonomy is based on morphological and physiological characteristics, including the mode of glucose fermentation, the stereochemical configuration of the lactic acid produced, the optimum growth temperature, the growth pH range, salt tolerance, and resistance to acidic or alkaline environments.
Additional phenotypic data were later incorporated, such as fatty acid composition, motility, and cell wall or membrane antigens, which were used for the characterization of new genera of lactic acid bacteria.

However, molecular approaches have profoundly transformed bacterial taxonomy. Criteria such as DNA G+C content, DNA–DNA hybridization, and ribosomal RNA sequence analysis (16S rRNA) have become essential classification tools **(figure 47)**. These methods have led to major revisions in the systematics of lactic acid bacteria, with a significant increase in the number of described species over recent decades.

These reorganizations resulted either in the merging of certain species or in the creation of new genera, such as *Tetragenococcus halophilus* and *Vagococcus.*
Lactic acid bacteria include several bacterial genera, classically distributed into more than a dozen genera, including *Lactobacillus, Streptococcus, Lactococcus, Leuconostoc, Pediococcus, Oenococcus, Enterococcus, Tetragenococcus, Carnobacterium, Aerococcus, Vagococcus, Weissella,* as well as *Bifidobacterium.*

The principal genera used in dairy technology are notably *Lactococcus, Streptococcus, Lactobacillus, Leuconostoc, Pediococcus, Oenococcus,* and *Enterococcus.* These bacteria are involved in the fermentation of dairy products and mainly produce lactic acid from carbohydrates, although some genera may also generate secondary compounds (CO_2, acetate, ethanol) depending on their metabolic type.

The genus *Bifidobacterium* is often associated with lactic acid bacteria because of functional and technological similarities, particularly in probiotic products. However, it possesses a specific metabolic pathway (the bifid pathway) and a high G+C content (approximately 55–67%) **(figure 48)**. It belongs to the phylum Actinobacteria, class Actinobacteria, and order Bifidobacteriales, which clearly distinguishes it from classical lactic acid bacteria of the order Lactobacillales.

Axelsson (2004) conducted studies to highlight the phylogenetic relationships among the different genera of lactic acid bacteria and proposed a phylogenetic organization of these groups, as shown in the figure.

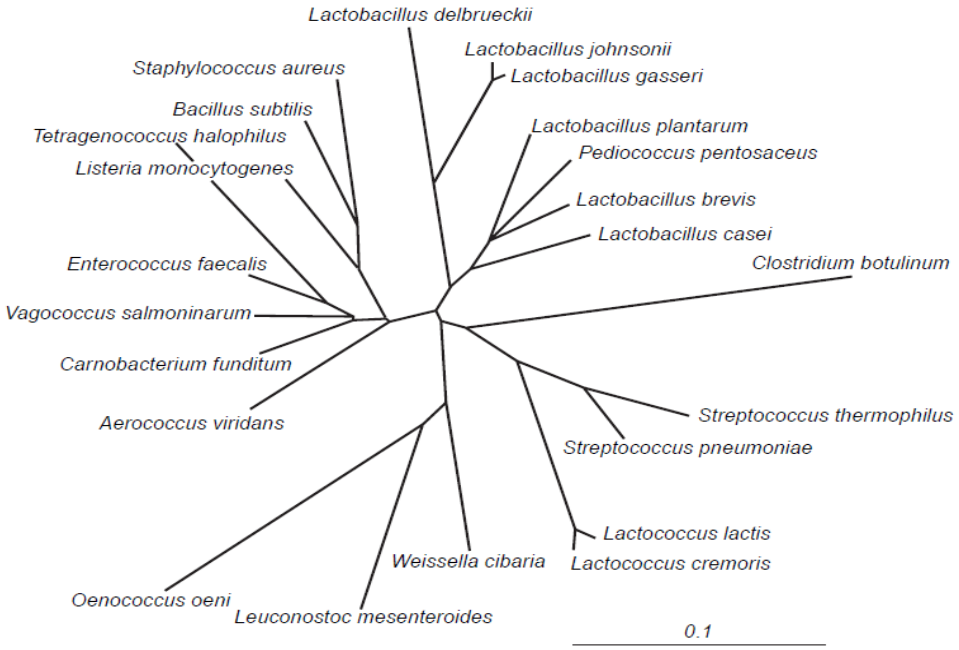


Figure 47 : Phylogeny of lactic acid and other Gram positive bacteria (based on 16s rRNA). The tree (un-rooted) was generated using the neighbor-joining method.

With the development of phylogenetic analysis in the 1980s, several modifications were introduced to their classification. Today, 16S rRNA sequencing has become the reference method, not only because of its high accuracy, but also due to the availability of an extensive database of reference sequences.

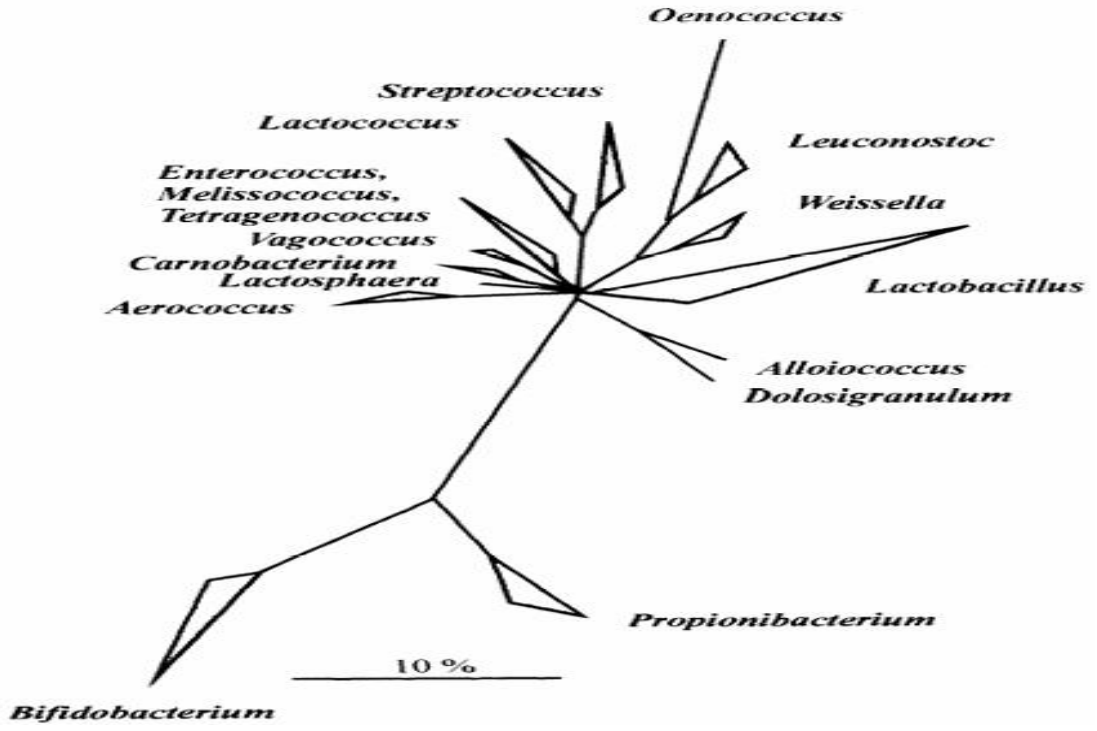


Figure 48 : Major phylogenetic groups of lactic acid bacteria and related taxa, classified according to their DNA G+C content (high or low)

I.2.1.2.1. *Lactobacillus*

The genus *Lactobacillus* is the principal genus of the family *Lactobacillaceae* **(figure 49).** It represents an important group of lactic acid bacteria both at the industrial level and within the infant commensal microbiota. The heterogeneity of the species is reflected by the G+C content, which may vary from 32 to 53%. The revised classification proposed by Kandler and Weiss (1986) subdivided this genus into three groups according to their fermentative type, as indicated in the **table 1.**

The genus *Lactobacillus* (Lb.) includes Gram-positive, rod-shaped bacteria that are facultatively anaerobic or microaerophilic, non-spore-forming, and non-motile. It is one of the largest bacterial genera, comprising more than 180 species. These bacteria convert lactose and other sugars mainly into lactic acid, with smaller amounts of acetic acid, ethanol, and acetaldehyde.

Table 1: Classification of *Lactobacillus* based on glucose fermentation.

Characteristic	Group 1 Thermobacterium	Group 2 Streptobacterium	Group 3 Betabacterium
Growth at 15°C	−	+	+
Growth at 45°C	+	−	±
Pentose fermentation	−	+	+
CO_2 from glucose	−	−	+
CO_2 from gluconate	−	+	+
Phosphoketolase	Absent	Inducible by pentose	Present
FDP aldolase	Present	Present	Absent
Examples	*Lb. Helveticus* *Lb. acidophilus*	*Lb. Plantarum* *Lb. casei*	*Lb. Brevis* *Lb. fermentum*

Legend: + = growth; − = no growth; ± = variable growth.

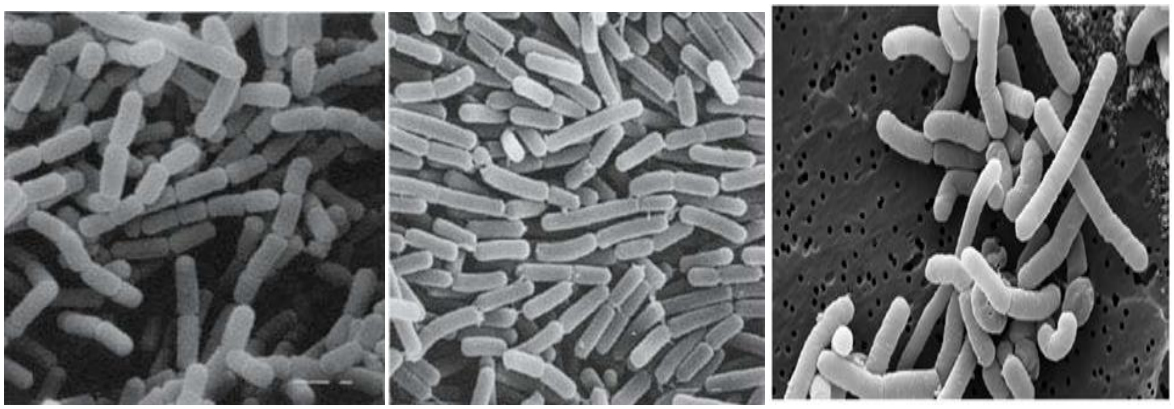


Figure 49: Morphology of A) *Lactobacillus casei* and B) *Lactobacillus acidophilus* C) *Lactobacillus paracasei* (electron microscopy, ×7000)

I.2.1.2.2. *Carnobacterium*

Carnobacterium are heterofermentative bacilli commonly found in vacuum-packed beef, fish, and poultry stored at low temperatures. Morphologically similar to Lactobacillus (small rods occurring singly, in pairs, or in short chains), they are distinguished by the production of L(+)-lactic acid and their inability to grow on acetate-based substrates. In general, Carnobacterium species can grow at relatively high pH values (pH 9), whereas lactobacilli cannot. Growth is possible at 0°C and 10°C but not at 45°C, nor in the presence of 8% NaCl. Their G+C content ranges from 33 to 37%.

This genus includes four species frequently associated with foods: Carnobacterium divergens, Carnobacterium piscicola, Carnobacterium mobile, and Carnobacterium gallinarum. They are isolated from meat products and seafood such as smoked salmon, although some strains have also been isolated from cheese.

I.2.1.2.3. *Enterococcus, Lactococcus, Streptococcus* and *Vagococcus*

The genera *Enterococcus, Lactococcus, Streptococcus* and *Vagococcus* were previously included within a single genus: Streptococcus. They are cocci occurring in pairs or chains, and their metabolism is homofermentative, producing mainly L-lactic acid. These species are primarily differentiated by the presence of a group-specific antigen known as the Lancefield antigen, designated by letters from A to V, with each group associated with a specific antigen type.

I.2.1.2.3.1. The genus *Streptococcus*

The genus Streptococcus **(figure 50)** includes gram-positive homofermentative cocci that usually form pairs or chains. In 1937, Sherman separated the genus into four general groups according to physiological and growth characteristics, especially with regards to temperature limitations on growth. This categorization has become somewhat obsolete as relation ships between species have been shown to overlap.

The only species used as starter culture is Str. thermophilus, a homo fermentative facultatively anaerobic strain. It is called yogurt culture, which is thermophilic in nature with an optimum growth temperature of 38–42°C. Some strains of *Str. thermophiles* were explored to produce folic acid at significantly high level. Some species of streptococci are pathogenic such as *Str. agalactiae* and *Str. dysgalactiae* are most common isolates in clinical mastitis, an udder disease of milch animals.

The Lancefield classification differentiates streptococci based on cell wall carbohydrate antigens and physiological characteristics, including growth at 10°C and 45°C. Pyogenic streptococci (groups A, B, C, F, H, and I) are pathogenic and do not grow at either temperature. *Streptococcus thermophilus* is thermophilic and grows at 45°C, whereas group D enterococci grow at both temperatures. Group N lactic cocci (***Lactococcus*** spp.) are mesophilic, growing at 10°C but not at 45°C, and are widely used as starter cultures in dairy fermentation **(table 2).**

Table 2 : Groups of genus *Streptococcus* and their major characteristics

Lancefield Group	Group	Example(s)	Growth at 10°C	Growth at 45°C
A, B, C, F, H, I	Pyogenic	*Streptococcus agalactiae, Str. pyogenes*	No growth	No growth
–	Viridians	*Str. thermophilus*	No growth	Growth
D	Enterococcus	*E. faecium, E. faecalis, E. durans*	Growth	Growth
N	Lactic cocci	*S. lactis, S. cremoris*	Growth	No growth

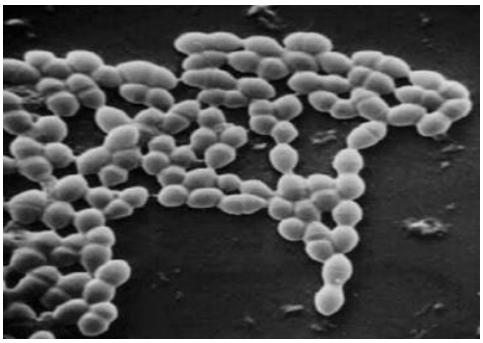


Figure 50: Electron microscopy morphology of *Streptococcus thermophilus*

I.2.1.2.3.2. The genus *Lactococcus*

Lactococcus belongs to Lancefield group N and represents the so-called « lactic streptococci. » Based on molecular criteria **(figure 51),** Schleifer *et al.* (1985) proposed separating mesophilic lactic streptococci from the genus *Streptococcus* and establishing the genus *Lactococcus.*

Lactococci occur as cocci in pairs or in chains of variable length. They are facultative anaerobic, homofermentative bacteria producing only lactic acid; only *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* **(figure 52)** is capable of producing diacetyl. Their optimal growth temperature is close to 30°C; they can grow at 10°C but not at 45°C.

Lactococci are associated with numerous food fermentations and do not exhibit any pathogenic traits. However, only *Lactococcus lactis* is currently used in dairy technology. The genus *Lactococcus* comprises five species; the most well-known is *Lactococcus lactis*, which includes three subspecies: *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Lactococcus lactis* subsp. *hordniae*. Only the first two subspecies are involved in most dairy products.

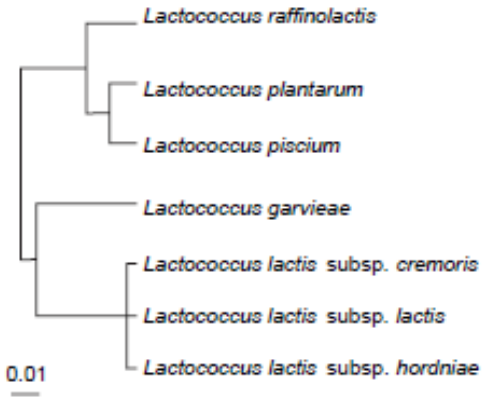


Figure 51 : Phylogeny of *Lactococcus* based on 16S rRNA sequence analysis.

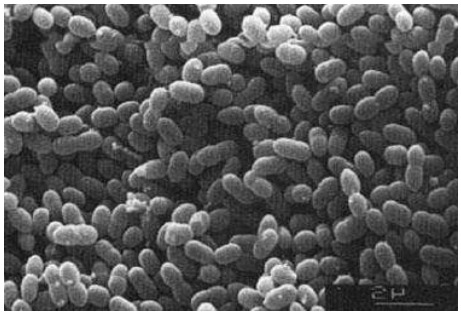


Figure 52 : Electron microscopy morphology of *Lactococcus lactis* subsp. *diacetylactis***.**

I.2.1.2.3.3. The genus *Enterococcus*

Enterococcus comprises most species of Lancefield group D (fecal streptococci). They exhibit γ- or α-hemolysis and are characterized by their ability to grow at 10°C and 45°C, their capacity to grow in the presence of 6.5% NaCl, and their high resistance to environmental stresses.

The species encountered in foods are mainly *Enterococcus faecalis* (formerly *Streptococcus faecalis*) **(figure 53)** and its variants (*Enterococcus durans* and *Enterococcus bovis*). Their habitats are highly diverse, including the intestinal tract of humans and animals, plant products, soil, and dairy products. Enterococci play an important role in cheese ripening, with *Enterococcus faecium* frequently found in raw-milk cheeses.

The species *Enterococcus italicus*, described a few years ago, has been identified as being involved in the production of certain traditional Italian cheeses. Interestingly, unlike other species of the genus Enterococcus, its ecological niche appears to be mainly, or even exclusively, associated with fermented dairy products.

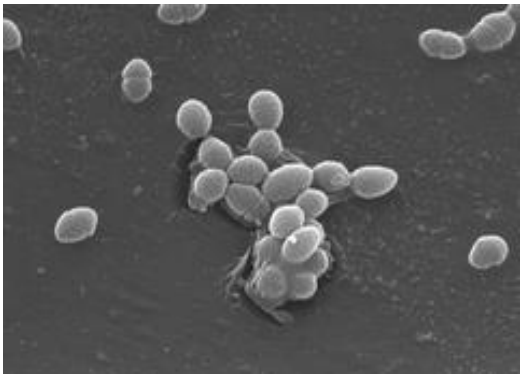


Figure 53: Scanning electron microscopy morphology of *Enterococcus faecalis*

I.2.1.2.3.4. The genus *Vagococcus*

Species of the new genus Vagococcus are easily confused morphologically with lactococci; however, the two genera are clearly distinguished by differences in membrane fatty acid composition.

I.2.1.2.4. *Leuconostoc, Oenococcus* and *Weissella*

These genera comprise lenticular cocci occurring in pairs or chains. They are mesophilic and exhibit a strongly heterofermentative metabolism, with production of D(-)-lactic acid **(table 3).**

The genus *Leuconostoc* **(figure 54)** has an optimal growth temperature between 25°C and 30°C, and a relatively homogeneous G+C content (approximately 37–45%). Their growth is generally slow. They are mostly non-hemolytic and non-pathogenic.

Leuconostocs play an important role in the manufacture of various cheeses, where they contribute to eye formation in the curd through CO_2 production. They are also involved in dairy industries (butter and cream), particularly *Leuconostoc mesenteroides* subsp. *cremoris,* as well as in silage production and vegetable fermentations (olives).

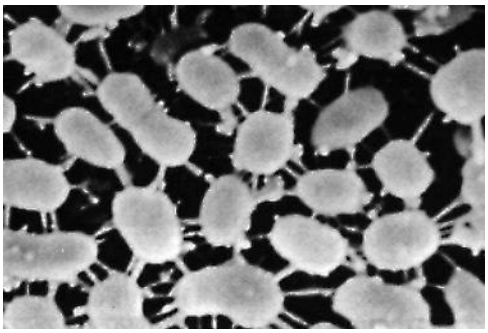


Figure 54: Attachment of *Leuconostoc* cells on glazed stoneware molds (scanning electron microscopy, ×11,500).

Table 3 : Characteristics of *Leuconostoc* and *Oenococcus*

Property	*Leuconostoc mesenteroides* subsp. *cremoris*	*Leuconostoc mesenteroides* subsp. *mesenteroides*	*Leuconostoc mesenteroides* subsp. *dextranicum*	*Leuconostoc lactis*	*Oenococcus oeni*
% G+C	38–44	28–32	28–32	37–42	37–42
Growth at 37°C	−	±	+	+	±
Growth at pH 4.8	−	−	−	−	+
Dextran from sucrose	−	+	+	−	−
Growth in 10% ethanol	−	−	−	+	+
Acid production from:					
Arabinose	−	+	−	−	±
Fructose	−	+	+	+	+
Maltose	±	+	+	+	−
Melibiose	±	+	+	±	±
Salicin	−	±	±	±	±
Sucrose	−	+	+	+	−
Trehalose	−	+	+	−	+

Legend: + = positive reaction (growth or acid production); − = negative reaction; ± = variable reaction among strains.

I.2.1.2.5. *Pediococcus, Aerococcus* and *Tetragenococcus*

These are cocci occurring as cells arranged in pairs or tetrads. They are mesophilic, homofermentative, and most often unable to utilize lactose.

The genus *Tetragenococcus* contains only three species, *Tetragenococcus halophilus, Tetragenococcus muriaticus*, and *Tetragenococcus solitarius*. Based on 16S rRNA sequences, these bacteria are phylogenetically more closely related to *Lactobacillus* and *Enterococcus*, than to *Pediococcus* **(figure 55).** The genus was first recognized in 1990, when *Pediococcus halophilus* was re-classified as *T. halophilus*. A second species, *T. muriaticus*, isolated from salty fish sauce, was proposed in 1997, and in 2005, *Enterococcus solitarius* was reclassified as *T. solitarius*.

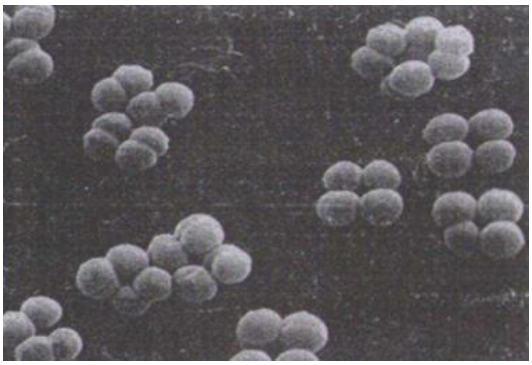


Figure 55: Electron microscopy morphology of *Pediococcus sp.*

I.2.1.2.6. *Bifidobacterium*

The genus *Bifidobacterium* (formerly known as *Lactobacillus bifidus*) consists mainly of rod-shaped cells; however, under unfavorable conditions, bifidobacteria may exhibit branching and pleomorphism, forming bifid (Y- or V-shaped) structures **(figure 56)**. They are distinguished from other lactic acid bacteria by their strict anaerobic nature, high G+C content (55–67%), and the presence of fructose-6-phosphate phosphoketolase (F6PPK), a key enzyme in their metabolic pathway **(figure 57)**, which serves as a taxonomic marker for the family *Bifidobacteriaceae.*

Bifidobacteria are Gram-positive, non-motile, non-spore-forming, and non-capsulated bacteria. They ferment hexoses mainly into acetic acid rather than lactic acid (acetate:lactate ratio of approximately 3:2), along with small amounts of ethanol and other organic acids. This fermentative metabolism has led to their association with lactic acid bacteria. Their optimal growth temperature ranges between 37°C and 41°C.

They are commonly isolated from the intestinal microbiota of newborns. *Bifidobacteria* are non-gas-producing anaerobic microorganisms; some strains may tolerate oxygen. They are typically found in the oral cavity, intestine, vagina, and gastrointestinal tract of mammals, including humans and various animals, as well as in food products and wastewater.

Bacteria of the genus Bifidobacterium are widely used as probiotics in fermented dairy products and functional food formulations. Although they are not the primary starter cultures in traditional yogurt, their incorporation into these food matrices is associated with beneficial effects on gut health, particularly through stimulation of their growth via the bifidogenic effect induced by certain non-digestible food substrates.

To date, more than 50 species of the genus *Bifidobacterium* have been described, including several medically and food-relevant species such as *Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium adolescentis, Bifidobacterium dentium,* and *Bifidobacterium animalis* subsp. *lactis.*

From a metabolic standpoint, bifidobacteria are chemo-organotrophic organisms with strictly fermentative metabolism. They exhibit a strong capacity for carbohydrate catabolism and can utilize various sugar sources. All studied species are capable of fermenting glucose, fructose, and galactose via a specific pathway known as the bifid shunt (fructose-6-phosphate phosphoketolase pathway).

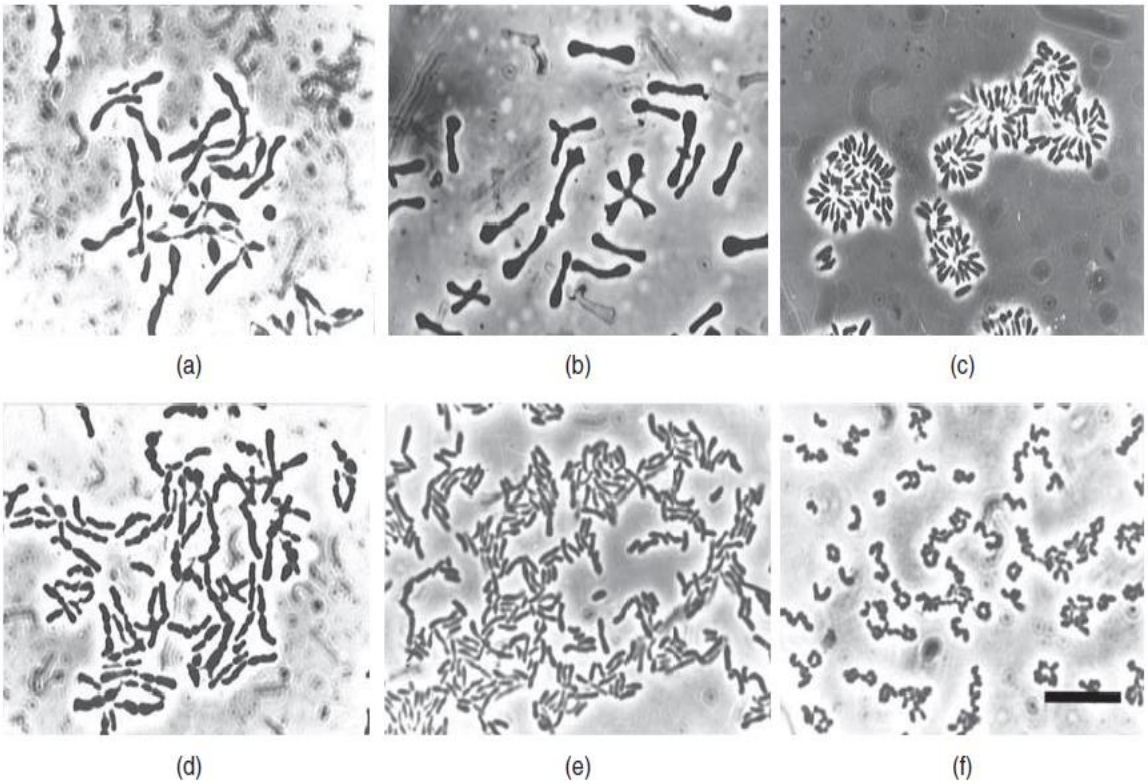


Figure 56: Cellular morphology of (a) *Bifidobacterium bifidum,* (b) *Bif. animalis* subsp. lactis, (c) *Bif. asteroides*, (d) *Bif. catenulatum,* (d) *Parascardovia denticolens* and (f) *Scardovia inopinata* . Phase-contrast photomicrographs. Bar = 10 μm

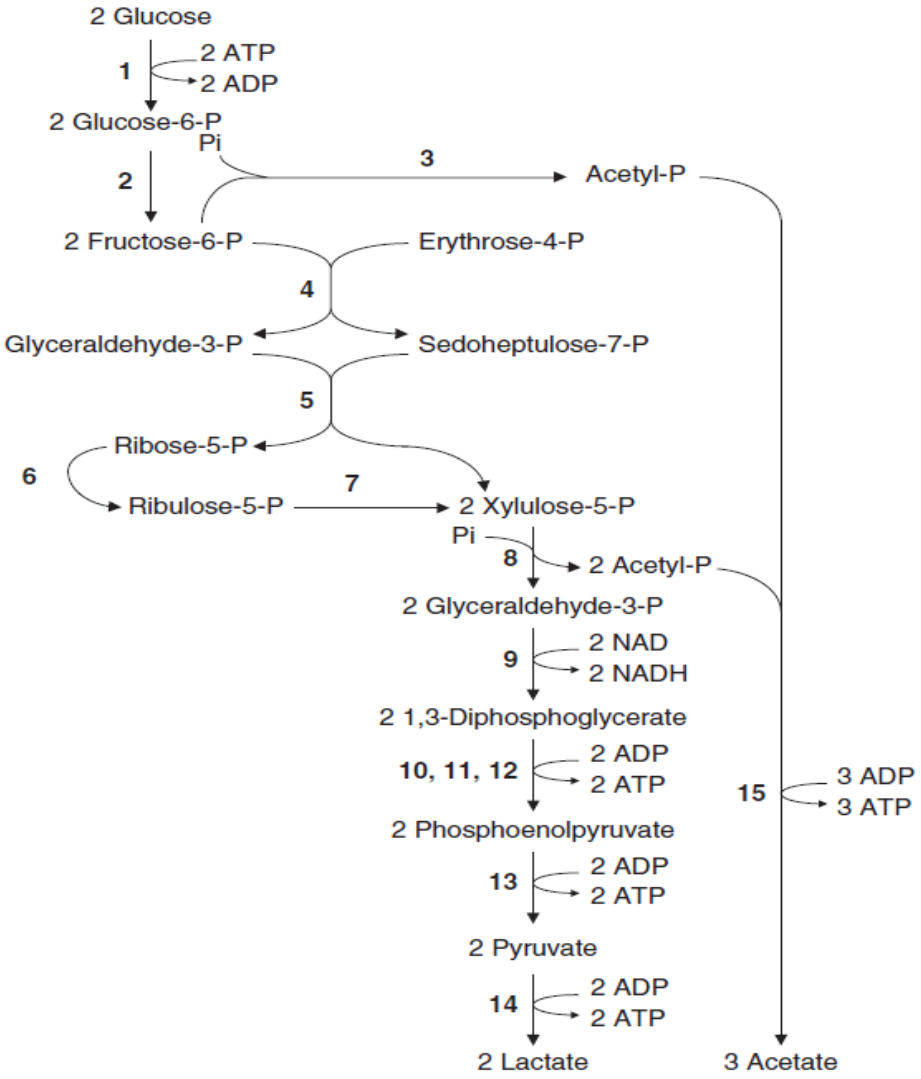


Figure 57: Fructose-6-phosphate shunt ('bifid pathway').
Bold numbers represent enzymes as follows: **1**, hexokinase ; **2**, glucose-6-phosphate isomerase ; **3**, fructose-6-phosphate phosphoketolase ; **4**, transaldolase ; **5**, transketolase ; **6**, ribose-5-phosphate isomerase ; **7**, ribulose-5-phosphate epimerase ; **8**, xylulose-5-phosphate phosphoketolase ; **9**, glyceraldehyde-3-P dehydrogenase ; **10**, phosphoglycerate kinase ; **11**, phosphoglyceromutase ; **12**, enolase ; **13**, pyruvate kinase ; **14**, lactate dehydrogenase ; **15**, acetate kinase.

I.2.2. Use of lactic acid bacteria in milk processing (yogurt and cheese)

Lactic acid bacteria constitute one of the most widely used groups of microorganisms in the production of various fermented foods.

I.2.2.1. Indigenous or native flora

Milk contains few microorganisms when obtained under aseptic conditions from a healthy animal; it should contain fewer than 5,000 microorganisms/mL and less than 1 coliform/mL. The indigenous flora of milk is defined as the community of saprophytic microorganisms originating from the udder and the galactophorous ducts **(table 4)**.

Table 4: Indigenous flora of raw milk

Microorganisms	Percentage (%)
Micrococcus	30–90
Lactobacillus	10–30
Streptococcus* or *Lactococcus	< 10
Gram-negative bacteria	< 10

The microbial flora of raw milk plays an important role in determining the organoleptic characteristics of cheeses, independently of the addition of starter cultures. Dairy product manufacturing relies heavily on microorganisms, particularly lactic acid bacteria.

I.2.2.2. Fermented Dairy Products

A wide range of fermented dairy products is marketed worldwide. Numerous types of fermented milks exist across different countries, varying in raw material, microbial flora, processing technology, texture, flavour, and shelf life.

I.2.2.2.1. Fermented milk

Fermented milks are dairy products obtained through an essentially lactic fermentation process, which leads to milk acidification and gel formation **(figure 58, 59)**. Traditional Algerian fermented milks include *L'ben* and *Raïb*.

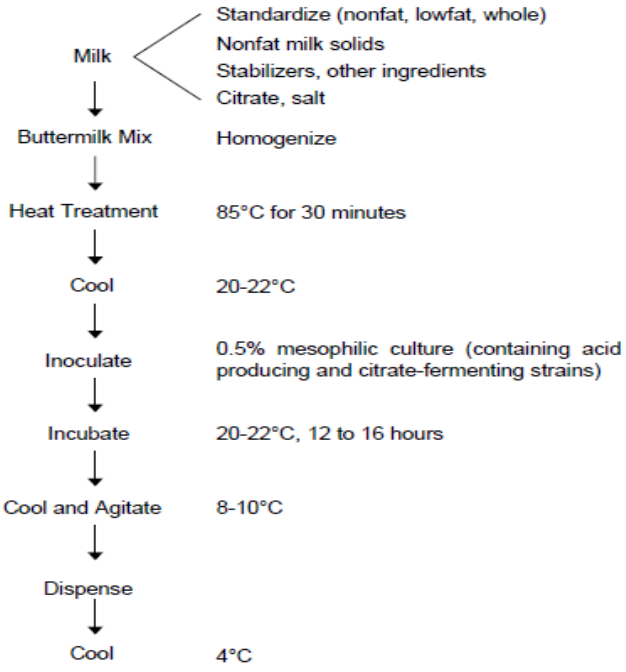


Figure 58 : Manufacture of cultured buttermilk.

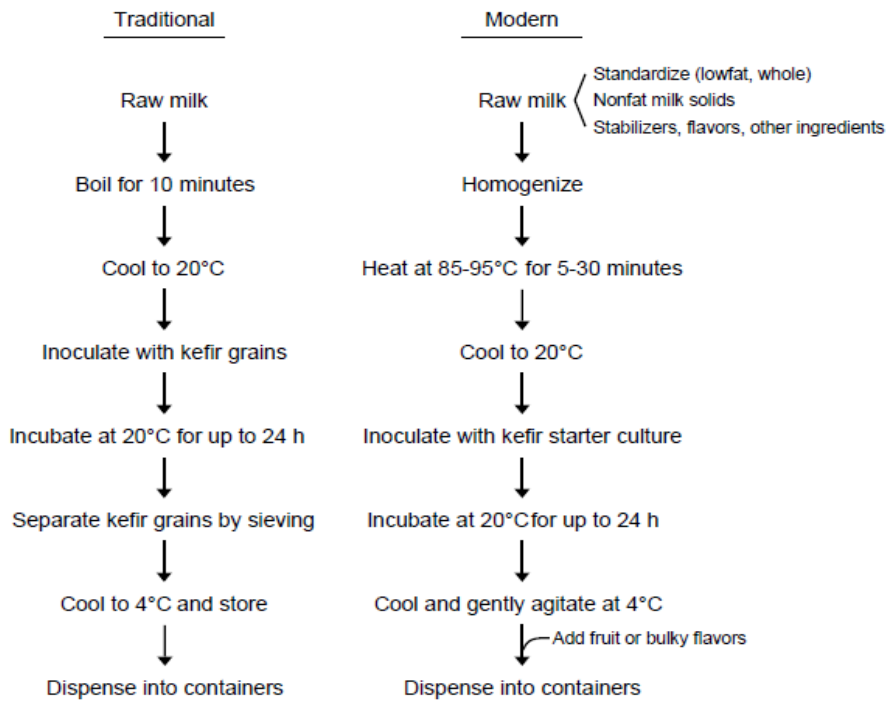


Figure 59 : Traditional and modern manufacturing process for kefir.

I.2.2.2.2. Yogurt

Yogurt is a fermented milk obtained exclusively by milk coagulation through the action of two bacteria: Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. These bacteria must remain viable in the final product, and their population must exceed ten million per gram of yogurt at the end of its shelf life.

a. Use of lactic acid bacteria in yogurt manufacturing

The main concern of industrial producers is to obtain a consistently high-quality product. This objective requires:

- Adjustment of the quality of the milk used;
- Knowledge of the properties of the bacterial cultures employed;
- Control of the different stages of yogurt manufacture.

During yogurt fermentation, the temperature is maintained between 40 and 45°C, which corresponds to the optimum for the mixed culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* **(figure 60)**.

In general, yogurt fermentation is based on a symbiotic association between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus.*

Streptococcus thermophilus initiates fermentation rapidly by hydrolyzing lactose and producing lactic acid, leading to a decrease in pH and milk gelation. It also promotes the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* by providing growth-stimulating compounds.

Lactobacillus delbrueckii subsp. *bulgaricus* develops more slowly but persists longer due to its acid tolerance. It releases peptides and amino acids essential for the growth of Streptococcus thermophilus and contributes to yogurt flavor formation, particularly through acetaldehyde production.

b. Synergistic effect on texture and viscosity

- The use of bacterial strains producing exopolysaccharides (EPS) can significantly influence yogurt texture and viscosity.
- EPS may also improve yogurt stability during storage, reducing syneresis and extending shelf life.

The two main types of yogurt are stirred yogurt and set yogurt **(Figure 60).**

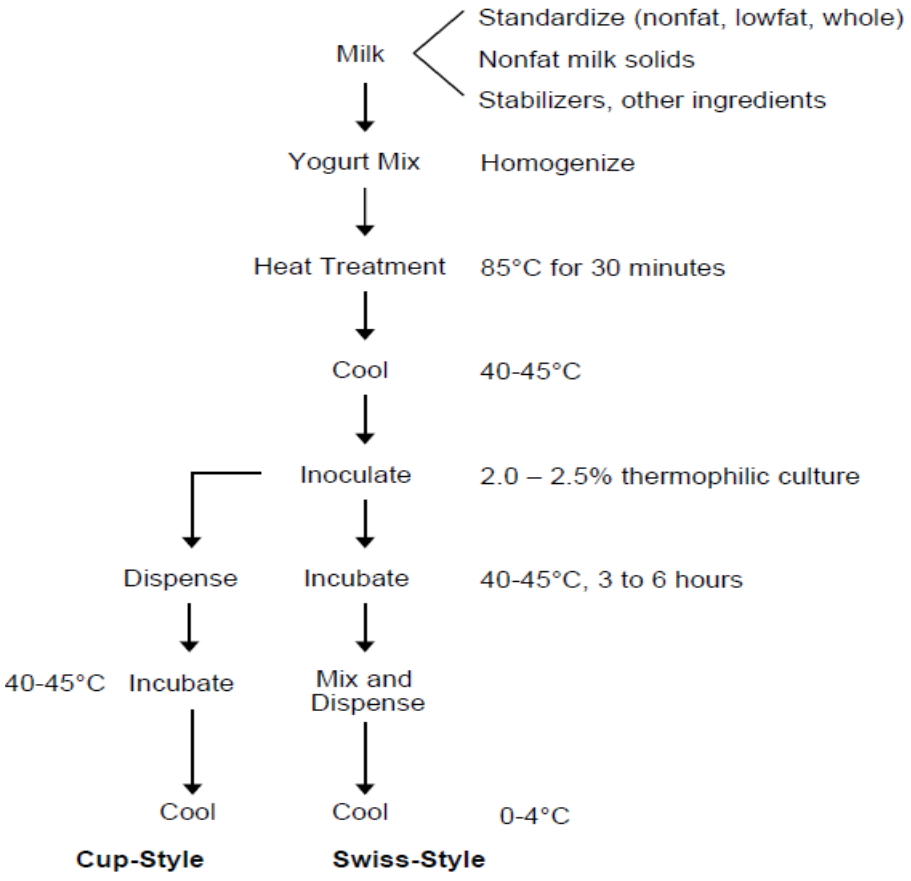


Figure 60 : Manufacture of yogurt.

I.2.2.3. Use of lactic acid bacteria in cheese manufacturing

Their role in cheese production is mainly associated with two aspects of their metabolism: Lactic acid production and Proteolytic activity.

I.2.2.3.1. Milk acidification

Lactic acid bacteria, particularly mesophilic homofermentative species such as Lactococcus lactis, are among the first microorganisms to develop in milk. Their primary function is to initiate acidification from the lactose present in milk. This acidification is essential for several reasons:

- **Coagulation:** Lactic acid induces the coagulation of milk proteins, forming curd. This is essential for separating curd from whey.
- **Food safety:** Acidification inhibits the growth of undesirable and pathogenic bacteria, thereby improving safety and shelf life.
- **Texture:** Acidification influences cheese texture by contributing to firmness and consistency.

I.2.2.3.2. Proteolytic activity

Lactobacilli such as *Lactobacillus helveticus* and *Lactobacillus casei* play an important role in the hydrolysis of curd proteins:

- **Flavor development:** Protein degradation releases amino acids and peptides that serve as flavor precursors, contributing to the complex aromatic profile of cheese.
- **Texture and ripening:** Proteolytic activity influences cheese texture and is essential for cheese ripening, allowing the development of desired flavors and textures over time.

I.2.2.3.3. Cheese

The goal of the cheese industry is to transform milk into a product with extended shelf life and distinct sensory properties through microbial and enzymatic activities. Milk coagulation and curd draining represent a form of concentration that contributes to preservation. This is further enhanced by acidification resulting from lactic fermentation, which inhibits spoilage bacteria.

This dual process of dehydration and acidification is present, to varying degrees, in all cheese-making processes. The transformation of milk into cheese involves four essential stages **(figure 61).**

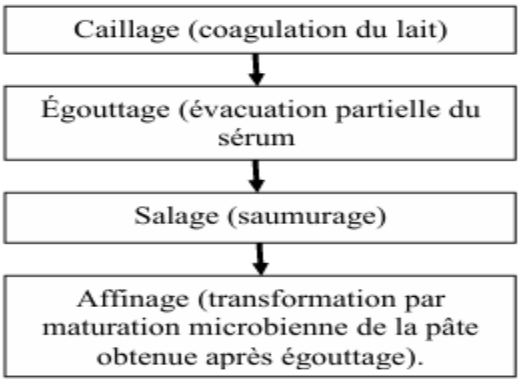


Figure 61 : Essential steps in the transformation of milk into cheese

There are approximately 4,000 varieties of cheese worldwide, all produced through a four-step process following the same general principle. Specific interventions applied at certain stages determine the unique characteristics of each cheese type. The different categories of cheeses are presented in the **table 5** below:

Table 5 : Microorganisms involved in the manufacture of cheeses and fermented milks

Product	Principal acid producer	Intentionally introduced secondary microbiota
Cheeses		
Colby, Cheddar, cottage cheese, cream cheese	*Lactococcus lactis* subsp. *cremoris* or *lactis*	None
Gouda, Edam, Havarti	*Lactococcus lactis* subsp. *cremoris* or *lactis*	*Leuconostoc* spp., *Lactococcus lactis* subsp. *lactis*
Brick, Limburger	*Lactococcus lactis* subsp. *cremoris* or *lactis*	*Geotrichum candidum*, *Brevibacterium linens*, *Micrococcus* spp.
Camembert	*Lactococcus lactis* subsp. *cremoris* or *lactis*	*Penicillium camemberti*, sometimes *Brevibacterium linens*
Blue cheeses	*Lactococcus lactis* subsp. *cremoris* or *lactis*	*Lactococcus lactis* subsp. *lactis*, *Penicillium roqueforti*
Mozzarella, Provolone, Romano, Parmesan	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*	None; animal lipases added to Romano for piquant or rancid flavor
Swiss	*Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*	*Propionibacterium freudenreichii* subsp. *shermanii*
Fermented milks		
Yogurt	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*	None
Buttermilk	*Lactococcus lactis* subsp. *cremoris* or *lactis*	*Leuconostoc* spp., *Lactococcus lactis* subsp. *lactis*
Sour cream	*Lactococcus lactis* subsp. *cremoris* or *lactis*	None

The cheese starter cultures consist mainly of lactococci, leuconostocs, lactobacilli, and streptococci. The starter cultures used in the fermentation of different cheeses are summarized in the table below.

I.2.3. Lactic starter cultures

I.2.3.1. General overview of lactic starter cultures

Lactic starter cultures are added to milk to initiate the fermentation process. They are used in the production of a wide range of dairy products such as cheese, yogurt, fermented milk, butter, and cream **(table 6).**

Table 5 : Starter cultures used in the production of major fermented dairy products

Culture	Culture function	Product use
Propionibacterium freudenreichii	Flavor and eye formation	Emmental (Swiss) cheese
Lactobacillus delbrueckii* subsp. *bulgaricus	Acid and flavor	Bulgarian buttermilk, yogurt, kumiss, and Emmental (Swiss) and Italian cheeses
Lactococcus lactis		
Lactobacillus helveticus		
Lactobacillus brevis		Kefir
Lactobacillus acidophilus	Acid	Acidophilus buttermilk
Streptococcus thermophilus	Acid	Emmental, Cheddar, and Italian cheeses, and yogurt
Lactococcus lactis* subsp. *diacetylactis	Acid and flavor	Sour cream, ripened cream butter, cheese, buttermilk, and starter cultures
Lactococcus lactis* subsp. *lactis	Acid	Cultured buttermilk, sour cream, cottage cheese, all types of cheese (domestic and foreign), and starter cultures
Leuconostoc cremoris	Flavor	Cultured buttermilk, sour cream, cottage cheese, ripened cream butter, and starter cultures
Streptococcus faecium*, *Streptococcus faecalis	Acid and flavor	Soft Italian, Cheddar, and some Swiss cheeses

Since the natural lactic microflora of milk is either inefficient, uncontrollable, unpredictable, or destroyed by the heat treatments applied to milk, the addition of starter cultures after the pasteurization step ensures a more controlled and predictable fermentation process.

A lactic starter culture is a preparation containing a large number of microorganisms (either a single species or multiple species) that is added to milk to produce a fermented food by accelerating and directing the fermentation process **(table 7).**

Table 7 : Lactic acid bacteria used as starter cultures.

Organism	Application
Lactococcus lactis* subsp. *lactis	Cheese, cultured dairy products
Lactococcus lactis* subsp. *cremoris	Cheese, cultured dairy products
Lactococcus lactis* subsp. *lactis* biovar *diacetylactis	Cheese, cultured dairy products
Lactobacillus helveticus	Cheese, cultured dairy products
Lactobacillus delbrueckii* subsp. *bulgaricus	Cheese, yogurt
Lactobacillus sanfranciscensis	Sourdough bread
Lactobacillus casei	Cheese, cultured dairy products
Lactobacillus sakei	Sausage
Lactobacillus plantarum	Sausage, fermented vegetables
Lactobacillus curvatus	Sausage
Streptococcus thermophilus	Cheese, yogurt
Pediococcus acidilactici	Sausage, fermented vegetables
Pediococcus pentosaceus	Sausage
Tetragenococcus halophilus	Soy sauce
Oenococcus oeni	Wine
Leuconostoc lactis	Cheese, cultured dairy products
Leuconostoc mesenteroides* subsp. *cremoris	Cheese, cultured dairy products, fermented vegetables

I.2.3.2. Taxonomy of microorganisms used in the dairy industry

I.2.3.2.1. Yeasts

Yeasts are widely used in the production of dairy products, particularly cheeses (soft cheeses with bloomy rind, washed-rind soft cheeses, blue-veined cheeses, and pressed cheeses), as well as in certain fermented milks (e.g., *Candida kefir*, *Torulopsis kefir*). Yeasts mainly contribute through ethanol production.

I.2.3.2.2. Moulds

Moulds are also used in the production of a wide range of cheeses (bloomy-rind soft cheeses, washed-rind soft cheeses, blue-veined cheeses, and pressed cheeses). The most commonly used species are Penicillium camemberti and Penicillium roqueforti.

I.2.3.2.3. Bacteria

Lactic acid bacteria used in the dairy industry are mainly restricted to five principal genera: *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris; Streptococcus thermophilus; Leuconostoc* species (including *lactis*, *mesenteroides* subsp. *cremoris* and *dextranium*, and *pseudomesenteroides*); *Enterococcus;* and several species of *Lactobacillus*, including *delbrueckii* subsp. *bulgaricus*, *casei*, *brevis*, *helveticus*, *rhamnosus*, *acidophilus*, *fermentum*, *curvatus*, *johnsonii*, and *gasseri*.

In addition to lactic acid bacteria, a wide variety of bacteria, molds, and yeasts are intentionally employed as starter or adjunct cultures in food fermentation. These microorganisms perform specific technological functions, including acidification, flavor and aroma development, pigment production, surface ripening, eye formation, and carbon dioxide generation. Their metabolic activities are essential for improving the safety, preservation, texture, and sensory characteristics of fermented foods. **Table 8** summarizes some of the principal microorganisms used in the food industry and their major applications.

Table 8 : Other organisms used as starter cultures.

Organism	Application
Bacteria	
Brevibacterium linens	Cheese: pigment production, surface ripening
Propionibacterium freudenreichii* subsp. *shermanii	Cheese: eye formation in Swiss cheese
Staphylococcus carnosus* subsp. *carnosus	Meat: acid production, flavor development, color formation
Molds	
Penicillium camemberti	Cheese: white surface ripening
Penicillium roqueforti	Cheese: blue veins, protease and lipase production
Penicillium chrysogenum	Sausage
Aspergillus oryzae	Soy sauce, miso
Rhizopus microsporus* subsp. *oligosporus	Tempeh
Yeasts	
Saccharomyces cerevisiae	Bread: carbon dioxide production
Saccharomyces cerevisiae	Ale beers
Saccharomyces pastorianus	Lager beers
Saccharomyces cerevisiae	Wine

I.2.3.3. Types of starter cultures

Starter cultures can be classified based on their function, growth temperature, or composition. Before the advent of modern biotechnology, traditional (artisanal) starters were widely used. Although they are still in use today, their microbiological instability has led to the development of defined lactic acid bacterial blends in order to achieve more stable acidification activity and consistent quality in final products.

Two main categories of starter cultures are distinguished: traditional artisanal starters used in conventional processes and commercial starters used in modern industrial processes.

I.2.3.3.1. Artisanal starters

All currently available starter cultures are derived from artisanal starters with undefined composition (containing mixtures of different, non-characterized strains and/or species). The production of such cultures, also referred to as “natural starters,” originates

from an ancient practice known as “back-slopping,” which consists of using a portion of a previous fermented batch to inoculate a new batch.

I.2.3.3.2. Commercial starters

Commercial starter cultures are generally marketed in freeze-dried form and can be used for direct inoculation of fermentation tanks.

Depending on their composition, commercial starters are classified into three categories:

- **Pure starters**, consisting of a single well-characterized strain of one species;
- **Mixed starters**, with partially or non-defined composition;
- **Selected mixed starters**, containing multiple well-defined strains from several species.

I.2.4. Beneficial and detrimental effects of lactic acid bacteria

I.2.4.1. Industrial applications

Lactic acid bacteria are widely used in food fermentation, one of the oldest food preservation methods. They are involved in the production of dairy products (yogurt, cheese), meat products, wine, and silage. Their main function is the production of lactic acid, which lowers pH and inhibits undesirable microbial flora **(figure 62)**. Some strains also produce bacteriocins, heat-stable antimicrobial peptides that contribute to food microbiological safety. They also strongly influence organoleptic properties (taste, texture, aroma).

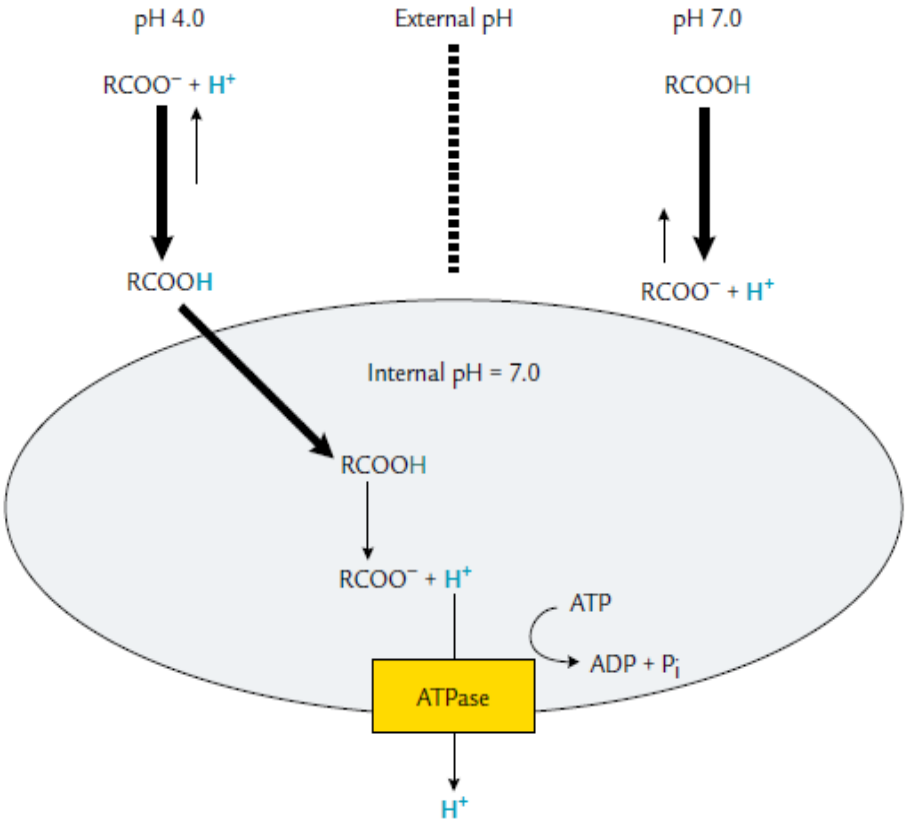


Figure 62 : Organic acids are effective inhibitors only at low pH, where their protonated form can cross the cell membrane. At the higher pH inside the cell, the acid dissociates and the cell must use energy to pump out the proton to deacidify the cytoplasm. At high external pH, the dissociated acid cannot cross the membrane and get into the cell and therefore is not effective.

I.2.4.1.1. Fermentative and preservative role

Lactic acid bacteria convert sugars into lactic acid, enabling:

- Acidification of the medium,
- Inhibition of undesirable microorganisms,
- Stabilization and preservation of foods.

They also enhance food safety through the production of antimicrobial compounds such as bacteriocins.

I.2.4.1.2. Technological role

Lactic acid bacteria play a major role in the quality of fermented foods:

- **Flavor production:** formation of volatile compounds (acetaldehyde, diacetyl, acetoin, ethanol, acetate, etc.) from lactose, citrate, amino acids, and lipids, which are essential for the flavor of fermented dairy products.

- **Exopolysaccharide (EPS) production:** these polymers improve texture, viscosity, and homogeneity of products such as yogurt, resulting in improved stability and sensory quality.

I.2.4.1.3. Probiotic role

Probiotics are live, non-pathogenic microorganisms which, when administered in adequate amounts, confer health benefits to the host. Several lactic acid bacteria have probiotic status, including *Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus casei,* and *Streptococcus thermophilus.*

They are used in human and animal nutrition for their beneficial effects on gut microbiota balance and digestive health **(table 9).**

Table 9 : Examples of human probiotic species and strains with health claims

Bifidobacterium adolescentis BA 02
Lactobacillus delbrueckii subsp. *bulgaricus*
Lactobacillus reuteri
Bifidobacterium breve BR 03
Lactobacillus fermentum
Lactobacillus rhamnosus LR 04
Bifidobacterium longum BL 03
Lactobacillus plantarum LP 01
Lactobacillus salivarius
Lactobacillus acidophilus LA 02
Bifidobacterium breve BR 03 and *Lactobacillus plantarum* LP 01
Streptococcus thermophilus

I.2.4.2. Therapeutic applications of lactic acid bacteria

Certain lactic acid bacteria are used as probiotics due to their beneficial health effects. The most commonly used species include Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus reuteri, and *Lactobacillus delbrueckii* subsp. *bulgaricus.*

They are used for both preventive and therapeutic purposes, particularly in the management of different types of diarrhea (infectious, antibiotic-associated, or nosocomial diarrhea in infants). Some strains also exhibit anti-allergic activity, related to their proteolytic properties.

Other reported benefits include anti-infective effects (against *Clostridium* and *Helicobacter pylori*), immunomodulatory and anti-inflammatory properties, as well as potential anti-carcinogenic and hypocholesterolemic effects, particularly in the prevention of chronic inflammatory bowel diseases **(figure 63).**

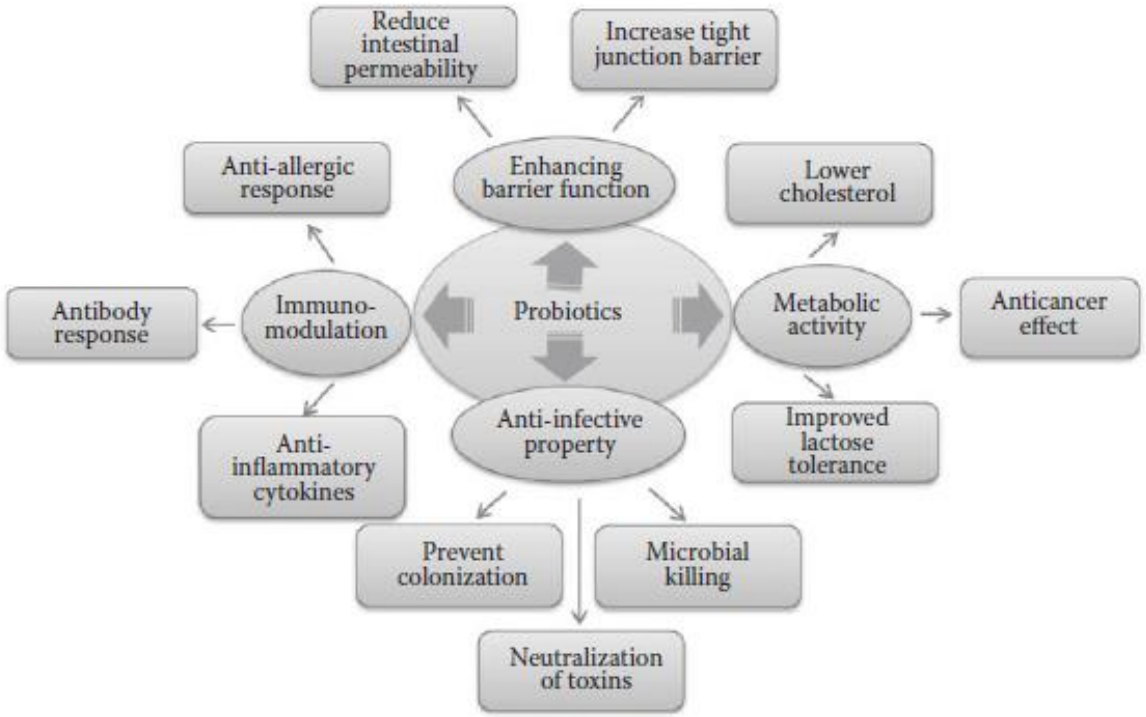


Figure 63 : Diagram summarizing probiotic function.

I.2.4.2.1. Effects on intestinal functions

I.2.4.2.1.1. Intestinal digestion

Yogurt improves lactose digestion in individuals with lactase deficiency (hypolactasia). In cases of lactose maldigestion, unabsorbed lactose is fermented in the colon, producing gases and organic acids responsible for bloating, abdominal pain, and diarrhea.

Studies have shown that yogurt bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) improve lactose tolerance through their lactase activity, promoting lactose hydrolysis in the small intestine.

In addition, certain probiotics contribute to digestion by providing digestive enzymes or stimulating their activity, thereby improving nutrient absorption and reducing digestive disorders associated with enzymatic deficiencies.

I.2.4.2.1.2. Intestinal motility and transit time

Clinical studies in healthy volunteers have shown that daily consumption of yogurt containing *Bifidobacterium animalis* DN-173010 significantly reduces colonic transit time (≈ 20%) compared with yogurt without probiotics. This effect is particularly pronounced in women with initially slower intestinal transit.

These results have been confirmed in older subjects (50–75 years), with a dose-dependent reduction in oro-fecal transit time. The beneficial effect may persist for several weeks after cessation of probiotic consumption.

I.2.4.2.1.3. Strengthening of the epithelial functional barrier

a. Intestinal permeability

Probiotics such as *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* reduce intestinal permeability and strengthen the gut barrier. They stabilize tight junctions between intestinal epithelial cells and protect against pathogenic bacteria such as *Escherichia coli* by maintaining mucosal integrity.

b. Stimulation of defensins

The probiotic strain *Escherichia coli* Nissle 1917 interacts with the intestinal epithelium and modulates the expression of numerous genes. It reduces pro-inflammatory cytokines and stimulates innate immune defenses by increasing the production and activity of defensins, thereby enhancing protection against pathogens.

c. Mucus production

Probiotics such as *Lactobacillus plantarum* and *Lactobacillus rhamnosus* GG stimulate the expression of mucin genes (MUC2 and MUC3) in the colon. This increases intestinal mucus production, reinforcing the protective barrier and preventing adhesion of pathogenic bacteria such as enteroinvasive *Escherichia coli*.

I.2.4.2.2. Barrier effect

The barrier effect is primarily the result of a beneficial modulation of the intestinal microbiota, as well as strengthening of the functional intestinal barrier.

I.2.4.2.2.1. Modulation of the intestinal microbiota

Probiotics are able to exclude pathogenic microorganisms through antagonistic properties, thereby maintaining intestinal balance. These antagonistic mechanisms include:

- **Nutritional and spatial competition:** due to their high nutritional requirements, lactic acid bacteria colonize the environment extensively, limiting the growth of other microorganisms.
- **Production of antimicrobial metabolites:** including organic acids (mainly lactic acid), hydrogen peroxide, carbon dioxide, diacetyl (2,3-butanedione), and bacteriocins.

I.2.4.2.3. Immunomodulation

Probiotic organisms produce several compounds that can influence the host immune system, such as cell wall components, DNA, and various metabolites. Similar to those produced by pathogenic bacteria, these components are recognized by the immune system as potentially harmful, triggering an immune response. However, unlike pathogen-induced responses, probiotics activate T and B lymphocytes without causing inflammation or neutrophil infiltration.

Effects on both innate and adaptive immunity have been widely reported in the literature. More specific studies on antibody production have shown that consumption of *Lactobacillus rhamnosus* GG reduces the duration of rotavirus-induced diarrhea while simultaneously stimulating the production of antibodies against these viruses.

I.2.4.2.4. Carcinogenesis

Colonic microbiota may contribute to carcinogenesis by activating pro-carcinogens into carcinogens through specific bacterial enzymes. Conversely, it may also exert a protective effect by inactivating or inhibiting carcinogenic compounds in the gastrointestinal tract and by reducing the activity of bacterial enzymes such as β-glucuronidase, azoreductase, and nitroreductase.

I.2.4.2.5. Reduction of serum cholesterol and bile salt deconjugation

Some lactic acid bacteria are able to deconjugate bile salts, converting conjugated forms into less inhibitory compounds for intestinal bacteria. This mechanism may contribute to the reduction of serum cholesterol by promoting hepatic utilization for the synthesis of new bile salts. However, excessive deconjugation may be detrimental, leading to impaired

lipid and fat-soluble vitamin absorption, as well as a potential increased risk of colon cancer development.

Although lactic acid bacteria are generally recognized for their beneficial roles, particularly in food fermentation and as probiotics, they may also exhibit undesirable effects under certain conditions. They are also recognized as spoilage agents in a wide range of products.

In meat products, the growth of lactic acid bacteria can lead to the development of sour or rancid odors and flavors, slime formation, and greening. Species such as *Carnobacterium divergens* and *Lactobacillus sakei* have been identified among the spoilage flora of refrigerated beef. Similarly, *Leuconostoc mesenteroides* has been reported as a major spoilage organism in vacuum-packed meat products such as ham.

In wines, phenomena such as “lactic spoilage” (increased acidity at the end of fermentation), increased viscosity (due to exopolysaccharide production), and the formation of off-flavors have been associated with the presence of bacteria from the genera *Oenococcus*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus*.

Chapter II : Microbial Food Spoilage and Control Measures

II.1. Food Spoilage

II.1.1. Natural Contamination

The normal microbial load of most foods is approximately **10^4 CFU/g**.

Microbial death occurs when microorganisms do not encounter the conditions required for growth within the food matrix, including suitable nutrient composition, storage conditions, and the absence of antimicrobial treatments.

Microbial survival occurs under conditions that prevent cell death but do not permit microbial multiplication, such as unfavorable food composition or refrigeration.

Microbial proliferation occurs when microorganisms encounter favorable environmental conditions that support growth. Under these conditions, saprophytic microorganisms cause spoilage and deterioration of the commercial quality of foods, whereas pathogenic microorganisms compromise food safety and may also reduce commercial quality.

II.1.2. Impact on Commercial Quality

Microbial proliferation in foods results in changes to their organoleptic properties, which generally become detectable when microbial populations exceed 10^6 CFU/g**.**

Alterations in appearance (color changes and slime formation), texture, odor, and taste are usually undesirable. Spoilage is commonly associated with microorganisms such as *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Aspergillus*, *Rhizopus*, *Flavobacterium*, and *Clostridium*.

In some cases, however, microbial growth produces desirable changes that are intentionally exploited in the manufacture of fermented foods and beverages, including beer, wine, fermented sausages, butter, cheese, yogurt, and sauerkraut.

II.1.2.1. Interactions Between Microorganisms and Food Components

Microorganisms transform foods by metabolizing their major constituents, thereby affecting texture, flavor, aroma, nutritional value, and safety.

II.1.2.1.1. Carbohydrates

Structural polysaccharides, such as starch and cellulose, are hydrolyzed, resulting in changes in food texture. Simple sugars (glucose and sucrose) undergo microbial

fermentation, producing organic acids, alcohols, and carbonyl compounds that influence flavor and aroma.

II.1.2.1.2. Proteins

Proteins undergo enzymatic hydrolysis, leading to modifications in food texture. The amino acids released during proteolysis may subsequently undergo decarboxylation, deamination, or other catabolic reactions, producing compounds that alter flavor and odor and may generate toxic metabolites, including biogenic amines.

II.1.2.1.3. Lipids

Lipids may undergo oxidation or enzymatic lipolysis, generating compounds responsible for undesirable rancid flavors and odors.

II.1.2.2. Odor Changes

Microbial growth in foods is often first detected through changes in odor because the human olfactory system is highly sensitive to volatile organic compounds, which can be detected at concentrations ranging from **10^{-6} to 10^{-9} g**.

The production of a specific odor cannot generally be attributed to a single microorganism, as odor formation depends on several factors, including food composition, storage temperature, microbial strain, and environmental conditions. Nevertheless, molds frequently produce characteristic musty or rancid odors.

II.1.2.2.1. Meat

Surface microbial growth at **10°C** produces stale or off-odors when microbial populations reach approximately **10^7 CFU/g**. At ambient temperature, the production of ammonia and hydrogen sulfide (H_2S) results in putrefactive odors. In certain products, controlled putrefaction is intentionally induced during meat aging or game hanging.

II.1.2.2.2. Fish

Microbial spoilage of fish produces ammoniacal odors owing to the formation of compounds such as trimethylamine and mercaptans. For example, mackerel initially develops a sour odor associated with lactic acid production, followed by an ammoniacal odor as spoilage progresses.

II.1.2.2.3. Cheese

Clostridium butyricum produces butyric acid, which imparts an unpleasant rancid odor. Advanced proteolysis may also lead to the development of ammoniacal odors.

II.1.2.3. Taste Alterations

Taste alterations result from the accumulation of both volatile and non-volatile microbial metabolites. The most common change is acidification caused by lactic acid production, resulting in souring of the product.

Such changes are desirable in fermented foods, including cheeses, sauerkraut, and fermented sausages. In vinegar production, acetic acid is the principal fermentation product responsible for its characteristic taste.

II.1.2.4. Changes in Appearance and Color

Changes in the appearance and color of foods caused by microbial activity generally occur after the development of off-odors.

II.1.2.4.1. Early manifestations

Initially, small colonies of various shapes (round, flat, or convex) appear with different surface characteristics (opaque or glossy) and colors (white, yellow, black, etc.). These colonies, together with extracellular polysaccharides and mucous secretions, frequently produce a slimy surface commonly referred to as slime formation**.**

II.1.2.4.2. Mold proliferation

Molds develop as colored colonies that spread centrifugally and exhibit a variety of textures, including velvety, cottony, or rough growth.

Color changes in foods mainly result from:

- microbial synthesis of pigments (e.g., by *Micrococcus* and *Pseudomonas*);
- transformation of endogenous pigments, such as carotenoids and myoglobin;
- cellular disruption, allowing enzymes such as polyphenol oxidase (PPO) to interact with their substrates, particularly in plant tissues;
- production of reactive chromogenic compounds, including hydrogen sulfide (H_2S), which reacts with metal ions to form black sulfides.

II.1.2.5. Structure and Texture

The structural integrity of foods depends on macromolecules such as pectins, cellulose, hemicelluloses, starch, and proteins in plant tissues, and primarily proteins in animal tissues.

When contaminating microorganisms secrete hydrolytic enzymes, including pectinases and proteases, softening of the food occurs. For a given microorganism, the extent of softening generally increases with microbial population density.

Examples include:

- **Desirable changes:** meat tenderization during controlled aging and clarification of fruit juices through the action of pectinases.
- **Undesirable changes:** loss of firmness, tissue breakdown, deformation, and reduced commercial quality.

Microbial gas production, mainly carbon dioxide (CO_2), can generate cracks, swelling, and package deformation. Certain microorganisms, such as *Leuconostoc* spp., synthesize extracellular polysaccharides (dextrans from sucrose), thereby increasing the viscosity of syrups and fruit juices.

II.1.2.6. Changes in Nutritional Value

In most foods, surface microbial populations preferentially metabolize simple carbohydrates and non-protein nitrogen compounds.

Consequently, in meat and fish, superficial spoilage characterized by slime formation and the development of characteristic off-odors is generally not accompanied by significant proteolysis and therefore has little effect on nutritional value, even when microbial populations reach 10^9 CFU/cm².

Once extensive proteolysis occurs, amino acid degradation products accumulate, producing undesirable odors, flavors, and textural defects that ultimately render the food unacceptable and unfit for consumption.

II.2. Factors influencing the spoilage flora of foods

Numerous physicochemical characteristics of food products and their environment determine the growth and development of microorganisms.

II.2.1. Intrinsic Factors (relative humidity, water activity, temperature, etc.)

II.2.1.1. Biological Structures

Natural coverings such as husks, shells, skins, and other biological structures provide effective protection against microbial proliferation in certain foods. For example, seed coats, fruit peels, nut shells, eggshells, and animal skin act as natural barriers.

Damage to these natural protective structures may often lead to contamination or microbial growth. Food packaging is primarily designed to protect foods, whether stabilized or not, against contamination. It should also be noted that edible packaging materials may provide additional protection.

II.2.1.2. Natural Antimicrobial Agents

Fresh milk contains lactenins and anti-coliform factors, although their effectiveness is time-limited. Eggs contain lysozyme, which is active against Gram-positive bacteria. Cranberries contain benzoic acid, which is effective against yeasts and molds.
In addition, compounds such as thymol (present in thyme), eugenol (present in clove), and cinnamaldehyde (present in cinnamon) also possess antimicrobial properties.

II.2.1.3. Chemical Composition of Food

To grow and multiply, microorganisms require essential nutrients available in the food matrix. It is important to note that most hazardous microorganisms are heterotrophic chemo-organotrophs**,** meaning that they obtain their energy from the organic constituents of food. They also require water, a nitrogen source, minerals, and for some species, vitamins and growth factors.

The more diverse the chemical composition of a food product (such as animal-derived foods, including meat and meat products, as well as milk), the more likely it is to serve as a favorable culture medium for microbial growth.

II.2.1.4. pH

For a specific microorganism, the growth rate as a function of pH reaches an optimum value. In general, enzymatic activities, which are highly sensitive to pH, determine the limits of microbial growth.

The growth of a microorganism depends on the pH of the medium because the conformation adopted by proteins at a given pH determines their biological activity **(table 10)**.

Table 10 : Typical pH values of foods

pH	Food(s)
> 7.0	Egg albumen, crab, sweet corn
7.0–6.5	Milk, ham, bacon, poultry, fish, shrimp
6.5–5.3	Raw beef, vegetables, vacuum-packed meat, melons
5.3–4.5	Cottage cheese, fermented vegetables, fermented meats (e.g., summer sausage), many sauces and soups
< 4.5	Tomatoes, fruits and fruit juices, yogurt, pickles, sauerkraut

If, at a certain pH, protein conformation becomes inactive, microbial growth will cease when that activity is essential for the survival of the microorganism **(figure 64).**

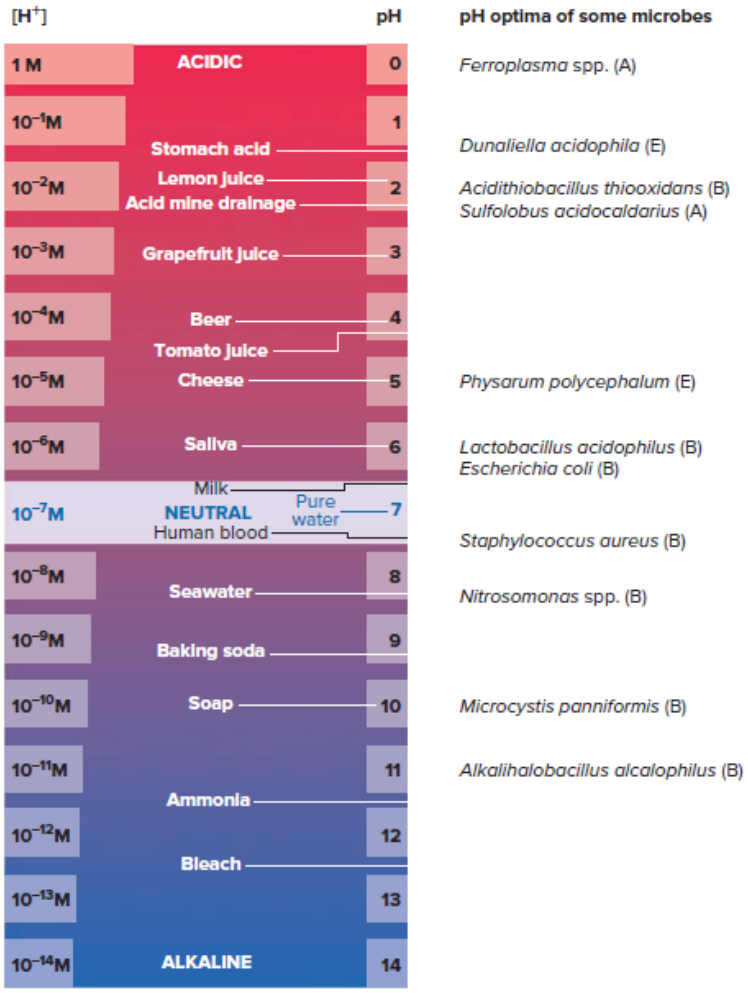


Figure 64 : The pH Scale. Examples of substances with different pH values. Several microorganisms are placed at their growth optima. *A, archaeon; E, eukaryote; B, bacterium.*

With regard to pH, foods are commonly divided into two groups: those with a pH below 4.5 and those with a pH above 4.5. In the first group, hazardous microorganisms generally do not multiply, and *Clostridium botulinum* does not produce toxin.

Acidophily is a characteristic mainly observed in yeasts, molds, and certain bacteria, which are classified according to the type of acid they produce (acetic, lactic, propionic bacteria, etc.).

In foods with a pH ranging from 4.5 to 9.5, numerous spoilage processes may occur, and most pathogenic bacteria are able to grow under these conditions. Food pH promotes microbial proliferation more effectively when it is close to the organism's optimal growth pH.

II.2.1.5. Water Activity (aw)

Microorganisms require available water for growth. Water availability is expressed as water activity (aw), which represents the fraction of free (unbound) water in a food that is available for biological, chemical, and microbial processes **(table 11).**

Table 11 : Typical aw_s of various foods

Foods	Water activity (aw)
Fresh, raw fruits, vegetables, meat, and fish	**> 0.98**
Cooked meat, bread	**0.95–0.98**
Cured meat products, cheeses	**0.91–0.95**
Sausages, syrups	**0.87–0.91**
Rice, beans, peas	**0.80–0.87**
Jams, marmalades	**0.75–0.80**
Candies	**0.65–0.75**
Dried fruits	**0.60–0.65**
Dehydrated vermicelli, spices, milk powder	**0.20–0.60**

Microorganisms capable of growing at low water activity are referred to as xerophiles**.** Those that grow in high-sugar environments are called osmophiles**,** while those that develop in high-salt environments are referred to as halophiles **(table 12).**

Several methods can be used to reduce water activity:

- **Physical methods:** freezing, drying
- **Addition of solutes:** salting, sugaring

These processes allow the production of various preserved foods such as frozen products, dried foods, salted products, brines, jams, and confectionery items.

From a microbiological perspective:

At **aw < 0.65**, no microbial growth is possible.

At **aw < 0.85**, the growth of pathogenic bacteria is generally inhibited, with the exception of certain molds capable of producing mycotoxins.

Table 12 : Minimal **aw_s** required for growth of foodborne microbes at 25°C[a]

Group of microorganisms	Minimum aw required
Most bacteria	**0.91–0.88**
Most yeasts	**0.88**
Regular molds	**0.80**
Halophilic bacteria	**0.75**
Xerotolerant molds	**0.71**
Xerophilic molds and osmophilic yeasts	**0.62–0.60**

The high mortality rate of microorganisms observed during dehydration, salting, sugar addition, or freezing is mainly attributable to the reduction in water activity (aw) **(figure 65).**

Water activity, temperature, and food composition are interrelated in their influence on microbial growth. Low water activity can inhibit microbial growth even under optimal temperature conditions; however, an abundance of nutrients can partially compensate for this effect by allowing microorganisms to survive and grow at lower water activity levels.

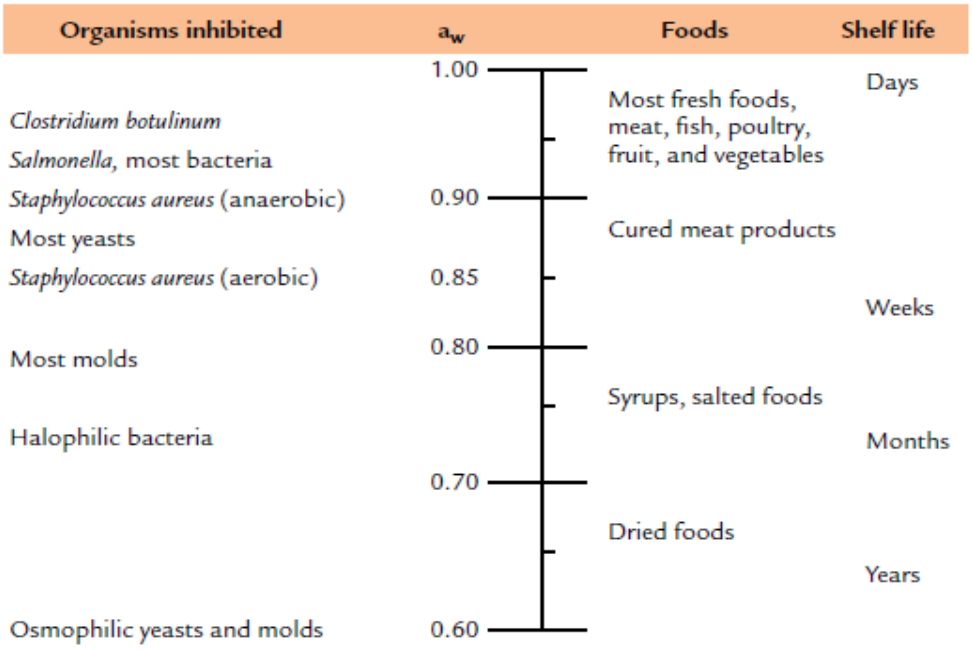


Figure 65 : Illustration of the effect of aw on shelf life and microbial growth.

II.2.1.6. Redox Potential

The oxidation-reduction potential (Eh) is an essential parameter influencing microbial growth in foods. It results from the balance between oxidized and reduced forms of compounds present in the food matrix.

a. Types of microorganisms

- **Strict aerobes:** Require positive Eh values for growth, as they depend on oxygen.

- **Strict anaerobes:** Require negative Eh values, as they grow in the absence of oxygen.
- **Facultative anaerobes:** Can grow under varying Eh conditions.
- **Microaerophiles:** Grow under reduced oxygen levels, requiring intermediate Eh values.

b. Oxidized and reduced foods

- Oxidized foods exhibit positive Eh values, favoring the growth of aerobic microorganisms.
- Reduced foods exhibit negative Eh values, favoring the growth of anaerobic microorganisms.

c. Reducing compounds

Compounds such as ascorbic acid, cysteine, and glucose contribute to lowering Eh, creating a more reducing environment.

Examples of Eh in different foods:

- Plant juices: +300 to +400 mV
- Meat cuts: around -200 mV
- Minced meat: approximately +200 mV
- Meat after slaughter: about +250 mV, decreasing to -150 mV after 30 hours
- Cheeses: between -20 and -200 mV
- *Clostridium* spp.: growth only below -36 mV

The redox potential is a determining factor in microbial growth in foods. Eh values directly influence the types of microorganisms capable of developing: oxidizing environments favor aerobic microorganisms, whereas reducing environments favor anaerobic microorganisms. Reducing compounds play a key role in modulating Eh, thereby contributing to the control of microbial growth and food stability.

II.2.2. Extrinsic factors (temperature, additives, radiation, etc.)

These parameters are closely related to environmental conditions surrounding the food and significantly affect product stability as well as the behavior of resident microorganisms.

II.2.2.1. Storage temperature

Temperature is a key determinant of microbial growth in foods. It influences all microbial metabolic reactions, including anabolism and catabolism. Generally, growth rate increases with temperature up to an optimum specific to each species.

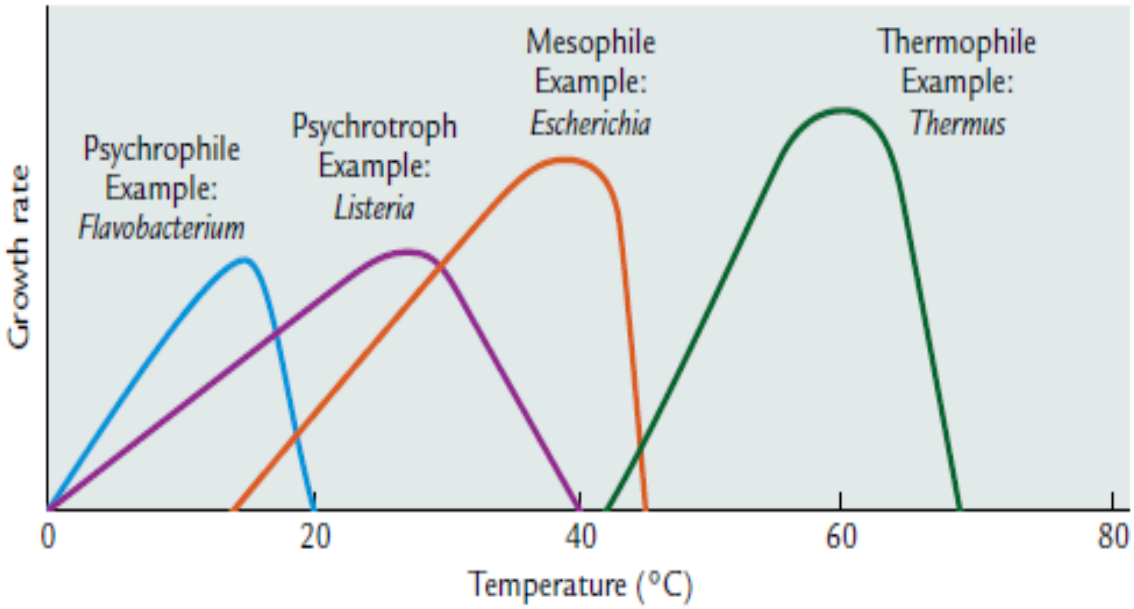


Figure 66 : Relative growth rates of bacteria at different temperatures.

However, excessive temperature leads to accelerated denaturation of bacterial proteins, particularly enzymes. When essential proteins involved in metabolic activity are denatured, microbial growth stops and the organism may die, especially if enzymes required for genome expression are inactivated. These denaturation processes are generally irreversible.

Conversely, at temperatures below the optimal growth range, metabolic reaction rates and microbial growth decrease. However, cold temperatures do not cause significant denaturation of microbial components; microorganisms can therefore resume activity once favorable temperatures are restored.

Microbial growth patterns as a function of temperature result from the interaction between enzymatic activation and protein denaturation. Growth curves vary among species and allow the identification of several physiological groups **(figure 67)**.

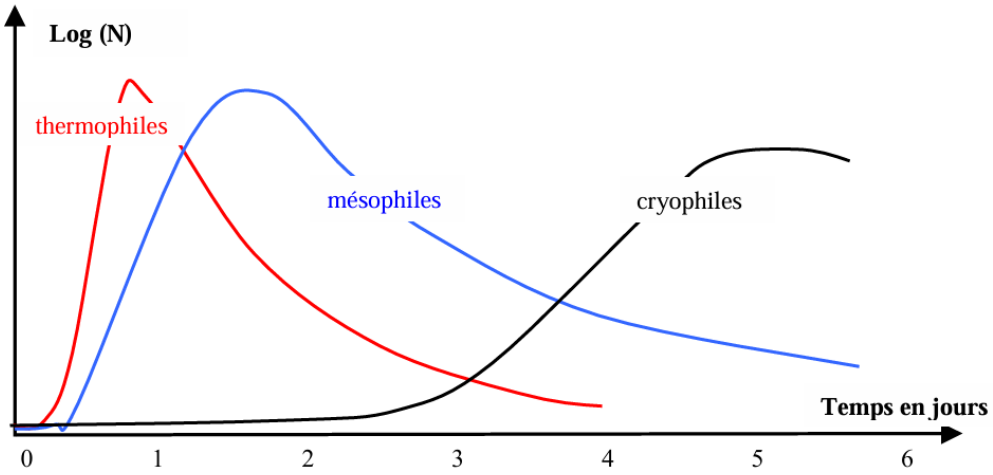


Figure 67: Microbial growth as a function of time for three categories of microorganisms

- **Thermophilic microorganisms:** They exhibit a very short lag phase, a rapid exponential phase, followed by a decline phase. They grow at temperatures above 45 °C, and some up to 80 °C. Among strict thermophiles, *Propionibacterium shermanii*, *Clostridium thermosaccharolyticum*, and *Bacillus stearothermophilus* are notable examples; these organisms grow only at temperatures above 37 °C.
- **Mesophilic microorganisms** constitute the majority of microorganisms. They exhibit an optimal growth temperature generally ranging between 20 and 45°C. Most human pathogenic bacteria belong to this group, as their development is adapted to human body temperature (≈ 37°C).
- **Psychrophilic microorganisms** are adapted to permanently cold environments. Their optimal growth temperature is generally ≤ 15°C, with an optimum often between 10 and 15°C. They can grow near 0°C, but their growth becomes very limited or absent above 20°C.

Refrigeration is a preservation method that strongly slows microbial growth. At 0–4°C, food shelf life is extended from a few days to several days depending on the product. However, microorganisms may still multiply slowly and cause spoilage. Freezing at −18°C does not destroy microorganisms but inhibits their growth, ensuring microbiological stability of foods.

Among pathogenic microorganisms capable of growth at low temperatures (psychrotrophic organisms), the following are commonly found: *Listeria monocytogenes* ; *Clostridium botulinum ; Bacillus cereus ; Yersinia enterocolitica ; Vibrio parahaemolyticus*

The extreme limits of microbial growth vary depending on species. Some bacteria can survive temperatures close to −12 to −20°C (dormant state without active growth), whereas the upper limit may reach approximately 80 - 121°C for heat-resistant spore-forming bacteria.

II.2.2.2.. Relative humidity

Environmental relative humidity influences both the water activity (aw) of food—with which it is in dynamic equilibrium—and microbial growth on its surface. Moisture exchanges may occur between food and ambient air until hygroscopic equilibrium is reached.

For example, a food with a water activity of 0.6 must be protected from excessively high relative humidity, as this may increase surface aw and create favorable conditions for microbial growth.

II.2.2.3.. Presence and concentration of gases

Atmosphere control around foods, by modifying CO_2 and O_2 levels or using nitrogen or vacuum packaging, is an effective method for extending shelf life by inhibiting microbial growth.

II.2.2.4. Antimicrobials produced during food processing

Antimicrobials generated during food processing play a crucial role in food safety and product quality preservation. They may be of natural origin or result from processing or fermentation.

II.2.2.4.1.Natural origin

Some foods naturally contain antimicrobial compounds. For example, garlic contains allicin, which has antibacterial and antifungal properties. Spices and herbs such as thyme, rosemary, and clove contain essential oils that can inhibit microbial growth.

II.2.2.4.2. Fermentation processes

During fermentation, certain microorganisms produce antimicrobial compounds. For instance, lactic acid bacteria used in fermented dairy products (e.g., yogurt and cheese) produce lactic acid and other substances that inhibit undesirable bacterial growth.

Fermentation may also produce bacteriocins, which are antimicrobial peptides synthesized by certain bacteria to inhibit competing microorganisms.

II.2.2.4.3. Food processing

Salting, smoking, and drying are traditional preservation methods that reduce water activity and create an environment unfavorable for microbial growth. These processes can also enhance sensory properties such as flavor and texture.

II.3. Food spoilage control methods

Many factors influence antimicrobial effectiveness. For example, the lethal efficiency of physical agents generally increases with their intensity of application. Similarly, chemical agents may exert bacteriostatic or bactericidal effects depending on their concentration. For certain chemical antimicrobials, stability is essential for their antimicrobial activity to be expressed, whereas for others—such as sodium hypochlorite and hydrogen peroxide—activity is enhanced during decomposition.

II.3.1. Physical methods

Low-temperature inhibition (refrigeration, freezing), thermal destruction (thermization, blanching, pasteurization, sterilization, etc.), radiation effects, bactofugation, and filtration.

Due to their broad spectrum of action, most physical antimicrobial agents are effective against a wide range of microorganisms. The relative sensitivity of microorganisms depends on their structure, composition, and environment. The observed microbicidal or, more rarely, microbiostatic effects generally result from reactions that damage the genome or functional and structural proteins.

II.3.1.1. Heat treatments

An increase in temperature initially accelerates the reactions that characterize microbial metabolism, resulting in faster microbial growth. However, the same temperature increase also induces conformational changes in macromolecules essential to microbial structure or metabolism, such as enzymes. This leads to a reduction in growth rate, followed by growth arrest when the level of alteration becomes incompatible with metabolic function. Eventually, when irreversible changes reach a critical threshold, microbial death occurs.

II.3.1.1.1. Thermization

Thermization is a mild heat treatment mainly used in the dairy industry to reduce the microbial load of dairy products without complete sterilization.

Thermization typically involves moderate heat treatment at temperatures between 57°C and 68°C for approximately 15 seconds. These conditions significantly reduce microbial load without ensuring complete destruction of microorganisms.

The main objective of thermization is to reduce the initial bacterial population, particularly psychrotrophic bacteria capable of growing at low temperatures and responsible for milk spoilage (production of heat-stable proteolytic and lipolytic enzymes). This treatment improves milk preservation prior to further processing steps.

II.3.1.1.2. Pasteurization

Pasteurization is a heat treatment primarily aimed at destroying vegetative forms of pathogenic microorganisms as well as those responsible for food spoilage. It significantly reduces microbial load, including many bacteria, yeasts, and molds.

However, it is not a sterilizing process: bacterial spores generally resist pasteurization, and some more heat-resistant bacteria, particularly Gram-positive bacteria such as *Streptococcus* and *Lactobacillus* present in milk, may partially survive the treatment.

II.3.1.1.3. Blanching

Blanching is a preliminary heat treatment mainly applied to plant-based foods to prepare them for further preservation processes such as freezing, drying, or canning.

It consists of exposing foods to hot water or steam at temperatures generally between 85°C and 100°C for a short duration ranging from 1 to 10 minutes, depending on the product. It is commonly used for vegetables and certain fruits prior to processing.

After blanching, foods are rapidly cooled by immersion in ice water or rinsing with cold water to stop cooking, preserve texture, color, and nutritional quality.

One of the main objectives of blanching is the inactivation of endogenous enzymes responsible for deterioration (browning, texture loss, nutrient degradation), as well as partial reduction of microbial load.

II.3.1.1.4. Sterilization

Heat sterilization is a thermal process aimed at eliminating all pathogenic microorganisms, including spore-forming organisms, as well as most other contaminating

microbes present in the treated product. Sterilized canned foods are therefore highly stable, and only chemical degradation can limit their shelf life.

Time/temperature combinations used for food sterilization typically range from 10 minutes at 115°C to 30 minutes at 121°C.

II.3.1.1.5. Tyndallization

Tyndallization consists of a series of mild heating cycles followed by cooling periods. Typically, the process involves three heating cycles at approximately 60–100°C for 30 minutes to one hour, with 24-hour resting intervals between each cycle.

II.3.1.2. Radiation

II.3.1.2.1. Electromagnetic radiation

Electromagnetic radiation is characterized by energy that is directly proportional to radiation frequency and inversely proportional to wavelength. This energy follows the principle that the shorter the wavelength, the higher the associated energy.

Wavelength ranges of different electromagnetic radiations are as follows:

- Infrared: > 800 nm
- Visible light: 400–800 nm
- Ultraviolet (UV): 10–400 nm
- X-rays: 0.1–10 nm
- Gamma (γ) rays: 0.1–0.001 nm
- Cosmic rays: < 0.001 nm

In the ultraviolet (UV) region, the biologically active range lies between 260 and 280 nm. Aromatic compounds generally absorb the energy of this radiation. In nucleic acids (DNA or RNA), purine and pyrimidine bases are responsible for the antimicrobial effects induced by UV irradiation. Weak but effective ionizing effects also contribute to antimicrobial activity, notably through the formation of ozone from oxygen.

UV radiation is more suitable for surface decontamination of rooms and equipment than for sterilization. When applied to water treatment, its effectiveness is often limited due to poor penetration depth, typically only a few millimeters. Its action is restricted to directly exposed areas. Mutagenic effects on skin and mucosal irritation, particularly ocular damage, require strict protective measures for operators handling this type of radiation.

- UV radiation can be used to disinfect the surface of fruits and vegetables, thereby reducing pathogenic and spoilage microorganisms.
- UV radiation can also be used to sterilize food packaging prior to filling, reducing the risk of contamination.

X-rays and gamma (γ) rays are considered "cold sterilization" methods used for the inactivation of microorganisms in foods and heat-sensitive products. Their effectiveness is based on their ionizing properties, which induce DNA damage in microorganisms, leading to cell inactivation or death.

However, their use in the food industry remains limited due to several factors, including high cost, negative consumer perception associated with irradiation, and concerns regarding the effects of ionization on certain food components.

In contrast, X-ray and gamma irradiation are widely used and well established in the medical, pharmaceutical, and cosmetic industries for sterilizing surgical instruments, packaging materials, and certain raw materials, in compliance with international safety standards.

Ultraviolet radiation is only weakly ionizing. In contrast, X-rays and especially gamma rays, which possess high energy, are classified as ionizing radiation.

II.3.1.2.2. Electron beam radiation

Electron beam radiations consist of high-energy electron beams used for sterilization and disinfection, mainly at the surface level, in the food, medical, and industrial sectors.

II.3.1.2.3. Sonic radiations (sound waves)

Ultrasound eliminates suspended microorganisms through a mechanical vibration effect that induces pressure variations within the medium. These radiations are mainly used to disrupt bacterial structures and/or extract intracellular components.

As mechanical waves, they propagate only through material media and do not travel in a vacuum. This allows them to reach areas that are difficult to access using other antimicrobial agents.

Their effectiveness depends on frequency: it increases with increasing frequency and therefore decreases with increasing wavelength.

II.3.1.3. Mechanical removal

II.3.1.3.1. Sterilizing filtration

Sterilizing filtration can be achieved using organic porous materials (such as cellulose derivatives) or mineral-based filters (such as asbestos, alumina, or porcelain filters), with precisely controlled pore sizes smaller than most microorganisms to be retained (diameter ≤ 0.5 μm).

These methods are limited to liquids containing low levels of suspended organic matter, such as beverages (beer, wine, and certain fruit juices). They have the advantage of preserving organoleptic properties, although in some cases aromatic compounds may be adsorbed by the filter material.

Industrial tubular or multi-tubular systems are available and offer high performance, capable of withstanding high flow rates and pressures, and can be cleaned using sodium hydroxide or acids.

Many manufacturers provide equipment suitable for sterilizing filtration, enabling cold sterilization of thermosensitive solutions or mixtures whose integrity is incompatible with thermal sterilization. These methods can also be used for microbial enumeration or for detecting microorganisms present in very low concentrations in large liquid volumes.

II.3.1.3.2. Centrifugation (bactofugation)

High-speed centrifugation (above 5,000 g) significantly reduces microbial load. In the dairy industry, this process is known as bactofugation and greatly enhances the efficiency of pasteurization.

- At 60°C, approximately 95% of spores are removed due to their interaction with agglutinins associated with fat globules, leading to their migration into the light phase.
- At 80°C, agglutinins are rapidly denatured and lose activity within about ten minutes; under these conditions, 98–99% of spores are removed in the sediment (bactofugate).

Bactofugation is mainly used in the dairy industry to reduce microbial load in milk, particularly bacterial spores and certain resistant bacteria.

- It involves high-speed centrifugation (generally > 5,000 g), enabling separation of components based on density.
- It improves the microbiological quality of milk and extends its shelf life.
- It enables the removal of bacterial spores, which are often resistant to thermal treatments.

- It helps preserve the organoleptic properties of milk by reducing the need for intense heat treatments.

II.3.1.4. Microwaves processing

The use of microwaves for food heating is based on the interaction of the electromagnetic field with polar molecules present in foods, primarily water.

-Microwave energy causes water molecules to oscillate, generating volumetric heating that is subsequently transferred to other food components via thermal conduction.

-Microwave heating is generally rapid and can be relatively uniform, although localized overheating (hot spots) may occur depending on food composition, water content, and geometry.

-Microwaves alone are not a reliable sterilization method; however, they can contribute to microbial reduction when combined with appropriate thermal treatment, particularly by damaging cell membranes and denaturing microbial proteins.

-Reduced heating time helps limit the degradation of heat-sensitive nutrients.

-Microwave penetration is limited in thick or heterogeneous foods, which may lead to non-uniform heating. Therefore, stirring, agitation, or appropriate process design is required to ensure better thermal homogeneity.

II.3.1.5. High Pressure Processing (HPP)

High hydrostatic pressure processing is a food preservation method based on the application of very high pressures, typically ranging from 3,000 to 6,000 bar (300 to 600 MPa). It reduces microbial load and extends shelf life while minimizing heat-induced alterations.

Its antimicrobial action results mainly from irreversible modifications of cellular structures, including:

- Protein and enzyme denaturation;
- Membrane permeability disruption;
- Impairment of metabolic functions;
- Alterations in ribosomal structures.

High efficiency is often achieved at pressures close to 5,000 bar, depending on food type, temperature, treatment duration, and microbial species present.

At these pressure levels, vegetative forms of bacteria, yeasts, and molds are generally sensitive. However, bacterial spores are more resistant and often require combination with moderate heat (pressure-assisted thermal processing).

II.3.2. Chemical methods (Antiseptic substances and antibiotics)

Not all chemical compounds with antimicrobial activity can be used as antiseptics, disinfectants, or food preservatives. Some, such as fluorides or cyanides, exhibit high cellular toxicity, which precludes their practical use. Others, such as antibiotics and sulfonamides, are generally distinguished because they are primarily intended for therapeutic use in human or veterinary medicine.

II.3.2.1. Antiseptics, disinfectants, and food preservatives

The choice of an antimicrobial agent depends on several criteria, including its efficacy, toxicity, stability, and physicochemical properties (corrosiveness, coloring ability, odor, and compatibility with the treated substrate).

To date, no ideal antimicrobial agent exists that simultaneously meets all these criteria.

II.3.2.1.1. Mode of action

The biochemical mechanisms responsible for the antimicrobial activity of antiseptics, disinfectants, antibiotics, and food preservatives are generally multiple. These agents may act either by damaging essential cellular structures or by inhibiting metabolic functions required for microbial survival and growth.

The main mechanisms of action include:

- Oxidation of cellular components (proteins, lipids)
- Coagulation or denaturation of proteins
- Formation of complexes with cellular macromolecules
- Damage to nucleic acids (DNA and RNA)
- Disruption of the cell wall and/or cytoplasmic membrane
- Inhibition of essential metabolic pathways

These mechanisms may act individually or synergistically depending on the nature of the antimicrobial agent and the target microorganism.

II.3.2.1.2. Classification

The classification of antimicrobial substances (based on chemical structure, mode of action, or application) remains limited, as a single compound may exhibit multiple mechanisms of action and variable effects depending on usage conditions.

II.3.2.1.2.1. Oxidizing agents

a. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a broad-spectrum antiseptic and disinfectant. In aqueous solution at 3% (≈ 10 volumes), its use is limited by its rapid decomposition into water and oxygen, especially in the presence of light, heat, or metal catalysts. It is colorless and odorless.

Its antimicrobial activity is primarily based on a strong oxidative mechanism. It oxidizes certain amino acids in proteins, particularly cysteine and methionine, thereby altering their structure and biological function. It also induces oxidation of unsaturated lipids (lipid peroxidation) and damages various cellular components.

b. Chlorine and its derivatives

Chlorine gas (Cl_2) and its derivatives are among the most widely used disinfecting agents. They are extensively applied in the treatment of drinking water and swimming pool water, as well as for the disinfection of surfaces, facilities, and contaminated equipment.
Chlorine dioxide (ClO_2) is a powerful oxidizing agent used particularly for disinfection and improvement of microbiological water quality. It is highly effective at low concentrations and exhibits a different activity profile compared to free chlorine.

Chlorine and its derivatives have been extensively studied for their potential toxic effects, including acute toxicity and possible mutagenic properties at high doses or through reactive by-products.

Chlorine rapidly penetrates microbial cells and reacts with cytoplasmic constituents. It forms reactive chlorinated compounds (N-chloro derivatives) and inhibits numerous essential enzymes through oxidation of sulfhydryl (–SH) groups in proteins.
In practice, sodium hypochlorite (NaClO) is commonly used for surface and industrial equipment disinfection at concentrations of approximately 100–300 ppm of active chlorine. Lower concentrations, generally 1–5 ppm, may be sufficient to reduce surface microbial loads on certain foods such as fruits and meat.

II.3.2.1.2.2. Heavy metals and their salts

Certain metals exhibit notable microbicidal activity, often referred to as the oligodynamic effect, which is expressed at very low concentrations. This effect is associated with the release of metal ions capable of strongly interacting with cellular constituents, particularly proteins. The main heavy metal salts used include those of silver, mercury, copper, and zinc. Their antimicrobial activity is generally higher than that of elemental metals due to the release of active ions. Examples of heavy metal salts include mercury chloride ($HgCl_2$) and copper sulfate ($CuSO_4$).

II.3.2.1.2.3. Alcohols

Short-chain alcohols are used as antimicrobial agents. At appropriate concentrations, they act primarily by protein denaturation and disruption of cell membranes. This denaturation of membrane and cytoplasmic proteins, particularly enzymes, leads to loss of biological function and microbial inactivation when vital cellular systems are affected. In some cases, denaturation effects may be partially reversible if cellular structures are not completely damaged.

Ethanol and isopropanol are the most commonly used alcohols for antiseptic purposes. Ethanol shows maximum efficacy at concentrations between 60% and 70%, where water enhances penetration and microbial protein denaturation. Alcohols are generally ineffective against spore forms and are mainly used as topical antiseptics with superficial action. Longer-chain alcohols (propanol, butanol, amyl alcohol) exhibit increasing bactericidal activity with increasing molecular weight; however, their low water solubility severely limits their practical use.

II.3.2.1.2.4. Phenols

Phenols are a group of antimicrobial agents, often characterized by their odor, and exhibiting microbicidal properties. They have been widely used in the past as antiseptics and disinfectants, and some derivatives are still in use today.

Their mechanism of action is mainly based on their lipophilic nature, which allows them to interact with and disrupt cellular membranes. For instance, derivatives such as thymol exhibit higher activity than phenol itself due to increased hydrophobicity, which enhances membrane interactions.

Phenol possesses bactericidal and fungicidal properties but shows limited activity against spore forms.

II.3.2.1.2.5. Food preservatives

The selection of an antimicrobial food additive is often complex. An effective preservative must meet several requirements to ensure both microbiological safety and food quality:

- **Antimicrobial efficacy:** It must exert sufficient microbicidal or microbiostatic activity to inhibit or eliminate spoilage and/or pathogenic microorganisms.
- **Spectrum of activity:** It must be effective against a broad range of microorganisms, including bacteria, yeasts, and molds involved in food spoilage.
- **Stability:** It must retain its activity under food processing and storage conditions (pH, temperature, time).
- **Safety (non-toxicity):** It must pose no risk to consumers at authorized doses and must not significantly affect nutritional value or organoleptic properties.
- **Regulatory compliance:** It must be approved by current regulations and used within limits established by health authorities.

a. Benzoic acid and benzoates

The antimicrobial efficacy of benzoic acid and its salts (benzoates) is optimal under acidic conditions, typically in acidic food products. The undissociated form (protonated benzoic acid) is the most active, as it can cross cell membranes and inhibit microbial growth, particularly of yeasts and molds.

b. Propionic acid and propionates

Propionic acid and its salts (propionates) are mainly used as inhibitors of mold growth and certain bacteria. Their effectiveness is also enhanced in acidic environments, where the undissociated form is more active. They are widely used in bakery products to prevent mold development.

c. Antibiotics

Antibiotics used as food preservatives include nisin, subtilin, tylosin, natamycin (pimaricin), certain tetracyclines, and polymyxin. Compounds active against spore-forming organisms are of particular interest for the preservation of non-sterilizable products.

- **Nisin :** Nisin is an antimicrobial peptide (bacteriocin) primarily active against Gram-positive bacteria and certain spore forms. Its mechanism of action involves pore formation in the cytoplasmic membrane, leading to cell lysis. Produced by *Lactococcus lactis*, it is widely used in the dairy and canned food industries.
- **Subtilin :** Subtilin is an antimicrobial peptide produced by Bacillus subtilis. It is stable over a wide pH range (approximately 2.5–7) and is mainly active against Gram-positive bacteria, with limited activity against some Gram-negative bacteria. It also exhibits inhibitory effects on spores, which explains its relevance in food technology.
- **Tylosin :** Tylosin is a macrolide antibiotic produced by Streptomyces fradiae. It is primarily active against Gram-positive bacteria, with variable activity against certain Gram-negative bacteria and mycobacteria. Its use is mainly veterinary, and its application as a food preservative is highly restricted or regulated depending on the country.

II.3.2.2. Gas sterilization

Gas sterilization includes processes used for the disinfection of equipment, objects, or facilities that are sensitive to heat. These methods are effective at low temperatures and rely on the chemical action of antimicrobial gases.

II.3.2.2.1. Formaldehyde

Formaldehyde vapors, generated by heating a formalin solution or its polymers, exhibit bactericidal, fungicidal, and sporicidal activity. Their effectiveness is highly dependent on environmental conditions, particularly temperature and humidity.

Antimicrobial activity increases with temperature and humidity. Effective disinfection can be achieved at approximately 20–25°C with relative humidity above 70–80%. Below 20°C, formaldehyde efficacy is significantly reduced.

Vegetative microbial forms are destroyed more rapidly than spore forms, which require longer exposure times. Treatment duration is generally several hours (approximately 8–12 hours), followed by a mandatory ventilation phase before reuse of the treated area in order to remove residual gases.

II.3.2.2.2. Ethylene oxide

Ethylene oxide is a gas at room temperature and a liquid below approximately 10°C. It is highly flammable and explosive, which prevents its use in pure form. For safety reasons, it is generally used in mixtures with an inert gas such as carbon dioxide or other non-flammable carrier gases.

It is a highly potent antimicrobial agent, with bactericidal, fungicidal, and sporicidal activity, widely used for low-temperature sterilization of heat-sensitive materials (particularly in the medical field).

In the food industry, its use is strictly regulated; however, it may be applied for the decontamination of certain dry materials such as spices to reduce microbial load.

II.3.2.2.3. β-Propiolactone

β-Propiolactone is a liquid compound at room temperature, whose vapors exhibit strong bactericidal, fungicidal, virucidal, and sporicidal activity. It is considered a very powerful cold sterilizing agent.

It is also non-flammable and has good penetration ability in certain materials. It has been used for the sterilization of heat-sensitive equipment, culture media, and certain biological products; however, its use is now highly restricted due to its toxicity and carcinogenic potential.

II.3.2.2.4. Volatile essential oils

Essential oils (volatile essences) are complex mixtures of natural compounds with antimicrobial activity. Their bactericidal effect is mainly due to the presence of phenols, alcohols, aldehydes, and oxygenated terpenes, which act on microbial membranes and cellular functions.

- **Eucalyptus oil:** traditionally used for its antiseptic properties on the respiratory tract, mainly due to eucalyptol (1,8-cineole).
- **Clove oil:** rich in eugenol, it has strong antiseptic activity and is widely used in dentistry.
- **Thyme oil:** rich in thymol and carvacrol, it exhibits antimicrobial activity against various microorganisms affecting the digestive and respiratory tracts.

These essential oils may be used in crude form or through their purified active constituents, such as eucalyptol, thymol, and eugenol, which are sometimes employed as natural antimicrobial agents or food preservatives.

II.3.2.2.5. Ozone

Ozone (O_3) is a highly oxidizing gas that is very effective for water disinfection. It acts against bacteria, protozoa, viruses, and certain spores. It also possesses decolorizing and deodorizing properties.

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Syllabus Food Microbiology

Semester: 6

Fundamental Teaching Unit 1 (UEF 3.2.1): Applied Microbiology

Subject 3: Food Microbiology

Credits: 5

Coefficient: 3

Teaching Objectives

This U.E. enables the study of:

- Different foods: dairy products, meats and derivatives,
- The behavior of microorganisms in food environments, the microbiological aspects of food safety and quality, food fermentations as well as the beneficial or harmful effects they cause:

  Lactic fermentations, bread fermentations, cheese fermentations, beverages, ... intoxications and toxico-infections alimentaires (of bacterial or fungal origin) spoilage of foods such as meats and derivatives, canned goods, ...
- The different control methods for the control, elimination, and inhibition of microbial growth in foods.

Recommended Prior Knowledge

Subject Content

I. Brief introduction to major food groups: (Classification of foods according to their constituents: proteins, lipids, carbohydrates, water, minerals, vitamins, etc.).

I.1/ Microorganisms and food (pathogens linked to intoxications, intoxication, toxico-infection, and virulent infection….).

I.2/ Lactic acid bacteria (*Lactococci, Lactobacilli*, *Leuconostoc*, *Bifidobacteria*….): The beneficial and harmful effects of lactic acid bacteria, lactic starters: pure, mixed, and natural; Use of lactic acid bacteria in milk processing (Yogurt and cheese).

II. Microbial spoilage of foods and control methods

II.1. Factors influencing the spoilage flora of foods

a. Intrinsic factors (Relative humidity, water activity, osmotic pressure, temperature).

b. Extrinsic factors (temperature, additives, radiations…).

II.2. Food spoilage: Milk and derivatives (Pasteurized, UHT, butter….); meats (red, fish, poultry…); cereals and derivatives.

II.3. Control methods

a. Physical methods: Inhibition at low temperature (refrigeration, freezing), thermal destruction (thermization, blanching, pasteurization, sterilization, etc.), the effect of radiations, the effect of bactofugation and filtration.

b. Chemical methods: Antiseptic and antibiotic substances.

Practical Work

TP 1: Microbiological analysis of pasteurized milk and cow's milk; Enumerate and identify the microorganisms present in these foods; Express the results according to Algerian standards.

TP 2: Enumeration of the flora of different dairy products: Observe, enumerate, and compare the microorganisms present in two different dairy products ; yogurt (classic or *bifidus*), cheese and monitoring of contamination by *S. aureus*.

TP 3: Analysis of a meat product: Observe and identify the flora potentially contaminating meat products composed mainly of meat such as merguezetc.

TP 4: Analysis of a cereal product: Observe, enumerate, and compare the microorganisms present in a cereal food such as flour…etc: Observation and identification of molds according to their morphological characteristics, identification of sulfite-reducing *clostridia*.

Evaluation Method

Continuous assessment and semester exam.