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POLYCOPIE

Food microbiology course

Intended for third year of the bachelor's degree (L3 Food Biotechnology)

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Preface

The course on "food microbiology" is a fundamental subject of semester 5, more specifically the training program for the third year of the academic bachelor's degree in Food Biotechnology (2024/2025), with a coefficient of 3 and a credit of 6, its overall hourly volume (VHS) is 45 (14-16 weeks), 1h30 of class and 1h30 of practical work per week.

The teaching of food microbiology aims to provide necessary information regarding the main micro-organisms of interest in the agri-food field, to assess the sanitary and hygienic qualities of foods, to show that the development of these micro-organisms can have health-related incidents in the case of pathogenic germs. On the technological level, it is also a question of showing the properties and capacities of micro-organisms to produce substances useful for the food industry and bio-industries.

This course handout consists of an introduction and five chapters. So, the first chapter deals with the major microbial groups relevant to food microbiology, the second chapter is intended to influence manufacturing techniques on microbes, and the third chapter concerns the microbiological problems of a food factory, then in chapter four, we will discuss the Biotechnological Processes and finally chapter five discusses poisoning and toxi-infections.

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Food Microbiology

Introduction

Microbiology is a rapidly developing science with applications in many varied fields, particularly in food and food industries. The microbiological aspect of food transformations and the incidence of microbiologically-originated incidents must be taken into account to ensure control of manufacturing processes as well as consumer health. Many industrial operators therefore need to possess and use basic microbiological knowledge and techniques.

Over the past ten years or so, food microbiology has experienced important upheavals, both in terms of concepts and methodologies and in techniques. New techniques have appeared or developed not only at the level of fermentation and preservation processes but also in analysis techniques, which have evolved toward greater sensitivity, specificity, and speed.

In the field of food microbiology commonly referred to as food microbiology, the objective is to apprehend the microbial phenomena occurring in foods, from the starting product to the dishes as they are consumed by consumers, across all technologies. This includes not only beneficial species but also those that are harmful.

I. Generalities

I.1. Definition of Food

Food may be called anything that is ingested and that can be assimilated by the organism to meet its energetic and physiological needs.

Foods are very numerous and exist in highly variable forms: water, milk, cheeses, meat (raw or cooked), fruits and vegetables, fish, eggs.

I.2. Definition of Microorganisms

The term “microbe” (synonymous with “microorganism”) relates to all living beings whose observation cannot be performed with the naked eye and whose cells are not differentiated nor assembled into tissues. Most are unicellular or have a coenocytic structure.

Microorganisms can be classified into five major groups: viruses, bacteria, algae, fungi (yeasts and molds), and protozoa. Viruses do not have a cellular structure; bacteria (and cyanophyceae) are unicellular organisms of primitive type (prokaryotes) whereas the other cellular organisms have cells of a higher type (eukaryotes) and are sometimes multicellular. Bacteria, yeasts and molds have a predominant place in food microbiology.

I.2.1. Origin of Food Microorganisms

Food microorganisms have three possible origins:

- They pre-exist in the raw material or the food before any handling or processing (endogenous).
- They are accidentally introduced during subsequent handling of the food (equipment, personnel, and environment: air, soil and water, insects...).
- They are deliberately added (e.g., lactic ferments for the manufacture of yogurts).

I.2.2. Use of Beneficial or Useful Microorganisms

The use of microorganisms to transform a food inevitably modifies the organoleptic qualities of the food: milk is not yogurt.

However, the initial aim of producing a new food is often the preservation of the original food.

- Yogurt and cheeses are easier to conserve when refrigerated even though milk is a very fragile food with very limited shelf-life (before UHT sterilization techniques).
- Cured sausage is easy to preserve for a long time, even though raw meat is particularly prone to spoilage.

Multiplication of useful microorganisms may be achieved in two compatible ways:

- Inoculation: addition of microorganisms in the form of a “starter” or a culture (ferments).
- Conditioning/orientation: combining various conditions that direct the flora present in the food toward the targeted purpose (e.g., salting and refrigerating curd during cheese production); addition of amylase in the form of germinated barley and yeasts to a mixture of water and starchy grains for beer manufacture.

Useful microorganisms include:

- Lactic acid bacteria (lactic ferments)
- Yeasts involved in alcoholic fermentation
- Acetic bacteria

- Useful microorganisms are not limited to this list: microscopic fungi frequently participate in the manufacture of “blue” cheeses, for example. In addition, numerous microorganisms can contribute to the aroma of a food (e.g., «*Brevibacterium*» in Camembert).

I.2.3. Harmful Microorganisms

Various microorganisms can cause food spoilage. Moreover, some microorganisms useful in one product can be harmful in another.

- Lactic bacteria useful for yogurt can be harmful in milk.
- Molds useful for blue cheeses can be detrimental in most other foods.

To be harmful, microorganisms must be present in large numbers so that the spoilage becomes perceptible.

Spoilage caused may include:

- Alteration of appearance or texture
- Alteration of taste and odor
- Deterioration of nutritional qualities

Chapter I.

Major microbial groups relevant to food microbiology

Chapter I

Major microbial groups relevant to food microbiology

Introduction

The relationship between food, microorganisms and humans is a long history. Foods are not only a nutrient value for the consumer but often constitute ideal culture media for microbial growth. Microorganisms can affect food quality during all phases of handling, from the producer to the final consumer. Microbial growth results either in the preservation or in the deterioration of food, depending on the microorganisms involved and the conservation conditions.

1. Bacteria

1.1. Generalities

Bacteria are unicellular prokaryotic microorganisms (without nucleus). Cyanophyceae (cyanobacteria or blue-green algae) are photosynthetic aerobic unicellular organisms or grouped in clusters or filaments: they are currently considered to be bacteria. Their genome consists of circular DNA (a single chromosome and possibly plasmids). Plasmids are homogeneous structures formed of circular double-stranded DNA. The bacterial cell presents different shapes, the most common of which are cocci (spherical), bacilli (rod-shaped) and intermediate forms or coccobacilli. We also find vibrio (curved rod, «comma-shaped»), spirilla (spiral-shaped rod), and other diverse shapes (club, filament, etc). Their size ranges from 0.2 to a few tens of micrometers(Fig.1). An important characteristic of bacteria is their high proliferation rate.

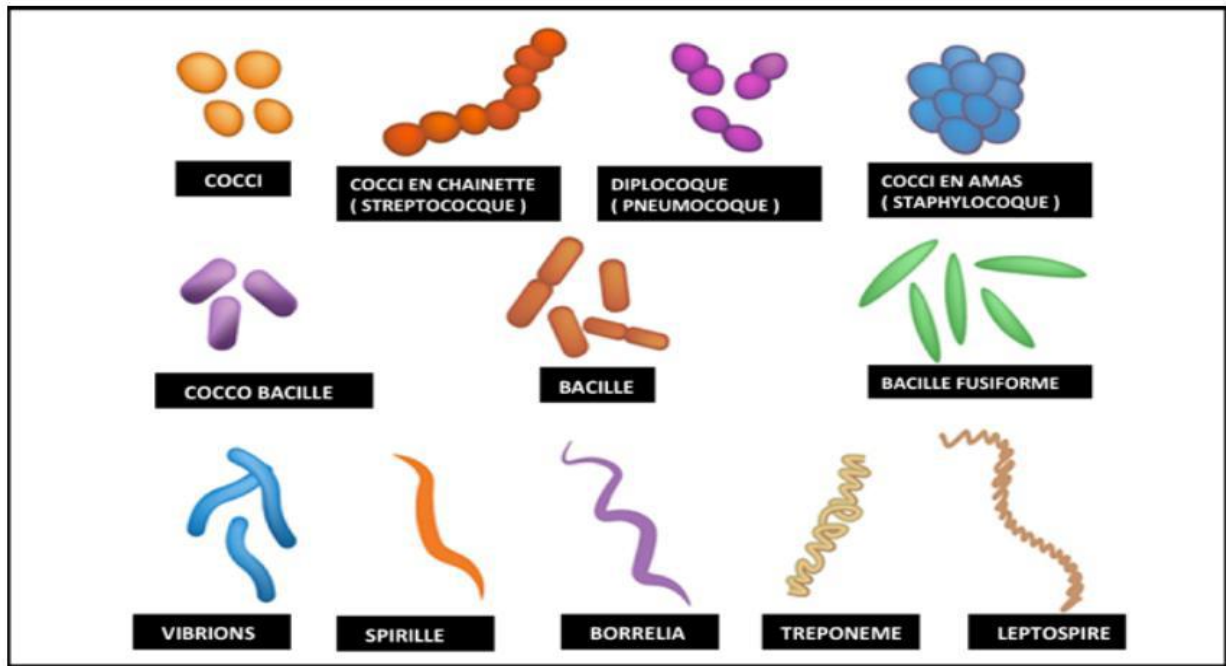


Figure 1. Microscopic morphology of bacteria.

Bacteria contaminate many food products and can constitute a serious danger to their quality and preservation. Several species present a sanitary risk. Others are useful fermentation agents and are involved in numerous industries.

From a physiological point of view there is a great diversity of trophic and respiratory types. Bacteria of interest in food microbiology are heterotrophs. That is, they require the presence of organic substrates. Some species are saprophytic, living freely in nature; others are commensals of humans and animals; others are parasites and have pathogenic potential. There exist species with infective power acting by host invasion (infection); species with toxigenic capacity which release toxins into the food (intoxination); species with mixed characters that can cause toxi-infections; finally other species act by transforming the substrate, rendering it toxic and thus producing intoxications. Saprophytic bacteria often have a degradative power toward foods. Useful bacteria are primarily agents of lactic, acetic, propionic and other fermentations.

Only some families grouping about forty genera are of interest regarding food bacteriology. The germs presented here are classified according to practical identification keys and their relevance within the food industry.

1.2. Enterobacteria

The family Enterobacteriaceae is a large family representing nearly three quarters of strains isolated from human clinical samples. This large family is composed of about 40 genera and more than 130 species. The species most commonly isolated from clinical specimens belong to the genera *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, and *Yersinia*.

The name “Enterobacteria” was given because these bacteria are generally normal or pathological inhabitants of the digestive tract of humans and animals. The environment (soils, waters, plants) can be contaminated by fecal matter and constitutes an indirect source of contamination.

1.2.1. Structure and cultural characteristics

Enterobacteria are Gram-negative bacilli, most often short (1 to 6 μm), straight, immobile or mobile thanks to peritrichous flagellation, facultative aero-anaerobes, fermentative, oxidase-negative, catalase-positive, nitrate reductase-positive (with rare exceptions). Several media are used for the culture and isolation of Enterobacteriaceae; the most common are:

- Violet red bile glucose agar with crystal violet and neutral red (VRBG or VRBGA)
- Lactose medium with bromocresol purple (BCP)
- MacConkey agar

They usually grow very easily on standard culture media at neutral pH such as Muller-Hinton (MH). The optimal growth temperature is generally 35–37 °C, with exceptions for some

genera such as *Yersinia*, *Pantoea* and *Erwinia*. They produce non-pigmented, smooth or rough colonies of 1 to 3 mm in diameter.

1.2.2. Habitat and pathogenicity

Most Enterobacteria are commensals of the digestive tracts of humans and animals (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella* sp.) but they can also be preferentially found in the environment (soil, water, plants) (saprophytic germs: *Serratia* sp., *Enterobacter* sp.).

The Enterobacteria set can be subdivided into two groups based on pathogenicity: (i) on the one hand enteropathogenic species whose ingestion almost systematically causes infection manifesting as diarrhea with or without accompanying signs (*Salmonella* sp., *Yersinia* sp., *Shigella* sp., certain strains of *E. coli*, *Salmonella typhi*); (ii) on the other hand commensal or saprophytic Enterobacteriaceae of the intestinal microbiota (*E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Proteus* sp., *Morganella* sp., *Providencia* sp., *Serratia* sp., *Citrobacter* sp.) responsible for extra-intestinal infections. They are also the bacteria most frequently involved in human infectious pathology.

These mainly include urinary infections, pulmonary infections (especially care-associated or nosocomial pneumonias), intra-abdominal infections including peritonitis, bacteremias and meningitis.

1.2.3. Classification and identification

Distinction between genera is made by studying biochemical characters, the most important of which are: IMViC tests (Indole, Methyl Red, Voges-Proskauer, Citrate), the urease test, and decarboxylase tests. Typically, *Escherichia coli*, *Klebsiella pneumoniae* and certain strains of *Citrobacter* ferment lactose, but this is not the case for *Salmonella*, *Shigella*, *Proteus* or *Yersinia*. Other genera in this family include *Enterobacter*, *Erwinia*, and *Serratia*.

Within each genus, species are individualized by studying biochemical or antigenic traits. Enterobacteria possess numerous antigens, some of which are used for diagnosis: O antigens (somatic wall antigen consisting of the polysaccharide component of lipopolysaccharide [LPS], heat-stable at 100 °C), H antigens (flagellar antigen, heat-labile, composed of proteins called flagellins), and envelope antigens (K, Vi...).

1.2.4. Principal groups

The main Enterobacteria encountered in the food industry have traditionally been classified into different tribes as follows:

Salmonelleae : *Salmonella*, *Citrobacter*, *Edwardsiella*.

Escherichieae : *Escherichia*, *Shigella*.

Klebsielleae : *Klebsiella*, *Hafnia*.

Proteae: includes the genera *Proteus* and *Providencia*.

Yersinieae : corresponds to the genus *Yersinia*, contains several pathogenic genera.

Erwiniae : usually classed among the Enterobacteria. Species of the genus *Erwinia* are frequently phytopathogenic and are often encountered in plant-origin foods where they cause degradation (soft rot). These bacteria are not dangerous from a public health viewpoint. They are ecologically close to Gram-negative saprophytic bacteria.

In food microbiology, «coliforms» are called Enterobacteria that ferment lactose with gas production at 30 °C. This is a disparate group from several tribes that includes the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*. *Serratia*, which ferments lactose slowly, is often included in this group. Except for some biotypes of *E. coli*, these species are of limited sanitary danger and are never highly enteropathogenic. However, in very high numbers, coliforms can cause foodborne intoxications. Thermotolerant coliforms (sometimes

called fecal coliforms) are coliforms capable of developing at 44 °C; this category mainly includes *Escherichia coli*; this flora is more specific for fecal contamination than total coliforms.

Principal genera include: *Salmonella*, *Edwardsiella*, *Arizona*, *Citrobacter*, *Escherichia*, *Shigella*, *Enterobacter*, *Obesumbacterium*, *Serratia*, *Proteus*, *Providencia*, and *Yersinia*.

Genus *Escherichia* : There are several species of *Escherichia*, the most important of which is «*E. coli*». It is lactose-positive, gas-producing, carries out a mixed fermentation, and produces indole. It is a normal inhabitant of the human and animal intestine and is very abundant in fecal matter (10^6 to 10^7 per gram in humans: 80% of the “aerobic” flora). Some serotypes can be pathogenic and cause specific digestive disorders, possessing one or more toxins (hemolysin, cytotoxin, and enterotoxin). We find:

Enteropathogenic *E. coli* (EPEC): a heterogeneous set of strains that sometimes possess Shiga-like toxins. Among these strains are *E. coli* responsible for infant gastroenteritis (headache, fever, vomiting, diarrhea).

Enterotoxigenic *E. coli* (ETEC): causes cholera-like syndromes. These strains can secrete thermostable and thermolabile toxins. It is the agent responsible for travelers' diarrhea.

Enteroinvasive *E. coli* (EIEC): these infectious strains are very rare. They cause acute diarrhea with fever. The strain adheres to and invades the mucosa. The presence of leucocytes in the stools testifies to the invasive process.

Enterohemorrhagic *E. coli* (EHEC): responsible for ordinary or hemorrhagic diarrheas; they can cause bloody diarrhea. The most dangerous strains have caused epidemic episodes with fatal cases. A contaminated food product can be the origin of epidemics (especially meat).

Enteroaggregative *E. coli* (EAggEC) : cause persistent chronic diarrheas.

Uropathogenic *E. coli* (UPEC): biotypes frequently encountered in urinary infections.

Animal-origin products (meats and certain dairy products) and sometimes water are implicated in contamination by *E. coli*.

1.3. *Pseudomonas*

The family Pseudomonadaceae belongs to a vast group of bacteria that in the past received the denomination of «non-fermenters» This family currently groups five genera: *Pseudomonas*, *Comamonas*, *Frateriuria*, *Xanthomonas*, and *Zoogloea*. The type genus is *Pseudomonas*, created in 1894 by Migula. This genus is one of the most complex among Gram-negative bacteria. The species grouped are mainly saprophytes of terrestrial and aquatic origin. They can also be isolated from plants, feces, secretions, skin surface, and food. By producing enzymes (proteases and lipases) which cause undesirable odors, certain *Pseudomonas* species are responsible for spoilage of refrigerated foodstuffs. In chilled meat, species such as *P. fragi* and *P. lundensis* are notably found. In hospital settings, *Pseudomonas* species are found in the patient's immediate environment (sinks, taps and sanitary equipment). This ubiquity is associated with their metabolic versatility and their ability to adapt to hostile environmental conditions (*P. psychrophila* can grow between -1 °C and 35 °C whereas *P. thermotolerans* grows up to +55 °C) as well as their high resistance to antimicrobial substances (antibiotics and antiseptics).

Infections by *Pseudomonas aeruginosa* range from minor external infections to serious diseases. These bacteria can infect blood, skin, bones, ears, eyes, urinary tract, heart valves and lungs as well as wounds (such as burns, injuries or surgical wounds). These infections are frequently contracted in hospitals.

The seminal work of Palleroni and his team, based on ribosomal RNA similarity, revealed the extreme heterogeneity of the genus *Pseudomonas* and allowed its reorganization into five rRNA homology groups. The “sensu stricto” *Pseudomonas* or the «true *Pseudomonas*» correspond to Palleroni's group I. This genus comprises about sixty species isolated from different environments. These species may or may not synthesize pyoverdins (yellow-green fluorescent pigments). Pyocyanins (blue pigment) and yellow pigments, ranging from pale yellow to orange-yellow, can be produced by various species.

1.3.1. General characters of the genus *Pseudomonas*

Currently, bacteria of the genus *Pseudomonas* correspond to Gram-negative bacilli, strict aerobes, generally motile thanks to polar flagella (monotrichous or multitrichous), chemotrophs, with oxidative metabolism, catalase-positive and oxidase-positive except for phytopathogenic species of the *P. syringae* complex. Strains grow easily on common culture media such as nutritive agar and develop between -1 °C and +55 °C.

The genera most frequently encountered in foods are *Pseudomonas* (e.g., *P. fluorescens*, *P. putida*, *P. fragi*, *P. lundensis*, etc.). They have a large metabolic activity, often exhibit proteolytic and sometimes lipolytic activity, and can degrade a wide variety of substrates.

These microorganisms are generally not pathogenic for humans and animals.

1.4. Acetic Acid Bacteria

These bacteria belong to the group of Gram-negative saprophytic aerobes and include the genera *Acetobacter* and *Gluconobacter* (formerly *Acetomonas*), which group species previously ascribed to *Acetobacter*. They contaminate alcoholic products and are used for vinegar production.

They are Gram-negative rods, sometimes elongated, non-sporulating, generally motile. They are oxidase-negative, aerobic and have an oxidative type metabolism. They are the agents of “acetic fermentation,” which is actually an incomplete oxidation of ethanol. Their main characteristic is the production of acetic acid from ethanol. Group characteristics can be demonstrated by tests such as Gram staining, respiratory metabolism on semi-solid meat-liver agar (VF), oxidase test, and production of acid from ethanol.

Acetic bacteria are frequent contaminants of alcoholic products which they acidify. In industry, they are used for vinegar production. The most used varieties are those of the species *A. aceti* (vinegar production by the Orléans method in shallow vats) and *A. pasteurianus* (vinegar produced in deep agitated and aerated vats). They are also used industrially for the production of gluconic acid.

1.5. Vibrios

These are curved bacilli (family Vibrionaceae).

1.5.1. *Vibrio*

The genus *Vibrio* includes species often saprophytic in waters and some highly pathogenic species transmitted by water or products in contact with water. *Vibrio* are non-sporulating Gram-negative curved bacilli and very motile. Aged cultures show a certain polymorphism. Fresh preparations show organisms moving rapidly in groups. *Vibrio* are oxidase-positive, catalase-positive, generally nitrate reductase-positive. They have a fermentative metabolism of sugars without gas production. They can be aerobic or anaerobic depending on the species. Their colonies are smooth, shiny or translucent. Their optimal temperature is 20–30 °C for saprophytes and 37 °C for pathogens. One of their characteristics is that they multiply well at alkaline pH (7 to 9).

Saprophytic species or fish parasites are often collectively called “water vibrios.” The species pathogenic to humans are essentially *Vibrio cholerae* (the cholera agent), *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.

Vibrio cholerae is the agent of cholera. These organisms contaminate water, shellfish and fish or various other products consumed raw (milk, vegetables, etc.) and have a fecal origin. They are robust and can survive for a long time in nature. Cholera is an intestinal toxico-infection manifested by abdominal pains, vomiting and, above all, intense, watery, rice-like diarrhea leading to severe dehydration. The infectious dose is 10^5 to 10^7 bacteria.

The disease results from oral ingestion of contaminated water or food. Once in the intestine, the vibrios secrete the cholera toxin, which is primarily responsible for the major dehydration characterizing the infection.

Vibrio parahaemolyticus is a halophilic marine vibrio (resistant to 3% NaCl) responsible for foodborne toxico-infections (gastroenteritis with vomiting, abdominal pain, mucous and sometimes bloody diarrhea, headache, fever): incubation is short (generally 12 hours) and the illness lasts 2 to 5 days. This bacterium resists freezing and refrigeration.

Vibrio vulnificus is close to *V. parahaemolyticus* but is lactose-positive. It causes severe infections through wounds: it is highly invasive. Transmitted by oysters, it can cause severe intestinal infection with septicemia in debilitated individuals (infectious dose: 10^4 to 10^6).

Among saprophytic species, *Vibrio costicola* sometimes contaminates brines and salted meats.

1.6. Brucella

Brucella are pathogenic bacteria often classified in the family Brucellaceae. They are small Gram-negative coccobacilli, non-motile, aerobic, and generally oxidase-positive. Their growth is weak and slow on ordinary media. They develop on media enriched with blood,

serum or growth factors. Trypticase-soy can, for example, be used. All species are pathogenic, causing an animal disease transmissible to humans. This is brucellosis or Malta fever, which can be chronic or acute. This febrile septicemic disease manifests through various symptoms (pain, chills, headache, weight loss): it is linked to an invasive character and the release of pyogenic endotoxins. Incubation varies from 1 to 3 weeks and convalescence is long. *Brucella* can be transmitted by meat products (composed primarily of meat), raw milk, cheeses (especially ovine).

The most important pathogenic species in the food industry context are: *Brucella suis*, *Brucella melitensis*, and *Brucella abortus*. There are other species, but they are rarer.

1.7. Micrococci

These are cocci (family Micrococcaceae), termed non-lactic by opposition to streptococci, although they can produce lactic acid by fermentation. They are commensal bacteria of animal and human skin, frequently contaminating foods and can cause spoilage and sanitary problems.

Micrococcaceae are Gram-positive cocci, non-motile, non-sporulating, usually grouped in irregular clusters. They are catalase-positive (except *Sarcina*), with respiratory type being aerobic or anaerobic on deep VF agar. Their colonies are smooth and often pigmented. Genera involved in food are essentially *Micrococcus*, *Staphylococcus*, and to a lesser extent *Sarcina*.

Micrococcus are not pathogenic: they sometimes play a useful role in industry, for example in the ripening of certain cheeses or in salted/brined products.

1.8. Streptococci

They are part of the lactic bacteria group which includes agents of fermentations producing lactic acid: bacilli (Lactobacillaceae) and cocci (Streptococcaceae). They are Gram-positive bacteria with sometimes complex nutritional requirements, found in rich food products, particularly milk.

The family Streptococcaceae groups genera very frequent in the food industry both as contaminants and principally as agents of lactic fermentation: *Streptococcus*, *Lactococcus*, *Enterococcus* (these three genera were formerly grouped under the single genus *Streptococcus*), *Leuconostoc* and *Pediococcus*. They are Gram-positive cocci, non-sporulating, non-motile, generally grouped in pairs and especially in chains of variable length. They are catalase-negative. They have fermentative metabolism producing lactic acid in variable but often significant amounts depending on the genus.

Differences between groups are based on cell arrangement and the type of lactic fermentation (homo- or hetero-lactic).

Streptococci were initially classified into four groups of *Streptococcus* species according to Shermann's criteria; we distinguish:

Pyogenic group: contains pathogenic hemolytic streptococci (β -hemolysis) belonging to Lancefield serogroups A, B, C, E, F, G, H. The type species is *S. pyogenes* (Group A). After 1 to 3 days of incubation, symptoms appear such as sore throat, headache, vomiting and fever. Product contamination is of respiratory origin and implicated foods include milk, eggs and ice cream, pastries. The species *S. agalactiae* (Group B), an agent of mastitis, is sometimes encountered in foods.

Viridans group: includes α or γ hemolytic streptococci (Group K) not groupable by Lancefield. *S. thermophilus* is a common acidifying agent in certain cheeses and especially in yogurts.

Lactic group: includes non-hemolytic streptococci (γ), belonging to serogroup N and contains species very important in cheesemaking, *S. lactis* and *S. cremoris* now called *Lactococcus lactis*, *Lc. cremoris*.

Enterococci group: fecal streptococci belonging to serogroup D. They are intestinal commensals with α , β or γ hemolysis.

The germs most commonly encountered in foods are essentially *S. faecalis*, *S. durans* and *S. bovis*, now classified as Enterococcus. They are contamination indicators of fecal origin and are only exceptionally pathogenic; when pathogenic they are opportunistic pathogens requiring high infectious doses (10^8 to 10^{10} cells). *Enterococcus faecalis* resists 6.5% NaCl and 30 minutes at 60 °C; most biotypes possess a hemolysin. Toxi-infections due to enterococci are very rare: they cause abdominal pains and diarrhea and are due to consumption of contaminated meats or pastries.

1.9. Lactobacilli

Lactobacillus is the principal genus of the family Lactobacillaceae. It contains numerous species that are agents of lactic fermentation involved in many industries or encountered as contaminants. They are often elongated bacilli or coccobacilli, Gram-positive, non-sporulating, sometimes grouped in pairs or chains, generally immobile. They are catalase-negative (some have pseudocatalase). They are microaerophilic or anaerobic. They have fermentative metabolism producing lactic acid. Some species are homolactic, others heterolactic producing volatile acids, ethanol and CO₂ alongside lactic acid. There are

obligate homofermentative species (e.g., *Lactobacillus delbrueckii*), obligate heterofermenters (e.g., *L. brevis*) and others that are facultative heterofermenters (e.g., *L. casei*). They are acidophilic and generally poorly proteolytic and poorly lipolytic.

They are found on vegetation, in the natural microflora of humans, and in various fermented food products.

1.10. Actinobacteria

This vast assemblage groups varied families of bacteria generally exhibiting particular morphologies. “Actinobacteria” are Gram-positive bacteria, but which sometimes stain poorly. They are non-sporulating that is, they do not form heat-resistant endospores. They have, depending on species, irregular bacillary forms and mycelium-like structures with branching. Some species form conidia called “spores” but these do not have the same biological significance as bacterial endospores. These bacteria are often aerobic, sometimes anaerobic, and catalase-positive. A few pathogenic bacteria belong to this group. The principal bacteria encountered in the food industry are propionic bacteria, “coryneform” bacteria, some *Streptomyces* and pathogenic mycobacteria (it should be noted that in recent classifications *Micrococcus* is attached to the actinobacteria).

Most actinobacteria live in the soil, but some are aquatic. The vast majority are aerobic. A distinctive feature of this group is the presence of several different peptidoglycans in the cell wall. The genus *Actinomyces* is a well-studied representative of actinobacteria.

Actinobacteria play a major role in ecosystems as they are involved in organic matter decomposition, bioremediation and bio-alteration and, more importantly, produce a large number of secondary metabolites with many therapeutic applications.

Actinobacteria have also been found to be the agents responsible for numerous human and animal infections, including a number of common diseases studied intensively, such as

diphtheria which is due to *Corynebacterium diphtheriae* producing a toxin. This is a highly contagious disease transmitted from person to person. Diphtheria chiefly manifests as an infection of the upper respiratory tract which can lead to paralysis of the central nervous system or of the diaphragm and throat, resulting in death by asphyxiation.

1.11. Aerobic spore-forming bacteria

These bacteria belong to the genus *Bacillus* (family Bacillaceae). They generally belong to the Gram-positive resident flora and contaminate many food products. *Bacillus* are Gram-positive bacilli, generally motile, capable of sporulation. A spore, a resistant structure, forms in the cell when conditions become unfavorable. The spore does not take up the Gram stain; it is spherical or oval, deforming or not depending on the species. *Bacillus* are characterized by their ability to sporulate and by being catalase-positive. Some are strict aerobes, others can develop under anaerobiosis.

Bacillus are widespread in nature, particularly in soil and on vegetation; they contaminate numerous food products and are often proteolytic. Because of their ability to sporulate, they resist adverse conditions and can be agents of food can spoilage. A few *Bacillus* can be agents of foodborne intoxication. *B. cereus* is an aero-anaerobic thermophilic bacterium. It is a species frequent in soil, vegetation, and particularly cereals and animal skins. It is often implicated in toxico-infections linked to its excessive multiplication in food (from 10^4 to 10^7 germs per g).

B. cereus produces several toxins: hemolysin, phospholipase C, cereolysin (a cytolyisin), an emetic toxin, and two protein enterotoxins. Foods involved include meats, cooked dishes, milk and milk-based desserts, pastries, and certain vegetable products.

Another species, *Bacillus thuringiensis*, pathogenic to insects, is used in biological control.

1.12. Anaerobic spore-forming bacteria

These bacteria belong to the genus *Clostridium* (family Bacillaceae). They are commonly found in soil, sewage waters and the intestine. They can contaminate and degrade food products under anaerobic conditions (canned goods). *Clostridium* are Gram-positive bacilli, often of large size, isolated singly or in chains. These bacteria are generally motile. They are capable of sporulation. The form and position of the spore are important in taxonomy (the spore resists several minutes at 100 °C). They are catalase-negative and strict anaerobes; oxygen is toxic for these species due to H₂O₂ formation. *Clostridium* are generally mesophilic and tolerate fairly large variations of pH and temperature. They multiply readily on ordinary media. In anaerobiosis they are proteolytic or saccharolytic depending on species and are frequently gas-producing. They contaminate many products: water, milk, fish, fermented or frozen foods and especially canned foods. They have a strong degradative power toward sugars (polysaccharides, di- and monosaccharides) and proteins by releasing butyric acid or H₂S. A few species are responsible for intoxication or gastroenteritis (*C. perfringens*) or for severe often fatal intoxications (*C. botulinum*).

Characterization of *Clostridium* is based on their capacity to sporulate, as well as their anaerobic character (by deep culture on meat-liver (VF) agar or meat-yeast (VL)) and catalase-negative reaction.

Industrially, *Clostridium* are classified according to their metabolism and action on substrates, we distinguish:

- **Saccharolytic *Clostridium*:** *C. butyricum*, *C. tyrobutyricum*.

- **Proteolytic or putrefactive *Clostridium*:** *C. botulinum*, *C. putrefaciens*.

- *Clostridium* both proteolytic and saccharolytic (often called “sulfite-reducing” *Clostridium*): *C. perfringens* and *C. septicum*.
- Some species are neither proteolytic nor saccharolytic (specialist *Clostridium*), and they are not of interest to the food industry.

2. Fungi

Yeasts are unicellular fungi whereas molds are filamentous fungi, uni- or multicellular.

2.1. Molds

2.1.1. General characteristics

Filamentous fungi (or molds) are heterotrophs: some live in symbiosis with plants, others are plant parasites or animal parasites; others are saprophytes, developing on organic wastes and contaminating food products. Molds are aerobic, generally acidophilic (growth pH between 3 and 7) and mesophilic (optimal temperature 20–30 °C); however certain species are psychrophilic, developing at low temperatures (< 15 °C or even sometimes < 0 °C). Molds generally have a lower water requirement compared to other microorganisms and can develop on foods with low water activity (down to $a_w = 0.65$): some species are osmophilic or xerophilic. They often possess significant lytic properties (cellulolytic, pectinolytic, amylolytic, proteolytic, lipolytic, etc.) which make them dangerous agents of degradation but also sometimes useful allies (cheese ripening, enzyme production). Their metabolism may be oxidative or mixed.

The harmful involvement of filamentous fungi in the food industries occurs at several levels. There are phytopathogenic fungi that are very problematic for the production of raw food materials such as fruits and vegetables. Saprophytic molds contaminate foods and degrade

them qualitatively. Some of them are toxinogenic, releasing mycotoxins into the food that represent a serious sanitary danger.

2.1.2. Applications

Many molds are used in the manufacture of cheeses, dried sausages, enzymes, and antibiotics.

Aspergillus produces an enzyme similar to rennet.

Penicillium is at the origin of Camembert flora.

Penicillium roqueforti is active in Roquefort and blue cheeses.

2.2. Yeasts

2.2.1. General characteristics

Like molds, yeasts are microorganisms and heterotrophs, which depending on the species have an exclusively oxidative metabolism or a mixed oxidative/fermentative metabolism. They are aerobic and those possessing a fermentative metabolism cannot develop in strict anaerobiosis except in the presence of ergosterol and oleic acid (Tween 80). They are generally acidophilic and mesophilic, multiplying at pH between 3 and 7.5 and at optimal temperatures near 25–28 °C. However, psychrophilic strains exist, acidophilic strains (growing down to pH 1.5), and osmophilic strains. Yeasts assimilate numerous carbon substrates. The oxidative pathway leads to formation of CO₂ and H₂O. The fermentative pathway, which exists only in certain species, leads to formation of ethanol and CO₂ (e.g., *Saccharomyces*).

Among yeasts, only a few species (e.g. *Candida albicans*, *Cryptococcus neoformans*) are pathogenic but they do not cause food poisoning. Yeasts thus do not represent a sanitary

problem in foods. They do, however, frequently act as contaminants and agents of deterioration especially in acidic, sugary or alcoholic products.

2.2.2. Applications

Yeasts occupy an essential place in the food industry. They participate in the manufacture of many products (brewing, cider, winemaking, cheesemaking) and also in the valorization of agricultural and industrial wastes and in protein production. Their role is fundamental in the fermentation industries: yeasts transform sugar- or starch-rich substrates into alcohol-rich products (e.g., *Saccharomyces*, *Kluyveromyces*). Yeasts also participate in cheese ripening (e.g., *Debaryomyces*).

At present, new industries use yeasts. Thanks to their metabolic possibilities and chemical composition that makes them suitable as food (particularly in animal feed), yeasts are ideal microorganisms for revalorization of agricultural and industrial wastes and for production of supplementary proteins. Presence of yeasts is therefore frequent in many foods and many industry operators are led to handle them.

Chapter II.

**Influence of
Manufacturing
Techniques on
Microorganisms**

Chapter II: Influence of Manufacturing Techniques on Microorganisms

The use of antimicrobial agents makes it possible to control the development of microorganisms, particularly pathogenic microorganisms and/or those responsible for the deterioration of food products. There are numerous methods for combating microorganisms, which can be schematically classified into:

- Physical agents (temperature, radiation, etc.).
- Chemical agents (whose activity and toxicity affect both microbial cells and human or animal cells).

1. Factors Influencing Microbial Destruction

The methods of control are numerous and varied; they must take into account the microorganism itself, its environment, and the intensity of the desired action. Several factors intervene in microbial destruction:

1.1. The Microorganism

- Not all species are equally sensitive to a given antimicrobial agent.
- The physiological state of the bacterium affects its sensitivity: bacteria are less resistant during the exponential growth phase to the action of chemical antimicrobial agents, which are rapidly absorbed by the germ, whereas cells aged 24 hours or more are often more sensitive to physical treatments.
- Sporulated forms are much more resistant to physical or chemical agents than their corresponding vegetative forms.
- The higher the initial number of microorganisms (load), the longer the time required to reach a

given level of destruction.

1.2. Treatment Time

Antimicrobial action follows a kinetic law: the longer the exposure time, the greater the microbial destruction, depending on the intensity of the agent applied.

1.3. The Antimicrobial Agent

Numerous parameters control antimicrobial action. The lethal effect of physical agents generally increases with the intensity of their application; chemical agents may, depending on their concentration, have either bacteriostatic or bactericidal effects.

The stability of certain chemical antimicrobial agents is necessary for their antibiotic activity. For chemical agents, solubility in water is a determining factor in their effectiveness.

1.4. The Environment

The environment can greatly influence the effectiveness of physical or chemical antimicrobial agents. Among the main influencing factors are:

- pH of the medium
- Turbidity and viscosity (UV rays are only effective within a few millimeters of depth in clear media)
- Water hardness (calcium and magnesium ions, for example, decrease the antimicrobial activity of disinfectants such as quaternary ammonium compounds)
- Organic matter (proteins precipitate in the presence of alcohol, and the resulting precipitate prevents alcohol diffusion; hypochlorites react with organic matter to form less active chloramines, etc.)
- Temperature can modify the action of certain chemical antimicrobial agents (most antibiotics lose their activity at high temperatures).

2. Methods of Microbial Control

2.1. Physical Agents of Stabilization or Destruction

Due to their low specificity of action, most physical antimicrobial agents are effective against a wide range of microorganisms. The relative sensitivity of the microbes depends on their structure (species), composition, and environment.

In most cases, the observed microbicidal or, more rarely, microbiostatic effects result from reactions that affect the genome or functional/structural proteins of the microorganisms.

2.1.1. Heat

The use of heat is a widely employed method for the destruction of microorganisms. Thermal treatments form the basis of the preservation of many foods.

– Pasteurization

Pasteurization primarily ensures the destruction of the vegetative forms of pathogenic microorganisms and/or those responsible for certain types of spoilage present in the food. It results in the elimination of molds, yeasts, and Gram-negative bacteria. Most spore-forming organisms resist treatment, and some Gram-positive bacteria are only partially destroyed (e.g., *Streptococcus* and *Lactobacillus* in milk).

Pasteurization is generally carried out at temperatures below 100°C, but over time, the process has evolved toward shorter durations and higher temperatures. It is used for foods that are relatively heat-sensitive (such as ham, fruit juices, milk, butter, foie gras, etc.). Because viable microorganisms may remain in treated products, these are called semi-preserved foods; their limited shelf life is ensured immediately after pasteurization through refrigeration or freezing.

The time/temperature combinations used in pasteurization vary between:

- 30 minutes at 63°C (“low-temperature pasteurization”) used for milk, certain fruit beverages,

and ice cream.

- A few seconds at 90°C (high-temperature pasteurization) used for foods with medium or low water content.

- Ultra-High Temperature (UHT) treatment at temperatures above 100°C for a few seconds or fractions of a second. When the temperature reaches around 140°C, the process is no longer considered pasteurization but true sterilization.

– Sterilization

Sterilization by heat refers to a thermal treatment that eliminates all pathogenic microorganisms, including spores, as well as almost all other microbes capable of contaminating the treated product.

Sterilized canned foods therefore exhibit high stability, and only chemical reactions may reduce their storage life. The term “sterile” means that no revivable microorganisms can be detected using standard culture methods (or that the number of survivors is so small as to be insignificant under storage conditions).

Typical time/temperature combinations for sterilization range from 10 minutes at 115°C to 30 minutes at 121°C.

– Tyndallization

This method involves repeated pasteurizations separated by incubation periods at temperatures around 30–40°C for about 10 to 24 hours.

During pasteurization, only vegetative forms are inactivated, while during incubation, most heat-resistant spores germinate into vegetative cells, which are then destroyed by the following pasteurization cycle.

– Boiling

This commonly practiced method can be considered a form of “super-pasteurization.” It does not destroy spore forms, and it is often observed that spore germination is actually promoted during cooling.

2.1.2. Cold

Cold is an effective agent for stabilizing food products. Cooling (below 15°C) is of limited effectiveness, whereas refrigeration which maintains food at temperatures close to 0°C (typically 0–4°C) prevents the multiplication of most pathogenic bacteria. However, it does not inhibit the growth of psychrophilic microorganisms, some of which may be spoilage agents or even pathogens (*Listeria*, *Yersinia*, *Campylobacter*).

Freezing at –18°C and deep freezing (–40°C or even –80°C) ensure total stabilization against microorganisms and cause varying degrees of microbial death depending on the type of organism, the nature of the food, the cooling rate, and the storage temperature.

Cold treatment is widely used to preserve raw or pre-treated foods. However, refrigeration may promote the establishment and growth of psychrophilic flora, which may include pathogens (*Listeria*, *Yersinia*, etc.).

For effective cold treatment, the following conditions are required:

- The product must be microbiologically sound before treatment.
- The process must be rapid and initiated early.
- Continuity of refrigeration must be maintained — i.e., no interruption of the cold chain.

The effect of cold, particularly refrigeration, is limited: it only slows down microbial and biochemical processes, so the conservation therefore has a limited duration (expiration date).

2.1.3. Radiation

– Electromagnetic and Ionizing Radiation

Certain types of radiation are used to destroy microorganisms in food products.

The term “ionizing radiation” broadly includes both electromagnetic (from ultraviolet to cosmic rays) and corpuscular (e.g., β -electrons) radiations that can remove electrons and thereby ionize molecules.

The ionizing power of electromagnetic radiation depends on the energy carried and thus on the frequency of the radiation: the shorter the wavelength, the higher the energy.

The wavelengths of electromagnetic radiation are indicated below::

Infrared: > 800 nm

Visible: 400–800 nm

Ultraviolet: 10–400 nm

X-rays: 0.1–10 nm

Gamma (γ)rays: 0.1–0.001 nm

Cosmic rays: < 0.001 nm

Ultraviolet radiation (10–400 nm) has low penetrating power; it is useful for reducing the microbial load of air, surfaces, or thin liquid layers. When used for water treatment, it produces only moderate results due to its limited penetration (a few millimeters).

Gamma radiation (γ -rays) (0.1–0.01 nm) is widely used and exhibits strong antimicrobial action, allowing for “cold” pasteurization or sterilization.

X-rays are rarely used, as are corpuscular radiations (e.g., β -rays), which are expensive and difficult to apply.

The antimicrobial effect of radiation results primarily from nucleic acid denaturation.

Microbial inactivation depends on the dose absorbed, which is determined by both intensity and

exposure time.

Doses are expressed in Gray (Gy), representing energy per unit mass ($1 \text{ Gy} = 1 \text{ J/kg}$).

The dose rate corresponds to the rate at which energy is delivered, expressed in Gray per unit time.

A dose of 0.03 at 3KGy allows the inhibition of plant germination and disinfestation. The elimination of most pathogenic germs such as Salmonella or Listeria (radicalization) and spoilage germs like Pseudomonas (radurization) requires doses of 1 to 6 KGy (comparable to pasteurization), that of spores, viruses, and some resistant pathogens (Staphylococcus) doses of 10 to 50 KGy (radappertisation comparable to sterilization). In the latter case, product alterations are significant)

– **Electron Radiation**

Electrons, produced by thermo-electronic emission and accelerated by an electric field, possess sterilizing power similar to that of gamma rays.

Such radiation can be precisely directed, but its penetration depth is lower than that of gamma rays. Its main drawback lies in its tendency to degrade organic compounds exposed to it.

– **Sonic Radiation**

Ultrasound kills microorganisms in suspension through a mechanical vibration effect.

These radiations are primarily used for cell disruption and/or extraction of cellular components, rather than for industrial sterilization.

Antimicrobial effects occur only at short wavelengths (ultrasound range), and their industrial application remains limited.

– Microwaves

Microwaves, also a form of electromagnetic radiation, induce thermal agitation of polar molecules, particularly water.

The resulting rise in intracellular water temperature causes microbial death. Vegetative forms are more sensitive to microwaves than spores.

2.1.4. Pressure

High-pressure food treatment is an emerging technology. Its antimicrobial activity is significant: pressures of 100–300 megapascals (MPa) strongly reduce vegetative cells at ambient temperature; at 800 MPa, spores are denatured.

Using CO₂ under high pressure has also been considered: this gas is inherently toxic to many bacteria and may allow the use of moderate pressures (1–10 MPa). For example, *Listeria monocytogenes* can be destroyed at room temperature after 2 hours at 6 MPa.

2.1.5. Dehydration

This ancient stabilization technique is based on reducing the water activity (a_w) of the product.

Drying in general, and spray drying (for milk), involve the application of heat, though other antimicrobial factors may also contribute. Solar drying (for fruits, meat, fish) utilizes UV radiation, while smoking (for meats and fish) combines heat with the action of pyrolysis products (such as formaldehyde, organic acids, pyrolytic acids, alcohols, ketones, phenols, cresols, etc.). Smoking also modifies flavor and color.

Freeze-drying (lyophilization), which removes water by sublimation at low temperatures, is widely used and preserves the nutritional and sensory properties of foods.

2.1.6. Mechanical Elimination

– Sterilizing Filtration (Cold Sterilization)

It is called cold sterilization.

Sterilizing filtration is achieved using porous organic supports (e.g., cellulose derivatives, polyamide, Teflon) or mineral supports (e.g., asbestos, alumina, porcelain, sintered glass).

The pore size is carefully controlled and smaller than that of most microorganisms ($\leq 0.5 \mu\text{m}$).

These methods are mainly applicable to liquids with low suspended matter content, such as beer, wine, and some fruit juices.

They have the advantage of preserving organoleptic properties, although slight losses of aromatic compounds may occur due to adsorption on the filter surface.

– Centrifugation

Centrifugation at over 5000 g reduces microbial load — a process known as bactofugation in the dairy industry — making pasteurization more effective.

For non-sterilized milk:

- At 60°C, 95% of spores are removed through reaction with agglutinins associated with fat globules and are found in the lighter phase.
- At 80°C, agglutinins are rapidly denatured and lose activity within about 10 minutes; thus, 98–99% of spores are eliminated in the sediment (pellet).

2.2. Chemical Agents

Not all chemical compounds with antimicrobial properties can be used as antiseptics or disinfectants. Some, such as fluorides or cyanides, are powerful cellular poisons whose toxicity prohibits their use. Others, like antibiotics and sulfonamides, are treated separately because of their therapeutic applications.

2.2.1. Antiseptics, Disinfectants, and Food Preservatives

The choice of an antimicrobial agent depends on its intended use, activity, toxicity, stability, corrosive or staining power, odor, etc.

2.2.1.1. Oxidizing Agents

a) Hydrogen Peroxide

Hydrogen peroxide (H_2O_2 or oxygenated water) is an effective antiseptic. In aqueous solution at 3% (10 volumes), it is a good disinfectant. However, its use is limited due to rapid decomposition. It is a colorless and odorless compound.

Hydrogen peroxide oxidizes cysteine and methionine residues in proteins, causing them to lose their biological function. Other substances are also oxidized under these conditions: peroxides form in unsaturated lipids, and certain vitamins (especially A, D, C, and B_1) are destroyed during treatment.

b) Chlorine and Its Derivatives

Chlorine gas and, more commonly, its chemical derivatives are among the most widely used antiseptics and disinfectants. They are used in drinking water treatment, swimming pools, and for disinfecting rooms and contaminated objects.

Liquid compounds such as hypochlorites and chloramines are the most common. Sodium hypochlorite (NaClO), or bleach, is universally used and corresponds to chlorine dissolved in sodium hydroxide:



Applications:

For industrial disinfection of surfaces and equipment, sodium hypochlorite is generally used at concentrations of 100–300 ppm of active chlorine, whereas doses of 1–5 ppm are sufficient to

reduce surface microbial loads in certain products such as fruits or meats. To improve color and baking properties, flour is treated with gaseous chlorine at concentrations of 1500–2500 ppm. Antiseptic chlorine-based preparations such as Labarraque's or Dakin's solutions are partly prepared from diluted hypochlorite. Chloramines (e.g., chloramine T) have longer-lasting effects than hypochlorites, but are less effective overall. They are mainly used for disinfecting pool water.

c) Iodine and Its Derivatives

Iodine is one of the oldest disinfectants. Bactericidal and fungicidal, it is slightly soluble in water but readily dissolves in alcohol or aqueous solutions of potassium or sodium iodide. Iodine–iodide solutions (iodine tinctures or Lugol's solution) are used to disinfect superficial wounds. Certain detergents can solubilize iodine, forming iodophores. Iodophores are active at 150 ppm iodine, and 50% alcoholic iodine solutions (at 1500 ppm) exhibit a marked sporicidal activity. Other halogen derivatives such as fluorine and bromine also have antimicrobial effects but are seldom used because of handling difficulties or toxicity.

2.2.1.2. Heavy Metals and Their Salts

Some metals exhibit significant microbicidal effects—known as oligodynamic action—at very low concentrations. This effect can be attributed to their poor solubility, limited ionization, and strong affinity for cellular proteins. Silver and some of its derivatives are used for pool water sterilization and antiseptic dressings. The most commonly used heavy metal salts include those of silver, mercury, copper, zinc, and gold. They inactivate cells by precipitating proteins, particularly enzymatic ones, or by combining with SH groups.

2.2.1.3. Alcohols

Alcohols are bactericidal and fungicidal, though not sporicidal. They act mainly as solvents on the cytoplasmic membrane components.

Isopropanol is the most commonly used antiseptic alcohol in the USA.

Ethanol shows maximum antiseptic activity around 50% dilution and has long been known as a food preservative.

Methanol is less active and more toxic; it inhibits cerebral monoamine oxidase, causing irreversible damage. Higher alcohols (propyl, butyl, amyl) have increasing bactericidal power with molecular mass, but their water solubility decreases, limiting use.

Alcohol efficacy is enhanced when combined with formaldehyde (10%) or sodium hypochlorite (2000 ppm). Ethanol and propanol are most effective at around 70% concentration. They are ineffective against fungal spores and are rapidly inactivated by proteins.

2.2.1.4. Phenols

Phenols are a group of generally strong-smelling antimicrobials with potent microbicidal properties. Formerly, they were widely used as antiseptics and disinfectants. They are bactericidal and fungicidal but have limited activity on spore forms. Their action increases in the presence of sodium or potassium salts but decreases with alkalis and organic matter. Clear phenol solutions (1–5%) remain stable for about one week.

2.2.1.5. Soaps and Detergents

a) Soaps

Soaps are usually sodium or potassium salts of high-molecular-weight fatty acids, obtained by saponification. Their antiseptic power varies among microbial species, and their effect is

primarily mechanical: they lower surface tension and increase water's wetting power, enabling microbes to be removed by rinsing.

b) Detergents

Anionic detergents include sodium lauryl sulfate (SDS) and teepol. Cationic detergents consist mainly of quaternary ammonium compounds. Anionic detergents have weak antimicrobial activity, while quaternary ammonium derivatives show pronounced effects. They possess strong wetting and emulsifying power and act as bacteriostatic agents at low concentrations, used as disinfectants and skin antiseptics.

2.2.1.6. Dyes

Dyes exhibit varying degrees of antiseptic activity. They are used mainly for topical applications and for preparing selective culture media in microbiological analysis (Tab.1).

Table 1. Use of dyes

Family	Dye	Use
Thiazines	Methylene blue	Mild antiseptic
Triphenylmethane	Malachite green,	Wound disinfection
	Brilliant green	
	Methyl violet	urinary antiseptics
	Gentian violet	
Acridine	Trypaflavine,	Local disinfectants
	Gonacrine	

Certain dyes selectively act on Gram-positive or Gram-negative bacteria: crystal violet, malachite green, and brilliant green inhibit Gram-positive bacteria; ethyl violet inhibits *Bacillus* species; acridine derivatives inhibit Gram-positive bacteria. This property is used to design selective isolation or culture media.

The table (2) below summarizes the activity and potential use of some disinfectant agents

Table 2. Activity and potential use of some disinfectant agents

Activity Against

Agent	Yeasts and Molds	Gram-Negative Bacteria	Gram-Positive Bacteria	Mycobacteria	Bacterial Spores
Phenols	+++	+++	+++	++	-
Hypochlorites	+	+++	+++	++	++
Alcohols	-	+++	+++	+++	+++
Formaldehyde	+++	+++	+++	+++	+++
Glutaraldehyde	+++	+++	+++	++	+++
Iodophors	+++	+++	+++	++	+
Quaternary Ammonium	+	+++	++	-	-

2.2.1.7. Food Preservatives

A preservative is an additive incorporated into food to slow microbial growth, usually at low doses to avoid toxic effects.

The choice of an antimicrobial food additive is often difficult. A good preservative must meet the following criteria:

- it must be microbicidal rather than microbiostatic. It must be bactericidal and fungicidal rather than bacteriostatic or fungistatic.
- it must be active on pathogenic germs and those responsible for alterations.
- he must be stable.
- it must be harmless to consumption and not diminish the nutritional value of the food.
- it must be authorized and their list is published in the Official Journal

The table (3) below presents some molecules potentially usable as preservatives subject to their authorization by the competent authorities.

Table 3. Some molecules potentially usable as preservatives

Preservers	Tolerance	Sensitive microorganisms	Example of food
Benzoic acid*	0.1%	Yeasts and molds	Margarine, cider, etc.
Caprylic acid		Molds	Cheese packaging
Dehydroacetic acid	65 ppm	Insects	Strawberries
Propionic acid*	0.32%	Molds	Bread, cakes, cheeses
Sorbic acid*	0.2%	Molds	Jellies, cakes
Sulfur dioxide**	200 ppm	Insects, microorganisms	Dried fruits, wine
Antibiotics	10 ppm	Bacteria	Meats, poultry
Sodium chloride	None (Sans)	Microorganisms	Meats
Sodium diacetate	0.32%	Molds	Bread

- * and corresponding salts
- ** sulfur dioxide, sulfites, bisulfites, metabisulfites

Preservatives can be classified according to whether they alter food's organoleptic properties. For instance, adding sodium chloride or sucrose creates hypersalted (brines) or hypersweet (candies, jams) foods, allowing only halophilic or osmophilic microbes to develop (non-dangerous microorganisms). Smoking, the addition of vinegar, alcohol, and organic acids also preserve food but may alter sensory qualities. Non-altering methods include protective coatings, packaging, paraffin, or inert gases (N_2 , CO_2). Alkali silicates preserve eggs, fruits, and vegetables; diphenyl and orthophenylphenol are used to protect fruits and vegetables.

A number of chemical antiseptics or antibiotics may be added to foods to inhibit or suppress the multiplication of specifically altering or potentially dangerous microorganisms:

a) Benzoic acid and benzoates The antimicrobial activity (especially antifungal) of these derivatives is better at relatively low pH (acidic plant products), possessing excellent antimicrobial effects.

b) Propionic acid and propionates. These are inhibitors of mold growth. They are active at low pH.

c) Sorbic acid and sorbates. These compounds are especially active at low pH.

d) Sulphur dioxide (SO_2), sulphites (= SO_3), bisulphites (= HSO_3), and metabisulphites (= S_2O_5). These compounds are used in liquid or gaseous form, or as one of their salts for fruit juices, wines, molasses. Sulphur dioxide has antimicrobial activity and, in some foods, antioxidant properties. Lactic bacteria, many molds are more sensitive to sulphur dioxide than species with fermentative metabolism.

2.2.1.8. Antibiotics

Certain antibiotics serve as food preservatives, including nisin, subtilin, tylosin, pimaricin (natamycin), some tetracyclines, and polymyxins. Those effective against spores are especially useful for non-sterilized products.

Nisin: it is a polypeptide of thirty-four (34) amino-acid polypeptide active against Gram-positive bacteria. Soluble at pH 2, precipitates at pH 7, and is heat-stable under acidic conditions.

Produced by *Lactococcus lactis*, it causes cytoplasmic membrane lysis and is used in cheese and canned food production (500–5000 IU/g).

Subtilin: it is a polypeptide of thirty-two (32) amino-acid, stable between pH 2.5–7, active against Gram-positive and some Gram-negative bacteria, Its sporostatic activity makes it used in the canning industry. It is produced by *Bacillus subtilis*.

Tylosin (C₄₅H₇₇O₁₇N): Active against Gram-positive, Gram-negative, and acid-resistant bacilli; its sporicidal power makes it suitable for canning; produced by *Streptomyces fradiae*.

Pimaricin (Natamycin): A polyene antifungal produced by *Streptomyces natalensis*, used to preserve fruits, juices, vegetables, and cheeses.

3. Industrial Use of Microorganisms

Microorganisms are used in various industries, particularly in the food industry, for multiple purposes: obtaining fermented products, microbial cultures, or metabolites that can serve as food additives, purification processes, etc.

3.1. Fermentations

Fermentation transforms a product by modifying its properties in a favorable way. The nutritional value may be improved through the destruction of toxic or indigestible substances, the formation of microbial growth factors (such as vitamins and amino acids), or, more generally, a favorable modification of its chemical composition. Organoleptic qualities can also be enhanced through the formation or transformation of flavor and aroma compounds. Furthermore, the preservation potential of the product may be improved by eliminating substances that support the development of undesirable contaminants or by producing compounds with stabilizing or antimicrobial effects (such as acids, alcohols, etc.).

3.1.1. Alcoholic Fermentation

This fermentation, induced by yeasts (mainly *Saccharomyces* species), is involved in the production of wine, beer, cider, and various fermented beverages. Its main purpose is the production of ethanol; however, many other compounds that influence sensory characteristics are also formed. The alcohol content ensures the stability of the product under proper storage conditions. The strains and techniques used vary depending on the product. The contribution of bacteria to the production of alcoholic beverages or ethanol is marginal (*Zymomonas*). Alcoholic fermentation is also used in baking, where the essential factor is the production of CO₂, which causes the dough to rise.

Glucose ($C_6H_{12}O_6$) \rightarrow 2 Ethanol (C_2H_6O) + 2 CO_2 + 2 H_2O + Energy.

3.1.2. Lactic Fermentation

During this process, glucose is broken down into lactic acid, also known as lactate. The overall reaction is: Glucose ($C_6H_{12}O_6$) \rightarrow 2 Lactic Acid ($C_3H_6O_3$). This fermentation is carried out by lactic acid bacteria and is typically used in the production of dairy products such as cheese and yogurt. However, many other foods are produced through lactic fermentation, including coffee, olives, soy derivatives, cured meats, and sauces. Lactic acid contributes to the organoleptic properties and serves as an excellent acidifying agent, inhibiting the growth of spoilage microorganisms sensitive to low pH.

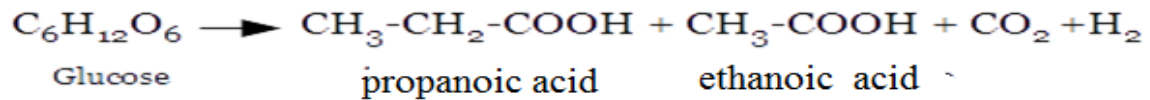
Homolactic fermentation: Glucose undergoes glycolysis and is converted into pyruvate, which is then almost entirely transformed into lactate by the bacterial enzyme lactate dehydrogenase. Since only one main product is formed, this process is termed homofermentative. It is carried out by *Streptococcus*, *Lactococcus*, and certain *Lactobacillus* species.

Heterolactic fermentation: In this case, glucose is catabolized through glycolysis and the pentose phosphate pathway, yielding multiple products such as lactate, ethanol, CO_2 , and acetate. Responsible bacteria include *Leuconostoc*, various *Lactobacillus* species, and some Enterobacteriaceae.

Bifidobacterium (*Lactobacillus bifidus*) performs a specific type of heterolactic fermentation characterized by non-gas production. These bacteria are used as additives in certain yogurts for their beneficial probiotic effects on intestinal flora (probiotic).

3.1.3. Propionic Fermentation

Propionic fermentation converts lactose into propanoic acid, ethanoic (acetic) acid, CO₂, and H₂.



Propanoic and acetic acids contribute to the flavor of cooked cheeses, while carbon dioxide is responsible for the holes in these cheeses (e.g., Comté, Gruyère, and Emmental). The anaerobic bacteria responsible for this process belong to the genus *Propioni bacterium*.

3.1.4. Acetic Fermentation

This process is unique because it is carried out by aerobic bacteria (Acetobacter, Gluconobacter). Oxygen is required for the formation of reduced coenzymes (NADH, H⁺). Despite involving oxidation, it is still termed fermentation because the bacteria cannot completely oxidize the substrate. Ethanol is partially oxidized to acetic acid:



The best-known application is vinegar production, which is made from alcohol-containing substances. Various methods exist: traditional barrel fermentation with large surface exposure to air, trickling of alcohol solutions over bacterial carriers, or industrial fermenters with strong aeration and agitation (Acetator systems). Vinegar is stabilized by its low pH and used as a preservative.

4. Determining Optimal Environmental Conditions for Microbial Growth

Bacteria grow only if their environment is suitable. When conditions are suboptimal, growth may be slow, absent, or lead to cell death, depending on the species and conditions. Numerous physicochemical characteristics of food and its surroundings determine microbial growth.

4.1. Nutritional Conditions

For microorganisms to live or grow, they must find in their environment the essential elements for synthesis. Most microorganisms relevant to the food industry are heterotrophic—they require organic compounds as energy sources. The main elements necessary for growth include C, O, H, and N in large amounts; P and S in smaller quantities; and trace elements (Ca, Co, Cu, K, Mg, Mn, Na, Fe, Zn, etc.) in minimal amounts. Energy-providing organic molecules simultaneously supply carbon, hydrogen, oxygen, and sometimes nitrogen.

Each microorganism has specific nutritional requirements. For a medium to support growth, it must contain all the necessary nutrients in forms accessible to the microorganism

Naturally, a medium must not contain inhibitors to allow growth: certain natural products play such a role (tannins, essential oils, phenolic compounds, lysozyme, etc.), as well as technological adjuvants.

4.2. Physicochemical Conditions

4.2.1. pH: The pH depends on proton concentration and strongly influences the ionic balance of the medium, membrane permeability, substrate availability, on extracellular enzymatic activities and to a much lesser extent on intracellular activities.

Acidophilic microorganisms have optimal growth below pH 5.5 or can grow under acidic conditions. Yeasts and molds are typical examples. From a hygienic standpoint, most pathogenic

bacteria cannot grow below pH 4.5, making acidic foods relatively safe. At pH 4.5, *Clostridium botulinum* toxin production ceases. Gram-negative bacilli are acid-sensitive and fail to grow at low pH values.

4.2.2. Temperature: Each bacterial species grows fastest at its optimal temperature. Growth slows as temperature deviates from this optimum, and ceases beyond specific minimum and maximum thresholds.

We distinguish different cases:

Psychrophilic (or psychrotrophic) microorganisms are capable of growing below 15-20 °C, including some down to negative temperatures. There are facultative psychrophiles and others that are obligate (strict psychrophiles): the latter are generally not capable of growing at 20 °C. Among facultative psychrophiles are found many Gram-saprophytic bacteria (*Achromobacter*, *Flavobacterium*, *Pseudomonas*, etc.), molds (*Cladosporium*, *Sporotrichum*, etc.) and pathogenic germs (*Listeria*, *Yersinia*),

Mesophiles: The majority of microorganisms; grow between 15–45°C.

Thermophiles: Grow above 45°C; extreme thermophiles tolerate up to 80°C or more. Examples: *Lactobacillus thermobacterium*, *Streptococcus thermophilus*, *Propionibacterium shermanii*, *Clostridium thermosaccharolyticum* spore-forming, and *Bacillus stearothermophilus*. Certain ascomycete fungi (*Aspergillus*, *Cladosporium*). Do not confuse thermophilic and heat-resistant microorganisms.

Thermophilicity differs from thermoresistance. Thermoresistance is the ability to withstand heat treatment while thermophilic is the ability to develop at high temperatures.

The action of temperature occurs at several levels: activation of metabolism according to Arrhenius' Law (Fig. 2), heat stress and denaturation of cellular constituents. cold slows down

and blocks microbial metabolism, usually without killing cells, however freezing (especially slow) can cause high mortality. Temperature also influences physical transfers.

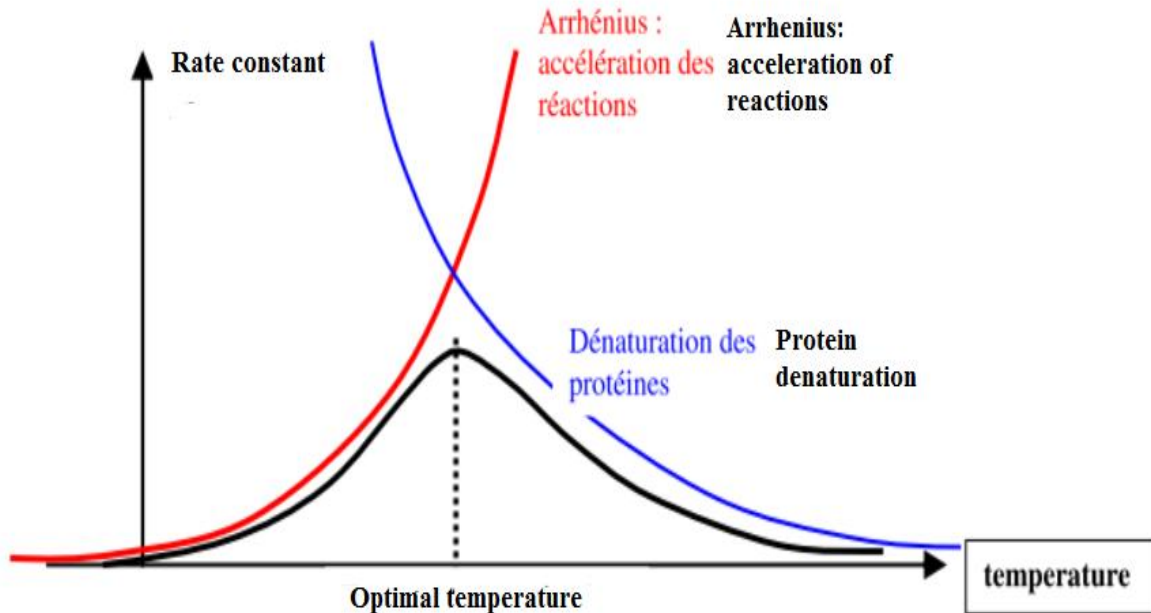


Figure 2. Influence of temperature on growth rate (Arrhenius law)

Water Activity (a_w): Microbial survival and growth require a certain amount of free water (otherwise there is plasmolysis and blockage of enzymatic activities).. Each species has specific requirements. The water activity (a_w) of a food is defined as the ratio of the vapor pressure of water in the food to that of pure water (P_s/P_e). In closed systems, a_w correlates with relative humidity (HR): $a_w = HR/100$. A decrease in a_w negatively affects growth below 0.97, inhibition occurs. Osmotic stress causes water loss, the nature of the solute influences the a_w : at equal concentrations, a sugar and a salt will not have the same effect.

Microorganisms that grow on dry products (some molds) are called **xerophilic**, those that grow on sweet environments (*Zygosaccharomyces rouxii*) are called **osmophilic**, and those that grow on salty products are called halophilic (*Vibrio*). In general, an $a_w < 0.7$ only allows the slow growth of rare osmophilic or xerophilic organisms and an $a_w < 0.62$ blocks all microbial development.

The lower the a_w , the more difficult it is for germs to survive. This explains the effectiveness of desiccation in food preservation (e.g., milk powder lyophilization).

Redox Potential (rH): Depending on their respiratory mode, microorganisms may be strict aerobes, strict anaerobes, facultative anaerobes, or microaerophiles. These traits explain the diversity of spoilage phenomena:

- Strictly aerobic molds and yeasts grow on the surface, forming layers of varying thickness.
- Fermenting yeasts multiply at depth, producing gas.
- *Clostridia* only grow in the absence of oxygen (in bulk, canned, etc.) (strictly anaerobic microorganisms).
- *Pseudomonas* only develop in the presence of oxygen (surface) (strictly aerobic microorganisms).
- *Lactobacillus* microaerophilis only develop at a reduced oxygen content (lactic bacteria).
- other aero-anaerobes or facultative anaerobes (Enterobacteria)

In food, the presence or absence of oxygen can be considered a fundamental parameter for microorganisms.

Chapter III.

Microbiological

Problems in the Food

Industry

Chapter III: Microbiological Problems in the Food Industry

It is of utmost importance to protect the food production process from contamination. Contamination can have various consequences, ranging from compromised product quality to regulatory challenges, and the first line of defense in protecting our food lies at the manufacturing stage.

1 - The Four Types of Food Contamination

There are four types of food contamination: chemical, microbial, physical, and allergenic. All foods are vulnerable to these four types. Food handlers are legally required to ensure that the foods they prepare and store are free from these contaminants in order to protect consumers.

The importance of contamination control in the food industry cannot be underestimated. Food contamination is dangerous, with consequences ranging from illness to death. Therefore, understanding the four types of contamination and knowing how to prevent them is essential to ensure the safety of food and consumers.

1.1. Chemical Contamination

Chemical contamination occurs when harmful chemicals infiltrate our food supply. The most common culprits are residues from cleaning products, pesticides present on fruits and vegetables, and various chemical additives. Pesticide residues, cleaning agents, and food additives fall under chemical contaminants.

Food preparers and handlers must prioritize preventing chemical contamination in the foods they serve. To ensure this, they must:

1. Ensure that fruits and vegetables are washed effectively to remove any pesticide residues.
2. Use containers and utensils specifically designed for food use to prevent harmful chemical leaching.

3. Carry out routine equipment inspections to detect any existing chemical residue or signs of wear.
4. Always store chemicals in a designated area, separate from food.
5. Keep food covered during cleaning operations.

1.2. Physical Contamination

Certain illnesses and injuries may result from the presence of foreign bodies in food. These physical hazards may arise from contamination or poor practices at several stages of the food chain, from harvest to consumption, including steps within the processing unit.

Physical contamination occurs when unwanted physical elements, such as glass or plastic, infiltrate the food supply. The sources of these contaminants may originate from raw materials, packaging, or even personal items in the production area. Common physical contaminants include glass fragments, metal pieces, plastic particles, and wood splinters.

You can prevent physical contamination by:

1. Thoroughly inspecting raw materials to detect any foreign bodies before incorporating them into the production process.
2. Using tools and equipment specifically designed to minimize breakage and splintering, including wearing appropriate personal protective equipment (PPE) in food production areas (following dress code principles such as removing jewelry and wearing hairnets).
3. Implementing a strong quality control system to identify and address any cases of physical contamination during production.

Integrating these preventive measures forms the first line of defense in food safety, effectively blocking contaminants and maintaining the highest hygiene standards. This ensures the protection of your products against any risk of physical contamination.

1.3 . Microbial Contamination

Microbial and biological contamination refers to living organisms such as bacteria and viruses that contaminate food and production processes. The most common causes are raw foods such as meat, unwashed vegetables, and contaminated hands.

Microbial contamination is the leading cause of foodborne illness outbreaks. To avoid this type of contamination, it is essential to follow strict and rigorous food hygiene practices, including:

1. Maintaining impeccable personal hygiene when handling food or contacting surfaces.
2. Ensuring thorough cooking to eliminate harmful bacteria.
3. Keeping raw and ready-to-eat foods separate at all stages of storage and handling.
4. Controlling pests and ensuring they are not present in the facility.

Integrating these preventive measures into contamination control strategies significantly reduces the risk of microbial transfer and improves overall hygiene.

1.4 - Allergenic Contamination

Allergenic contamination occurs when a food that triggers an allergic reaction comes into contact with another food. For example, when a surface used to prepare regular meals is later used to handle allergen-free dishes.

Another example is when the same knife used to cut regular bread is then used to cut gluten-free bread, or when pasta is stored in a container that previously held peanuts.

There are 14 named allergens that account for the majority of food allergies. This list includes allergens such as gluten, peanuts, eggs, dairy products, mustard, soy, and fish.

For individuals with food allergies, even trace amounts of an allergen can cause a fatal reaction. It is therefore essential to prevent allergenic contamination in the facility. To do this, you must:

1. Clearly label products containing allergenic ingredients.
2. Create separate zones for processing allergen-sensitive products.
3. Train staff on preventing cross-contact during production.
4. Conduct regular testing to ensure the absence of allergens in the production environment.

Regular allergen testing and validation of cleaning procedures are essential to maintaining a safe, allergen-free production environment.

2 - Manufacturing Incidents

-Example: Incident During Cheese Production

Incidents often arise from the nature of the milk itself: the milk may be too acidic and coagulate before renneting (due to an excess of lactic acid bacteria and/or poor storage conditions), and it may be contaminated by undesirable microorganisms. Additionally, bacteriophages present in milk may hinder or inhibit the proliferation and activity of lactic acid bacteria.

If the number of coliforms is very high, mixed-acid or butylene-glycolic fermentation will occur rapidly, resulting in swelling and a sharp flavor in fresh cheeses, and small holes in soft cheeses. *Enterobacter aerogeneses* is frequently implicated. Heterofermentative lactic acid bacteria and yeasts often play a similar negative role for many cheeses that should not have openings.

Manufacturing defects (swelling with gas bubbles and sharp flavor) observed in two fresh cheeses (such as petit-suisse and fresh cheese) are caused by heavy contamination with the yeast “*Torulopsis sphaerica*”.

Bacteria such as *Pseudomonas* or *Alcaligenes* may develop and cause viscosity. On the rind, contaminating microorganisms may appear and lead to undesirable appearance, color, or taste, including *Aspergillus*, *Penicillium*, *Mucor*, *Geotrichum*, *Cladosporium*, yeasts, and bacteria such as *Arthrobacter* and *Brevibacterium*.

Sometimes, ripening is excessive, leading to overly abundant proteolysis and lipolysis. Manufacturing defects do not always depend solely on the composition of the initial or contaminating flora; they depend greatly on ripening and production conditions. When carried out properly, these operations have a selective effect on the flora and prevent the development of harmful microorganisms.

Chapter IV.

Biotechnological

Processes

Chapter IV: Biotechnological Processes

Biotechnology, in its broadest sense, refers to any technique that uses living organisms to manufacture products, improve plants, or develop microbes for specific applications. This definition covers traditional techniques of plant selection, breeding, and fermentation, whose origins date back thousands of years, as well as modern biotechnology methods, such as the industrial use of recombinant DNA (deoxyribonucleic acid), cell fusion, and new biotransformation techniques.

1. Production of Amino Acids

1.1. Definition

Amino acids (AAs) are the fundamental components of proteins. More than 300 amino acids have been identified. They are classified as follows:

A -Standard or natural amino acids:

These are the 20 proteinogenic amino acids that constitute proteins and peptides.

B - Non-standard amino acids: These include:

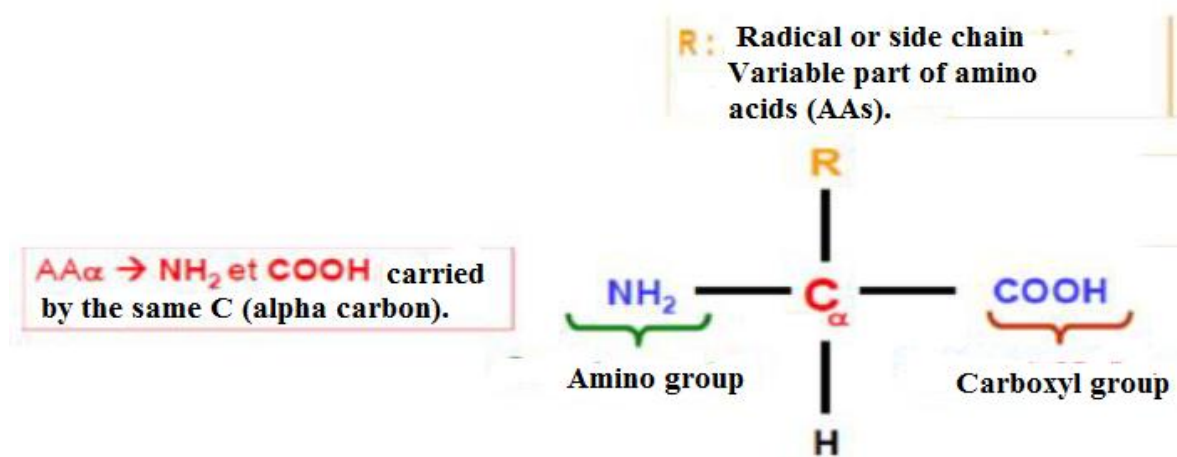
-Non-proteinogenic or modified amino acids: amino acids within a protein that have been chemically modified after translation. Examples: hydroxyproline, hydroxylysine, phosphoserine.

-Metabolic intermediates, structural elements of other molecules (lipids, coenzymes), or biologically active molecules. Examples: ornithine and citrulline, intermediates of the urea cycle.

1.2. Structure

Amino acids, or α -amino acids, share a common structural motif:

- An acid function: carboxyl group (COOH)
- A basic function: primary amine group (NH₂)
- A side chain or “R group” that varies from one amino acid to another
- An α-carbon (alpha carbon)



- The nature of the R group determines the identity of each amino acid.

Each amino acid has a hydrocarbon skeleton with two functional groups: an amine and a carboxyl group.

1.3. Origin of Amino Acids

Amino acids have a dual origin:

- Exogenous (dietary):** from animal and plant proteins
- Endogenous:** synthesized by the body or produced from degradation of endogenous proteins

1.4. Classification of Standard Amino Acids

According to essentiality:

- ❖ Essential amino acids: Not synthesized by the human body and must be supplied through diet.

There are 8 in humans: Leucine, isoleucine, methionine, threonine, lysine, valine, tryptophan, phenylalanine.

❖ Non-essential amino acids: These can be synthesized by the body: Glycine, alanine, cysteine, serine, glutamic acid, aspartic acid, arginine, histidine, tyrosine, proline, asparagine, glutamine.*

❖ Semi-essential amino acids: Under certain physiological conditions, non-essential amino acids may become indispensable:

- Histidine and arginine are essential for children (growth) and pregnant women.

- Arginine is essential for infants.

1.5. Synthesis of Amino Acids

Amino acids synthesized within the cell are mostly used for protein formation due to multiple cellular regulatory systems. From an industrial perspective, the most important amino acids are the essential or indispensable ones.

Amino acids may be produced through several methods:

-Chemical synthesis

-Enzymatic processes

-Extraction from amino-acid-rich natural sources

-Microbial fermentation

1.5.1. Metabolic Pathways of Amino Acid Synthesis

For the synthesis of an amino acid by a microorganism, it requires the presence of intermediates to form the hydrocarbon chain and others for the amine function.

The synthesis of amino acids by microorganisms is largely carried out from intermediate products of carbohydrate metabolism (source of the hydrocarbon chain): erythrose-phosphate, product of the pentose cycle), products of glycolysis (P-trioses: phosphoenolpyruvate, phosphoglycerate; pyruvate, acetyl-CoA) and products of the Krebs cycle (oxaloacetate, α -ketoglutarate) (Fig. 3).

The amine group comes from another amino acid (pre-existing amino acid) through a transamination reaction.

The form of nitrogen most readily used by microorganisms is the ammoniacal form, but other forms may be incorporated, including the N_2 molecular form.

- The use of nitrates (NO_3^-) and nitrites (NO_2^-) is carried out thanks to the existence of the corresponding reductases that transform these compounds into ammonium (NH_4^+).

- The use of molecular nitrogen (N_2) is only possible in a limited number of microorganisms (Azotobacter, Achromobacter, Klebsiella, Bacillus, Enterobacter, Actinomyces, certain Clostridium, photosynthetic bacteria, Cyanophyceae...), some of which are symbiotic (Rhizobium, Frankia).

The main amino acids obtained by microbial fermentation are L-glutamate (Micrococcus glutamicus), lysine, threonine (E.coli), isoleucine (Streptomyces rimosus, Serratia, Bacillus subtilis, Pseudomonas, E.coli, etc.), valine (Enterbacter, Micrococcus glutamicus).

2. Production of Organic Acids

2.1. Definition

An organic acid is a compound capable of releasing a positively charged ion (H^+) in an aqueous medium. The most common organic acids are carboxylic acids, such as: Lactic acid, acetic acid, propionic acid, butyric acid, citric acid, etc.

Organic acids are chemical compounds of natural origin (plant, animal, or microbial) that contain at least one acidic functional group, usually the carboxyl group ($-COOH$), and play an important role in the metabolism of living organisms as well as in many industrial processes.

They are characterized by their ability to release hydrogen ions (H^+) in solution, which gives them their acidic properties.

Examples:

- Lactic acid
- Acetic acid (vinegar)
- Citric acid (citrus fruits)
- Propionic acid

-General roles:

- Participation in cellular metabolism
- Use as food preservatives
- Involvement in fermentation processes
- Applications in the food, pharmaceutical, and chemical industries

-Role of Organic Acids in the Food Industry

Organic acids play several important roles:

- **Food preservation:** they lower the pH, which prevents the growth of microorganisms responsible for food spoilage and foodborne illnesses.
- **Flavor enhancement:** they provide a characteristic sour taste (e.g., citric acid in beverages).
- **pH regulation:** they stabilize the acidity level of food products.
- **Natural antimicrobial agents:** they inhibit the growth of bacteria, yeasts, and molds.
- **Participation in fermentation:** they are produced during fermentation processes (lactic, acetic, etc.).

-Main Types of Organic Acids

They can be classified according to their origin or structure:

a) Acids produced by fermentation

- Lactic acid (lactic fermentation)
- Acetic acid (acetic fermentation)
- Propionic acid (propionic fermentation)
- Butyric acid (butyric fermentation)

b) Acids naturally present in foods

- Citric acid (citrus fruits)
- Malic acid (apple)
- Tartaric acid (grapes)

c) Industrial food acids

- Benzoic acid (preservative)
- Sorbic acid (antifungal agent)

2.2. Applications of Organic Acids

Organic acids are widely used as additives in the food industry. They may function as:

- Preservatives
- Acidifying agents
- Antioxidants
- Emulsifiers

Applications of Organic Acids

Organic acids are widely used in many fields due to their antimicrobial, acidifying, chelating, and preservative properties. Their main applications include:

1. Food industry

This is the most important area of use:

- **Food preservatives:** they inhibit the growth of bacteria, yeasts, and molds (e.g., lactic acid, acetic acid, benzoic acid).
- **Acidity regulators:** they help adjust the pH of food products (e.g., citric acid, malic acid).
- **Flavor enhancement:** they provide a pleasant acidic taste (soft drinks, confectionery).
- **Fermentation processes:** used in the production of fermented foods (yogurt, cheese, fermented vegetables).

2. Pharmaceutical industry

- Used as **excipients** in drug formulations.
- pH adjustment in oral and injectable solutions.
- Preparation of stable forms and salts of active pharmaceutical ingredients.

3. Cosmetic industry

- pH regulation in cosmetic products (creams, shampoos).
- Mild exfoliating agents (e.g., alpha-hydroxy acids such as citric and lactic acid).
- Improvement of formulation stability.

4. Agriculture

- Soil and irrigation water acidification.
- Feed additives to improve digestion and reduce pathogens in livestock.
- Preservation of silage.

5. Chemical industry

- Intermediate compounds in the synthesis of solvents, plastics, and polymers.
- Chelating agents (e.g., citric acid) for binding metal ions.

6. Cleaning and detergents

- Natural descaling agents (citric acid, acetic acid).
- Removal of limescale and metal oxide deposits.

In summary

Organic acids are versatile compounds mainly used in the food industry, but also in pharmaceuticals, cosmetics, agriculture, and chemical industries due to their acidifying, preservative, and reactive properties.

2.3. Microorganisms Producing Organic Acids

Many microorganisms are capable of producing organic acids during their metabolism, especially through fermentation processes. The main groups include:

1. Lactic acid bacteria (LAB)

These are the most important producers of lactic acid:

- *Lactobacillus*
- *Lactococcus*
- *Streptococcus thermophilus*
- *Leuconostoc*

They mainly produce **lactic acid** from carbohydrates and are widely used in food fermentation (yogurt, cheese, fermented vegetables).

2. Acetic acid bacteria

- *Acetobacter*
- *Gluconobacter*

They produce **acetic acid** by oxidizing ethanol (used in vinegar production).

3. Fungi (molds)

- *Aspergillus niger* (very important industrial species)
- *Penicillium* species
- *Rhizopus* species

They produce various organic acids such as:

- citric acid
- gluconic acid
- fumaric acid

4. Yeasts

- *Saccharomyces cerevisiae*
- *Candida* species

They can produce small amounts of organic acids (e.g., acetic acid, succinic acid) during fermentation.

5. Bacteria producing mixed organic acids

- *Escherichia coli* (under anaerobic conditions)
- *Clostridium* species

They produce a mixture of acids such as:

- lactic acid
- acetic acid
- butyric acid
- formic acid

In summary

Organic acids are mainly produced by lactic acid bacteria, acetic acid bacteria, certain fungi (especially *Aspergillus*), yeasts, and some anaerobic bacteria through different fermentation pathways.

Microbial species involved in the production of organic acids differ depending on the type of industrially relevant acid (Tab.4).

Table 4. Organic acids produced by microorganisms and their uses in the food industry.

Organic Acids	Producing Micro-organisms	Examples of Use
Acetic acid	<i>Acetobacter aceti</i> (bacteria)	Preservative in mayonnaise, pastries...
Lactic acid	<i>Lactobacillus</i> (bacteria) <i>Aspergillus griseus</i> (mold) <i>Rhizopus</i> (mold)	Preservative and acidulant for jams, soft drinks, olives, fish...
Citric acid	<i>Aspergillus niger</i> (mold)	Acidulant and antioxidant in soft drinks, dairy products, frozen fruits...
Gluconic acid	<i>Aspergillus niger</i> (mold) <i>Penicillium chrysogenum</i> (mold)	Acidulant for meats, flavor enhancer in margarine...
Fumaric acid	<i>Rhizopus</i> (mold) <i>Mucor</i> (mold)	Acidulant in fruit juices...
Malic acid	<i>Penicillium brevicompartum</i> (mold) <i>Leuconostoc</i> (bacteria) <i>Aspergillus oryzae</i> (mold)	Acidulant in fruit juices, ice cream...
Tartaric acid	<i>Penicillium notatum</i> (mold)	Acidulant for beverages...
Propionic acid	<i>Propionibacterium spp</i>	Preservative for many foods, including pastries...

2.4. Metabolic Pathways for Organic Acid Production

Organic acid production begins with the degradation of glucose (glycolysis) and the formation of pyruvate. The conversion of pyruvate into an organic acid is called a «fermentation process» (see Chapter 2 on fermentation).

2.4.1. Lactic Fermentation

Lactic fermentation is an anaerobic fermentation reaction that occurs in certain bacteria and in animal cells such as muscle cells, break down sugars, mainly glucose, to produce **lactic acid** as the main end product..

This type of fermentation is very important in the **food industry**, especially in the production of yogurt, cheese, sauerkraut, and other fermented foods.

There are two types of lactic fermentation: Homolactic fermentation and Heterolactic fermentation (Fig. 4 and 5):

1. Homolactic fermentation

- Produces **only lactic acid** from glucose.
- Simplified equation: glucose \rightarrow lactic acid.
- It is carried out by bacteria such as *Lactobacillus* and *Streptococcus*.
- **Importance:** rapid acidification of foods; widely used in fermented dairy products (yogurt, fermented milk).

2. Heterolactic fermentation

- Produces **several compounds:** lactic acid, CO₂, ethanol, and/or acetic acid.
- Carried out by bacteria such as *Leuconostoc*.
- **Importance:** contributes to flavor formation and texture in some fermented foods (sauerkraut, certain cheeses).

In summary:

- **Homolactic** \rightarrow produces only lactic acid.
- **Heterolactic** \rightarrow produces lactic acid + gases + other compounds.

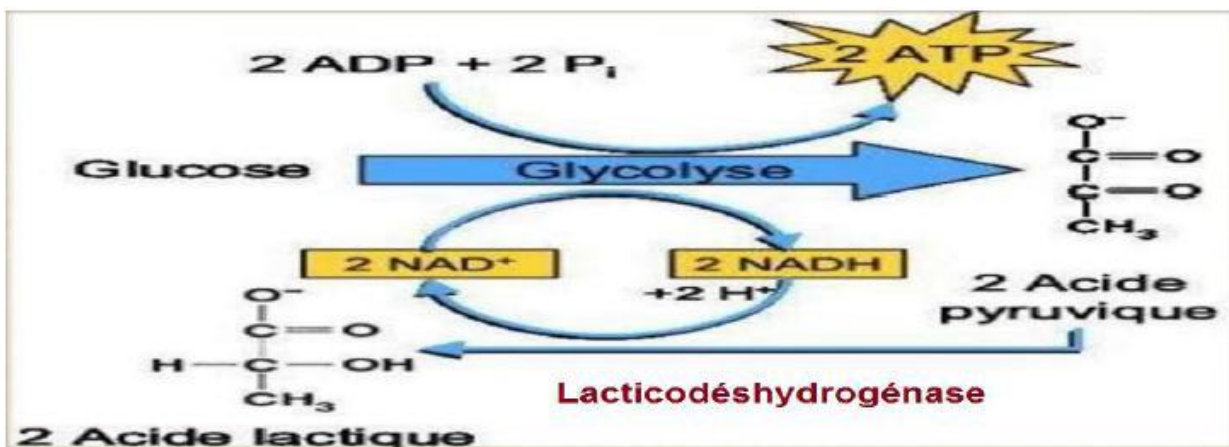


Figure4. Homolactic fermentation.

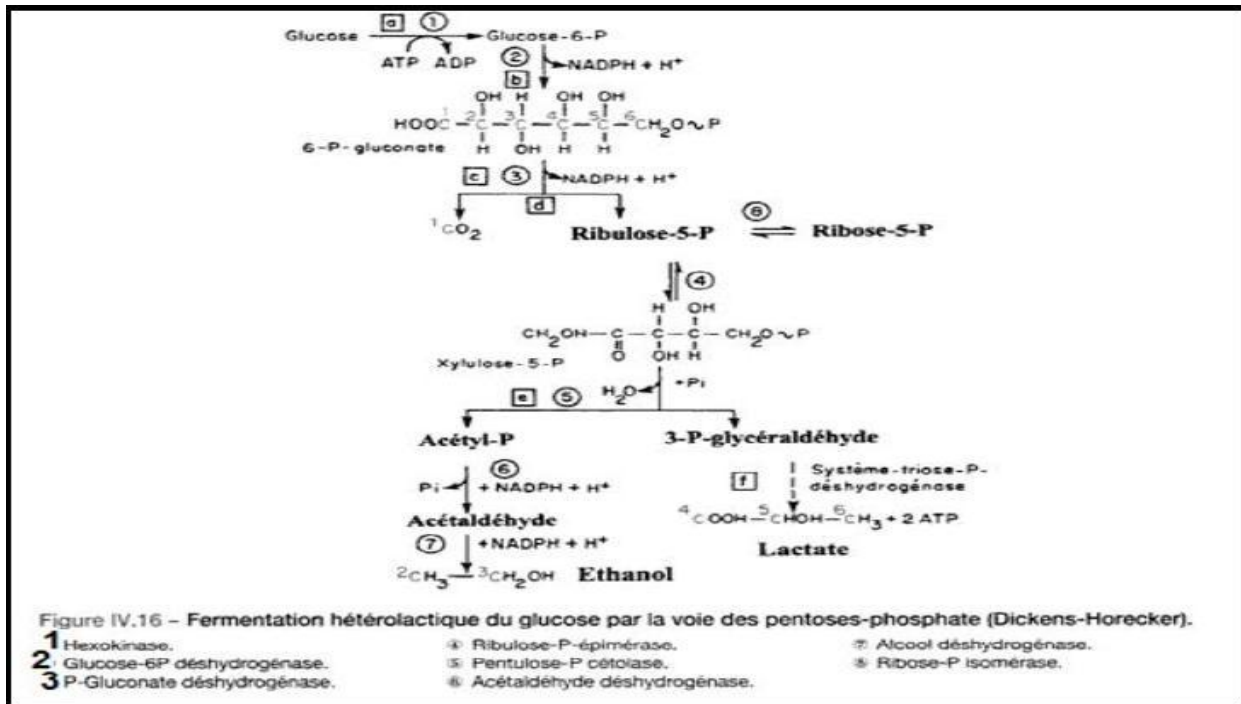


Figure 5. Heterolactic fermentation

Lactic fermentation plays a major role in the food industry, both in food processing, preservation, and quality:

1. Food preservation

Lactic acid produced lowers the pH, which inhibits the growth of pathogenic and spoilage microorganisms.

It therefore allows natural food preservation without the need for significant chemical additives.

2. Transformation of food products

It is used to produce many fermented foods, including:

- yogurt and fermented milk,
- cheese,
- sauerkraut,

- fermented olives,
- fermented cured meats.

3. Development of flavors and texture

Lactic fermentation improves:

- taste (pleasant acidity),
- aroma,
- texture (firmness of yogurts and cheeses).

4. Improvement of food safety

It limits the growth of pathogenic bacteria (e.g., *Salmonella*, *E. coli*).

Thus, it contributes to better food safety.

5. Nutritional value and health

Some fermented products have beneficial effects on the intestinal flora thanks to lactic acid bacteria (probiotics).

In summary:

Lactic fermentation is essential in the food industry because it ensures preservation, transformation, improvement of taste and texture, as well as the safety and nutritional value of food.

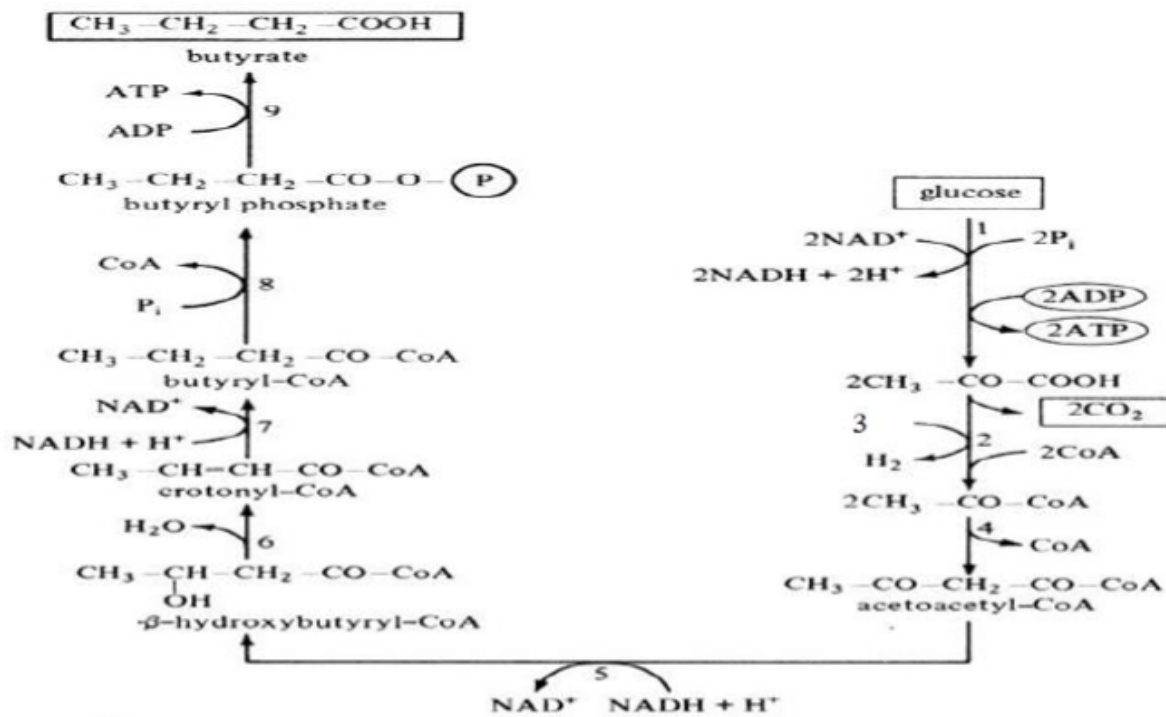
2.4.2. Butyric Fermentation

Butyric fermentation is an anaerobic metabolic process (occurring in the absence of oxygen) in which certain microorganisms mainly bacteria of the genus *Clostridium*, break down organic substrates (such as glucose) to produce primarily butyric acid, along with other compounds such as carbon dioxide (CO₂), hydrogen (H₂), and sometimes acetic acid (Fig.6).

Butyric acid can be:

- **Beneficial** → contributing to the aroma of certain fermented products,
- **Harmful** → when produced in excess, as it negatively affects the taste, odor, and overall quality of foods.

Butyric acid is naturally present in foods such as butter and certain cheeses.



Overall reaction: Glucose + 3 ADP + 3 Pi → Butyrate + CO₂ + 2 H₂ + 3 ATP

Enzymes involved: 1. Glycolytic enzymes, 2. Pyruvate dehydrogenase, 3. Hydrogenase, 4. Acetyl-CoA acetyltransferase (thiolase), 5. β-Hydroxybutyryl-CoA dehydrogenase, 6. Crotonase, 7. Butyryl-CoA dehydrogenase, 8. Phosphotransbutyrylase, 9. Butyrate kinase.

Figure 6. Production of butyric acid

Derivatives of butyric acid are chemical compounds obtained by modifying this acid (butanoic acid). They are highly important in food chemistry, biochemistry, and industry.

1. Salts of butyric acid (butyrates)

- **Examples:** sodium butyrate, calcium butyrate
- **Role:**
 - used as food additives or dietary supplements,
 - Play a beneficial role in intestinal health.

2. Esters of butyric acid

- **Examples:**
 - ethyl butyrate
 - methyl butyrate
- **Role:**
 - widely used as food flavorings,
 - provide fruity aromas (pineapple, apple, strawberry).

3. Amides of butyric acid

- Less common
- Used in organic chemistry and research.

4. Derived alcohols (reduction of the acid)

- **Example:** butanol
- **Use:**
 - solvents,
 - chemical industry applications.

5. Activated derivatives

- **Example:** butyryl-CoA
- **Role:**

- key intermediate in cellular metabolism (especially in fermentation).

Summary:

The main derivatives of butyric acid are:

- **salts (butyrates)** → health and additives,
- **esters** → food flavorings,
- **amides and metabolic derivatives** → biochemistry and industry.

A clear summary table(4) of the main derivatives of butyric acid is provided below:

Table 4: derivatives of butyric acid

Type of derivative	Example	Mode of formation	Main role / application
Salts (butyrates)	Sodium butyrate	Reaction with a base (NaOH, Ca(OH) ₂)	Intestinal health, food additives
Esters	Calcium butyrate	Reaction with an alcohol (ethanol, methanol)	Fruity flavoring agents (pineapple, apple, strawberry)
	Ethyl butyrate		
	Methyl butyrate		
Amides	Butyramide	Reaction with an amine	Chemical synthesis, research
Alcohol derivatives	Butanol	Reduction of the acid	Solvents, chemical industry
Metabolic derivatives	Butyryl-CoA	Enzymatic activation within the cell	Metabolism (fermentation, energy production)

2.4.3. Propionic Fermentation

Propionic fermentation is an **anaerobic metabolic process** carried out mainly by bacteria of the genus *Propionibacterium* (especially *Propionibacterium freudenreichii*), in which organic substrates «particularly lactic acid» are converted into **propionic acid**, **acetic acid**, and **carbon dioxide (CO₂)**.

This type of fermentation is especially important in the production of certain cheeses (such as Emmental), where the CO₂ produced is responsible for the formation of “holes,” while propionic acid contributes to the characteristic flavor.

Propionic acid is produced by certain bacteria capable of converting pyruvate through specific reductive pathways (Fig.7).

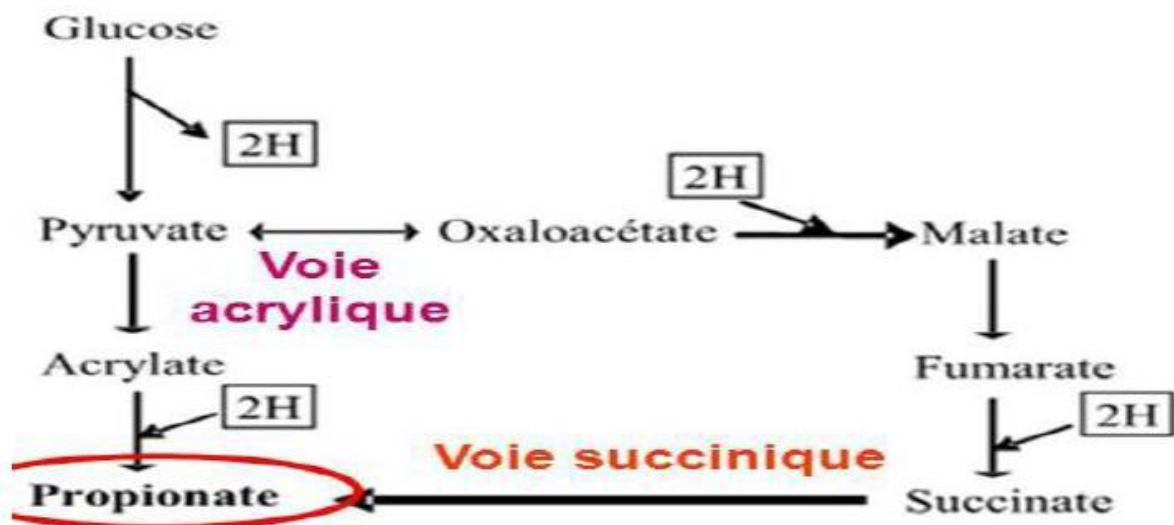


Figure 7. Production of propionic acid

Propionic acid plays several important roles in the food industry, mainly related to **preservation and product quality:**

1. Preservative agent

- It is widely used to **inhibit the growth of molds and certain bacteria.**
- It is particularly effective in bakery products (bread, pastries), where it extends shelf life.
- **2. Improvement of food safety**
- By limiting microbial growth, it helps **reduce the risk of food spoilage and foodborne illness.**

3. Role in cheese production

- Derived from propionic fermentation, it contributes to the **characteristic flavor development** of certain cheeses (such as Emmental).
- It also indirectly contributes to the formation of “holes” through CO₂ production.

4. Acidity regulator

- It can be used to **adjust the pH** of certain foods, improving their stability.

5. Use in salt forms

- It is often used in the form of **propionates** (calcium propionate, sodium propionate), which are easier to incorporate into food products.

In summary:

Propionic acid is mainly used as an **antifungal preservative**, but it also contributes to **flavor development** and **food stability**.

2.4.4. Mixed Acid Fermentation

This type of fermentation results in the production of multiple acids simultaneously.

Mixed acid fermentation is an **anaerobic metabolic process** carried out by certain bacteria (notably enterobacteria such as *Escherichia coli*), in which sugars, mainly glucose, are broken down into a **mixture of acidic and gaseous products**.

Unlike fermentations (Fig.8) that produce a single main end product, mixed acid fermentation generates several compounds, such as:

- **lactic acid,**
- **acetic acid,**
- **formic acid,**
- **ethanol,**
- **carbon dioxide (CO₂),**
- **hydrogen (H₂).**

This type of fermentation is characterized by a significant production of acids, which leads to a **strong decrease in the pH of the medium**. It is often used as a microbiological criterion for identifying certain bacteria in food and clinical analyses.

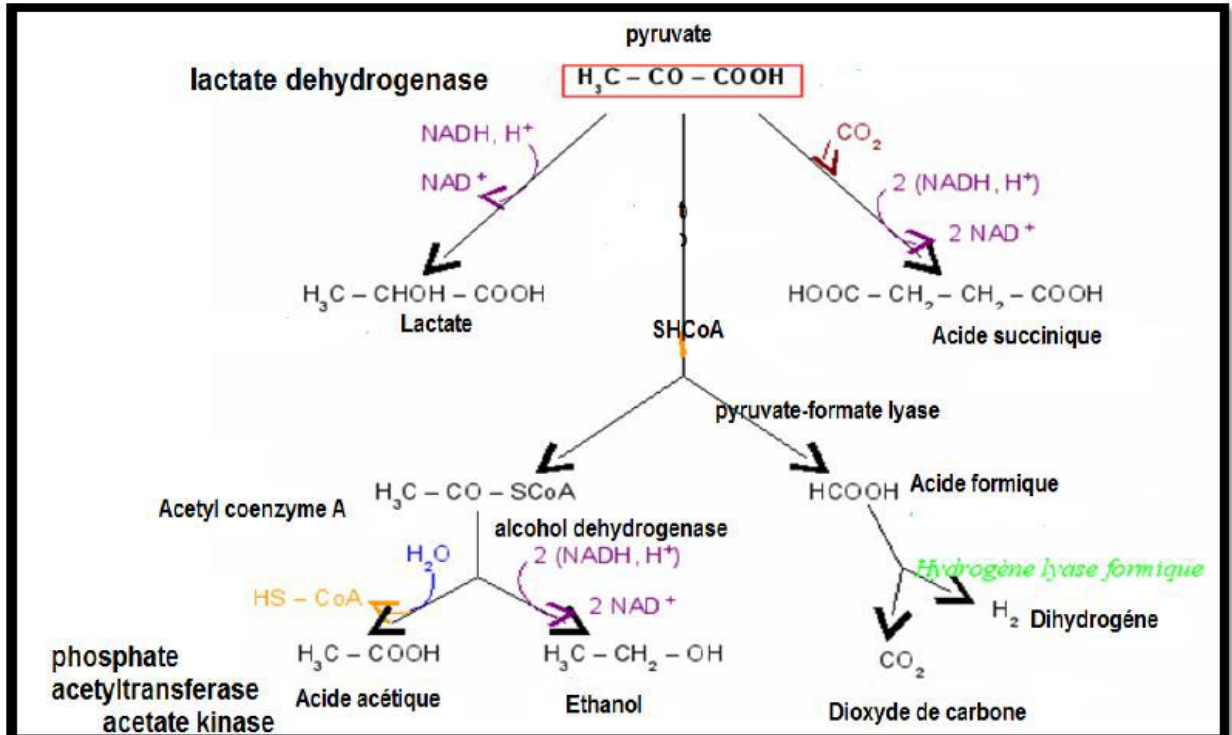


Figure 8. Mixed acid fermentation

-Role of mixed acid fermentation in the food industry

Mixed acid fermentation is not mainly used as an intentional industrial process in food production, but it plays several important roles in **food microbiology and food technology**:

1. Indicator of microbial contamination

- It is mainly used as an **identification test for enteric bacteria** (e.g., *Escherichia coli*).
- Its presence in food may indicate **fecal contamination or poor hygiene during food processing**.
-

2. Contribution to food spoilage

- The products of this fermentation (acids and gases) can cause:
 - **acidification of foods,**
 - **unpleasant odors,**
 - **changes in texture and taste,**
 - **package swelling** (due to CO₂ and H₂ production).

3. Role in food safety

- It helps detect potentially **pathogenic or hygiene-indicator microorganisms**, supporting food quality control and safety monitoring.

4. Importance in microbiological control

- It is used in food laboratories to **differentiate bacteria based on their fermentation products**, which is essential for food and water analysis.

In summary:

Mixed acid fermentation is not used to produce foods, but it is very important for **microbiological control, detection of contamination, and assessment of food safety and quality.**

3. Production of Vitamins

Vitamins are essential micronutrients that must be present in very small quantities in mammals.

Since not all vitamins are synthesized endogenously, mammals obtain them from plants and microorganisms. Vitamins are divided into two groups: Water-soluble: B vitamins and vitamin C, Fat-soluble: Vitamins A, D, E, and K

Prototrophic microorganisms are capable of synthesizing all required growth factors, including vitamins. By modifying their metabolism, microorganisms can be stimulated to produce significant quantities of various vitamins or provitamins. Common microorganisms used in vitamin production include:

Blakeslea trispora → Vitamin A

Ashbya gossypii → Vitamin B₂

Escherichia coli → Vitamin B₃

Corynebacterium glutamicum → Vitamin B₅

Vitamin B₁₂ is produced exclusively by microorganisms (bacteria and archaea). Certain vitamin B₁₂ producing microorganisms exist in the digestive systems of animals, allowing them to obtain this vitamin for growth.

In humans, the absorption of this vitamin (B₁₂) takes place at the level of the small intestine (ileum) while the bacterial production is carried out further away at the level of the large intestine (colon), but this vitamin is excreted in the feces without being used by the body.

Vitamin biosynthesis is complex and regulated by multiple genes. However, the biosynthesis of vitamins is complex and is conditioned by several genes that determine whether a microorganism can be considered as good producer or not. Current research still tends to optimize microbial productions of vitamins by playing on the constitution of culture media and using genetic modifications in order to over-express producer genes and remove repressor genes.

4. Production of Single-Cell Proteins (SCP)

The production of single-cell proteins involves using microorganisms such as bacteria and yeasts to produce proteins on a large scale. These proteins can be used in various applications, from medical research to animal feed.

Agriculture and agro-industrial sectors generate substantial quantities of by-products on a daily basis, which are generally regarded as sources of pollution (straw, bran, paper clarification sludge, wastewater from certain food industries, etc.). Therefore, the recovery and valorization of these by-products for nutritional purposes may represent an interesting alternative. This involves the production of biomass in the form of single-cell protein (SCP).

SCP is produced through fermentation processes involving microorganisms grown on an organic substrate. The interest in these microorganisms lies in their high content of amino acids. SCP can be obtained through the cultivation of bacteria, yeasts, fungi, and algae. Owing to their composition, SCP can be considered as a supplementary food source for both humans and animals.

The interest in filamentous fungi, particularly *Aspergillus niger*, has prompted us to investigate its performance in biomass production in both pure and mixed cultures with the yeasts *Candida utilis* and *Candida pseudotropicalis*, as well as its enzymatic activity.

4.1. Definition

Single-Cell Proteins (SCP) are microbial biomass rich in protein intended for human or animal consumption. They are not pure proteins; rather, they contain: Carbohydrates, Lipids, Nucleic acids, Minerals ; Vitamins

4.2. Microorganisms Used in SCP Production

Generally, four types of microorganisms are used. These are micro-algae, bacteria, yeasts, and molds. Below, some examples:

1. Bacteria:

Bacteria are unicellular prokaryotic organisms lacking a true nucleus.

Escherichia coli is widely used in protein production because:

- Its genetics are well known
- It grows easily in fermenters (the growth conditions are known).
- It expresses high levels of proteins (several grams/L)

Other bacteria used include «*Bacillus subtilis*» and «*Streptomyces*», which have strong secretion capacities but are less well-understood genetically. Species such as **Methylophilus** and **Methylomonas** are also employed.

2. Microscopic Fungi

These are single or multi-cellular eukaryotic organisms. They are found wherever there is a food source. There are two types of fungi: yeasts (single-celled organisms) and molds (multicellular organisms). *Saccharomyces cerevisiae* was the first yeast used in the production of proteins and several pharmaceuticals. *Saccharomyces cerevisiae* proteins already represent a well-established industrial process, ensuring production titles of up to hundreds of mg/L. As an alternative to *S. cerevisiae*, the yeasts of *Pichia pastoris* and *Kluyvcromyces lactis* can be used for the production of proteins. Among the molds used in the production of proteins, There is *Rhizopus oligosporus*, *Zygorrhynchus moelleri*, *Oospora lactis*, *Penicillium roqueforti*... etc

They are characterized by the production of proteins has a nutritional value for animals and even humans.

A wide range of microorganisms, including bacteria, filamentous fungi, and yeasts, can be mass-produced using various carbon-based substrates.

Why yeasts?

Since ancient times, yeasts have played a fundamental role in human nutrition, particularly in winemaking, breadmaking, brewing, and cheesemaking. They constitute a group of microorganisms whose growth rates are lower than those of bacteria, yet they offer significant advantages in terms of ease of implementation in industrial fermentation processes, as well as a highly valuable nutritional profile, notably rich in lysine and B-group vitamins [6].

Indeed, these microorganisms possess high nutritional value and may contain up to 60% protein. Moreover, they have a relatively low nucleic acid content (22.5%, including 4.8% DNA and 17.7% RNA). Their cultivation can be carried out under acidic pH conditions, which reduces the risk of contamination. In addition, their harvesting is straightforward, and the processing of their biomass is relatively simple. The incorporation rate of this biomass into dietary formulations can reach up to 20%.

3. Microalgae

Micro-algae are microscopic single-celled or multicellular species. They are eukaryotic or prokaryotic microorganisms. A micro-algae spirulina (*Arthrospira platensis* or, *Arthrospira maxima*) is known for developing a whole range of interesting products (via photosynthesis) with pharmaceutical, cosmetic and more particularly food interest. In terms of chemical composition, spirulines contain about 70% protein.

4.3. Criteria for Selecting Microorganisms for SCP Production

Before being used as a P.O.U source, the microorganism must meet the following criteria:

- * Non-pathogenic
- * High protein content
- * Rapid growth rate
- * Easy harvesting
- * Resistance to environmental variations

4.4. Production Process of Single-Cell Protein (SCP)

Several Single-Cell Protein (SCP) production processes are currently implemented in many countries (USA, Eastern Europe, Australia, France, Russia, Sweden, etc.). The first large-scale cultivation experiments of these microorganisms were conducted during the World War I, driven by issues of food scarcity. Among these processes, the following can be mentioned:

- The WHEAST process, which uses whey as the primary substrate and the strain *Kluyveromyces fragilis* as the inoculum;
- The SYMBA process, which involves the cultivation of yeast strains of the species *Candida utilis* on starch hydrolysates.

4.5. Methods of SCP Production

There are several methods for the production of single-cell proteins, notably:

1. Fermenter Culture: this method involves cultivating single-cell cells in a large-scale liquid medium in tanks called fermenters. The cells are fed with nutrients necessary for their growth and multiplication, and produce the necessary protein.

2. Semi-solid medium culture: this method involves culturing the cells in a semi-solid medium, (e.g., gel), which provides physical support for the cells, this method is often used to cultivate cells that do not develop well in a liquid medium, like the cells of fungi.

3- Solid medium fermentation: this method uses solid substrates such as cereals, oilseed cakes or agricultural waste to cultivate single-cell cells. The cells are cultured on the solid substrate until they reach a sufficient density, then the protein is extracted from the substrate.

4.6. Advantages of Single-Cell Protein Production

The production of proteins from single-celled organisms has several advantages over other methods of protein production:

-Speed: Microorganisms grow rapidly and produce large quantities of protein in short periods

-Efficiency: High-quality, active proteins can be synthesized

-Genetic modifiability: Allows production of modified or novel proteins

-Safety: Microorganisms generally do not produce toxic contaminants

-Low cost: Ability to use inexpensive substrates, including organic waste

Chapter V.

Food Poisoning and Toxi-Infections

Chapter V: Food Poisoning and Toxi-Infections

The terms « food poisoning» or «intoxication» refer to acute gastroenteritis caused by the ingestion of food contaminated with certain pathogenic microorganisms and/or toxins.

Toxin: a toxic substance of biological origin, i.e., synthesized by living organisms, such as bacterial toxins and mycotoxins. In fact, these substances are specific proteins and, in most cases, produce immediate effects.

The pathogenic potential of bacteria may depend on several factors. There are species with infectious potential that act by invading the host (infection); species with toxinogenic potential that release toxins into food (intoxication); species with mixed characteristics that can cause toxi-infections (infectious microorganisms producing secreted toxins); and finally, other species that act by transforming the substrate into toxic compounds, thus producing intoxications. The most widespread food intoxications include the following:

1. Botulism

Botulism is associated with the ingestion of a toxin secreted by the bacterium *Clostridium botulinum*. This bacterium survives in the environment (soil, runoff water) in the form of spores. When these spores encounter favorable conditions, they germinate and may secrete the toxin responsible for a severe disease.

Spores are present in soil, agricultural products, marine sediments, and in the intestinal tracts of animals, including fish.

1.1. Botulinum Intoxication

This intoxication is linked to the ingestion of botulinum toxin synthesized during the growth of *Clostridium botulinum* in food. This strictly anaerobic, spore-forming, soil-borne microorganism poses a very high risk of contamination to many foods, particularly canned foods (cans and bottles) subjected to insufficient heat treatment.

Botulinum toxin is one of the most potent poisons known. The LD₅₀ (dose lethal to 50% of exposed subjects) is estimated to be between 10⁻⁸ and 10⁻⁹ g per kg of body weight. For this reason, mortality remains high despite therapeutic measures such as antitoxic sera or toxoids.

Based on the serological specificity of their toxins, seven types (A, B, C, D, E, F, and G) of *Clostridium botulinum* have been identified. Types A, B, and E are most frequently involved in human botulism. Type E, referred to as the “fish-associated type,” is commonly found in marine and freshwater fish. The different types of «*Clostridium botulinum*» vary in their tolerance to salt and water activity, their minimum growth temperature, and the heat resistance of their spores.

Botulinum toxins are high-molecular-weight proteins. After ingestion, they are absorbed by the digestive lymphatic system, enter the bloodstream, and bind to the myoneural junctions of cholinergic fibers of the peripheral nervous system, where they inhibit the release of acetylcholine. This results in neurological disorders such as asthenia, headaches, dizziness, diplopia, nausea, vomiting, abdominal cramps, constipation, dryness of mucous membranes and skin, dry mouth, dilated pupils, dysphagia, dysphonia, and respiratory disorders with paralysis.

1.2. Implicated Food

Botulism is often associated with foods produced at home or by artisanal methods. It most commonly involves meats, hams, fish, pâtés, and sometimes vegetables such as beans or asparagus.

- ✓ It should be noted that acidic foods with a pH below 4.5 do not allow the development of the bacterium.
- ✓ Foods with a water activity (aw) below 0.94, such as many dried and salted products (often cured with nitrite salt), do not permit bacterial growth and therefore do not allow toxin synthesis.
- ✓ In the case of non-acidic products, the addition of nitrites, at concentrations of 20 ppm or higher, inhibits the germination and proliferation of the microorganism.

Due to the severity of botulinum intoxication, the hygienic quality of foods in this case relies exclusively on prevention and strict control of microbiological quality.

This prevention is based on the production of properly sterilized canned foods, cold storage of all foods that are not true canned products (semi-preserved, smoked products, etc.), and the addition of nitrites (at a maximum dose close to 200 ppm) to sensitive products such as ham.

It should be emphasized that botulinum toxins are denatured and therefore inactivated by heat. Data vary according to authors: at 80°C, inactivation requires 8 to 9 minutes, whereas at 100°C, only a few seconds are sufficient. Cooking food can therefore, in most cases, denature the toxin and render the food non-hazardous.

1.3. Transmission

1. Foodborne transmission: ingestion of contaminated foods in which the toxin has already formed (insufficiently heated canned foods, foods consumed without adequate cooking).
2. By inoculation: toxin production from spores that have contaminated a wound.
3. Endogenous route: infant botulism has been recognized since 1976. It is linked to the ingestion of bacterial spores that colonize the digestive tract and produce the toxin. This condition may be implicated in sudden infant death syndrome.

1.4. Incubation Period

From 12 to 72 hours after ingestion of the toxin.

2. Salmonellosis

Salmonellosis are diseases caused by enterobacteria of the genus *Salmonella*. Most *Salmonella* species inhabit the intestinal tract of vertebrate animals and are most often transmitted to humans through contaminated food. *Salmonella* may also be transiently present in soil and water. In human pathology, salmonellosis includes two main types of conditions: gastroenteritis and typhoid and paratyphoid fevers.

2.1. *Salmonella* Toxin-Infections

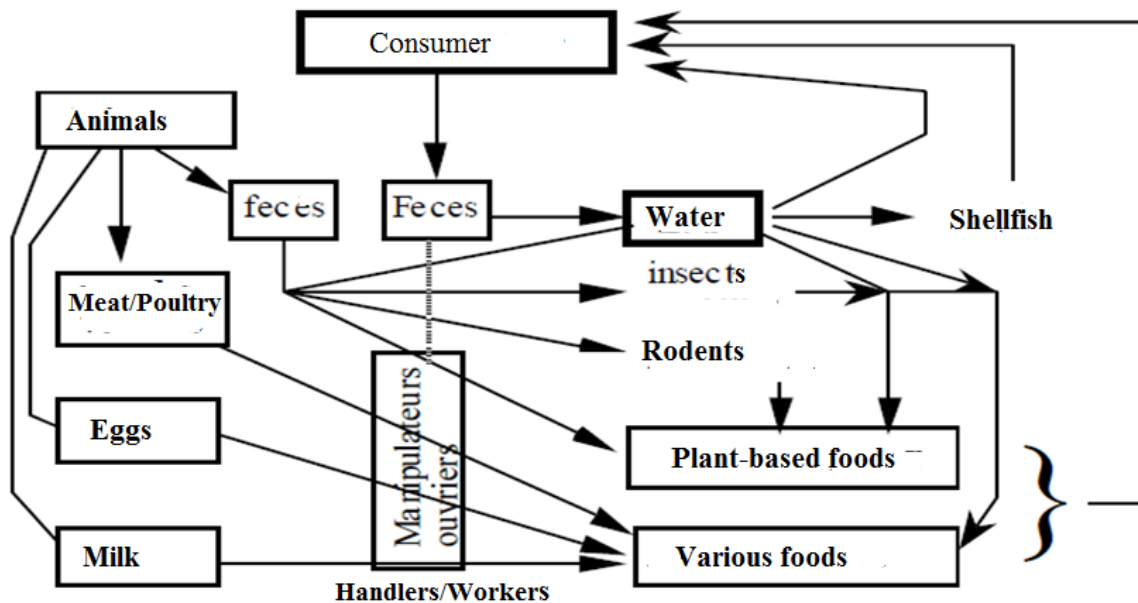
These enterobacteria are lactose-negative and H₂S-positive and are mainly present in the intestines of humans and animals. Within the genus *Salmonella*, more than 2000 serotypes have currently been described, all of which are presumed to be pathogenic to humans. Four of these serotypes, corresponding to the species *S. typhi* and *S. paratyphi* A, B, and C, are responsible for infectious diseases known as typhoid and paratyphoid fevers. The incidence of these diseases has markedly decreased, and their treatment with antibiotic therapy is well established (typhomycin or chloramphenicol). In addition, a vaccine (TAB-Typhim) is available and provides good protection.

The infectious dose is only a few cells for *Salmonella typhi*, *S. paratyphi* A, B, or C; approximately 10⁹ cells for *S. typhimurium*; and about 10⁶ cells for *S. anatum*.

With regard to foodborne toxin-infections, *Salmonella enteritidis* is the species most frequently involved.

Many other serotypes are responsible for toxin-infections. Among those most frequently encountered in our country are *S. typhimurium*, *S. heidelberg*, *S. java*, *S. panama*, *S. montevideo*, *S. goldcoast*, among others. Contamination of food products by microorganisms of the genus *Salmonella* may be primary (originating from infected animals), may result from

contact between contaminated environments and food, or may originate from infected food handlers or asymptomatic carriers.



All types of food may be contaminated by these microorganisms. If conditions of temperature, water activity, and pH are favorable, *Salmonella* can multiply. The foods most frequently implicated in salmonellosis include poultry (40%), meats particularly minced meat (10%), milk and dairy products (15%), eggs (5%, with a higher risk associated with duck and quail eggs), ice cream and pastry creams (5%), shellfish, and others.

The pattern of the most probable routes of food contamination by these bacteria shows that these processes may be vertical (direct) and/or horizontal (indirect).

Consumption of food in which the number of *Salmonella* has reached at least 10^6 organisms per gram will result in a toxin-infection. Clinical signs, which vary depending on the species involved as well as the age and physiological condition of the consumer, appear between 5 and 72 hours after ingestion. These signs are characterized by diarrhea, abdominal pain, chills, fever, vomiting, prostration, anorexia, headache, and general malaise. Enteritis or localized

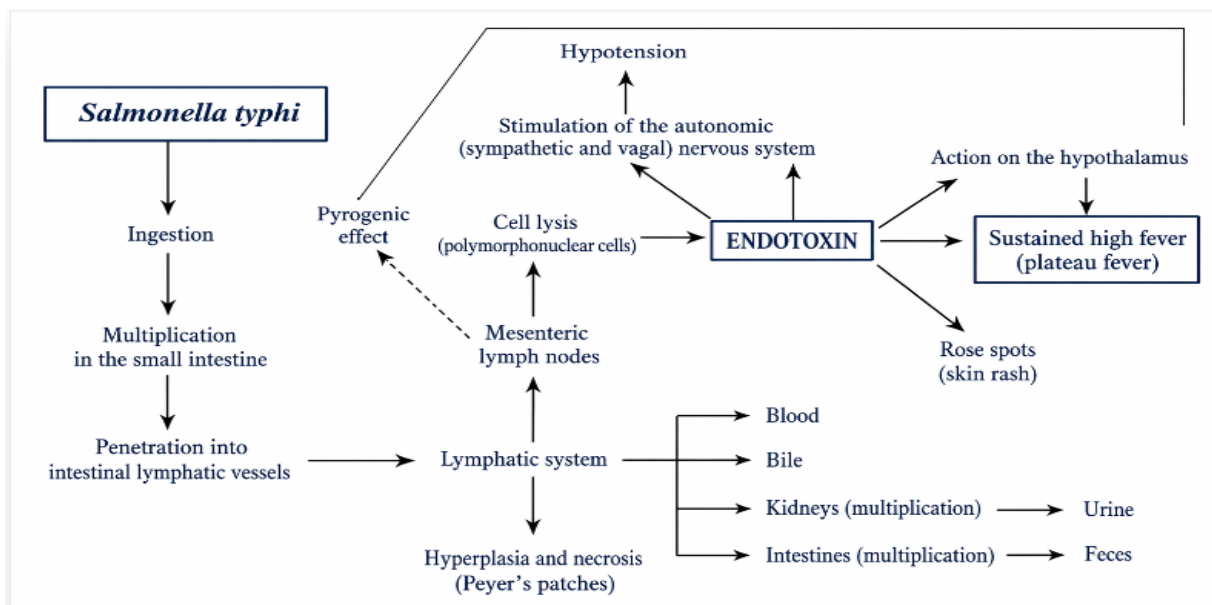
infection may sometimes occur. These clinical manifestations generally persist for several days. Children and elderly individuals are particularly susceptible to this toxin-infection.

An enterotoxin secreted in the intestinal tract by *Salmonella enteritidis* has been identified; this enterotoxin causes disturbances in hydro-mineral metabolism. Diagnosis is established through microbiological analysis of the patient's feces (stool culture). The patient may subsequently become an asymptomatic carrier.

2.2. Infectious Disease Caused by *Salmonella*

The infectious dose for species responsible for severe infectious diseases, such as *Salmonella typhi*, *S. paratyphi A*, or *S. paratyphi B*, consists of only a few bacterial cells.

For example, with *Salmonella typhi*, a pathogenic agent strictly adapted to humans, the pathophysiology of the disease known as typhoid fever results from in vivo multiplication of the bacterium and the release, within the lymphatic system—particularly in the mesenteric lymph nodes—of a neurotropic endotoxin. This molecule, released from the bacterial cell wall, has a molecular mass greater than 10^6 daltons. This endotoxin is a glucido-lipido-polypeptide complex, also referred to as lipopolysaccharide (LPS).



3. Staphylococcal Food Poisoning

Staphylococcal food poisoning is caused by enterotoxins produced by several species of *Staphylococcus*, mainly *Staphylococcus aureus*.

Staphylococcus aureus is a Gram-positive, mesophilic bacterium. Its optimum growth temperature is 37°C, although it can grow at temperatures as low as 7°C. Growth is inhibited at 30°C when the pH reaches 5. This bacterium tolerates a water activity (a_w) greater than 0.90. Media with a high salt content are favorable for its multiplication.

Staphylococcal enterotoxigenesis is a very common microbial disease in many countries. It results from the consumption of foods contaminated with toxigenic strains of *Staphylococcus aureus*.

This intoxication is characterized by a short incubation period (1–4 hours). The symptoms of this disease, sometimes referred to as “banquet disease,” are characteristic and include profuse salivation, nausea, vomiting, abdominal pain, severe diarrhea, sweating, headache, prostration, and occasionally fever. Symptoms generally disappear within 24–48 hours, and affected individuals do not develop specific immune defenses.

It should be noted that this intoxication represents only one of the possible manifestations of the pathogenic potential of *Staphylococcus aureus*. The almost constant presence of this microorganism on the skin and mucous membranes of humans and animals allows its wide dissemination. When food becomes contaminated, it must be stored for a sufficiently long time at a temperature that allows microbial growth for toxin production to occur. Since staphylococcal enterotoxin is a protein (molecular weight 26000–35000 Da), it is synthesized at the end of the exponential growth phase and during the stationary phase. The minimum number of bacteria required to produce enough toxin to cause poisoning is estimated, depending on the authors, at 5×10^5 to 5×10^6 cells per gram.

Enterotoxins A, B, C, D, E, and F are produced by enterotoxigenic *Staphylococcus aureus*, and a single strain may secrete several different toxins.

These proteins are not hydrolyzed by digestive proteases (pepsin, trypsin) and are highly resistant to heat treatments. Toxic (or serological) activity may persist even after sterilization-type treatments (15 minutes at 121°C).

It is therefore clear that if food has been contaminated, a heat treatment such as pasteurization (60°C for 30 minutes) will destroy the microorganisms, while the food may remain highly dangerous due to the possible presence of enterotoxin. The targets of staphylococcal enterotoxins are peripheral gastrointestinal sensory receptors which, after interaction, transmit nerve impulses via the vagus nerve to the intestinal motility center located in the hypothalamic region of the brain. These toxins therefore act as neurotoxins, inducing vomiting and intestinal hypermotility.

The foods most commonly implicated in this type of poisoning, in decreasing order of frequency, include meats and processed meats, pastries, poultry, cheeses, vegetables, and fish.

Food poisoning usually occurs through food contaminated by infected wounds on the hands, by the normal skin flora, or by microorganisms from the respiratory tract of food handlers. Foods with high salt and sugar concentrations provide a favorable environment for the growth of *S. aureus*.

4. Mycotoxins

Molds are ubiquitous filamentous microscopic fungi. Due to their heterotrophic nature, they contribute, along with other decomposer microorganisms, to the biodegradation and recycling of organic matter. Some molds are used to develop and enhance the organoleptic qualities of food products (*Penicillium roquefortii* and *P. camembertii* in cheese production), while others

are exploited in biotechnology for enzyme production (*Aspergillus niger* for protease and pectinase production), organic acids (citric and gluconic acids produced by *Aspergillus* and *Penicillium*), or antibiotics (penicillin produced by *P. chrysogenum*). Despite these beneficial aspects, fungal contamination of foodstuffs intended for human or animal consumption causes numerous economic and health problems. Undesirable mold growth may alter the appearance of food products (production of dark pigments such as melanin) and their organoleptic characteristics, and may also cause mycoses and allergies in consumers.

Toxigenic fungi are capable of producing secondary metabolites during their growth on food. These metabolites may be involved in serious health problems, giving rise to food intoxications (mycotoxicoses) due to the presence of mold toxins known as **mycotoxins**.

The term mycotoxin derives from the Greek “mycos,” meaning fungus, and the Latin “toxicum,” meaning poison. It refers to secondary metabolites produced by filamentous microscopic fungi (molds) toward the end of the exponential growth phase. These compounds have no biochemical significance for fungal growth, development, or competition.

Mycotoxins, which have highly diverse chemical structures, are exotoxins produced by lower fungi when growth conditions are favorable, particularly water activity, pH, and temperature. They are non-contagious and do not induce an immunological response in affected individuals. A given mold species may synthesize several mycotoxins, and a single mycotoxin may be produced by several species or genera. Moreover, toxin production depends on the strain. These toxins are secreted in very small quantities and persist even after destruction of the fungus.

The main toxigenic fungi producing mycotoxins belong primarily to the genera *Claviceps*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Fusarium* (tab. 5).

Table 5. The main mycotoxins likely to be present in food

Mycotoxin	Responsible Molds	Food Source	Syndromes / Effects
Ergotamine	<i>Claviceps purpurea</i>	Rye	Gangrene of the extremities
Aflatoxins	<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i>	Peanuts, cereals	Hepatic disorders
Ochratoxins	<i>Aspergillus ochraceus</i> <i>Penicillium viridicatum</i>	Maize, barley	Hepatic and renal disorders
Zéaralénone	<i>Fusarium graminearum</i>	Maize, food products	Estrogenic syndromes
Citrinin	<i>Penicillium citrinum</i>	Barley, rye	Nephrotic syndrome
Patulin	<i>Aspergillus clavatus</i> <i>Penicillium expansum</i>	Apples, apple juice, wheat	Neurotoxic effects; cutaneous syndrome (skin sarcoma); carcinogenic
Sporidesmin	<i>Pithomyces chartarum</i>	Grass	Eczema due to photosensitization
T2 toxin	<i>Fusarium tricinctum</i>	Cereals	Leukopenia
roquefortine	<i>Penicillium roqueforti</i>	Cheese	hepatotoxic

The primary metabolism of molds is identical to that of other living beings while their very important and very varied secondary metabolism depends on the strain considered, which leads to a great diversity of mycotoxins.

The different pathways of mycotoxin biosynthesis are extremely numerous. The polyketoacid pathway, however, remains the most common.

It is possible to classify mycotoxins according to the structure from which they are derived:

- Glucose derivatives: kojic acid
- Derivatives of amino acids: aspergillilic acid (leu, ile), fumitremorgenes (trp, pro), gliotoxin (phe, ser), roquefortine (trp, his), slafranine (lys), sporidesmines (trp, ala, cys)
- Amino acid and mevalonate derivatives: ergotamine, cyclopiazonic acid

- Derivatives of polyketoacids: aflatoxins, penicillic acid, citrinin, patulin, rubratoxins, sterigmatocystins, zearalenone
- Polyketoacid and amino acid derivatives: ochratoxin A, cytochalanase, erythrokyrin
- Derivatives of terpenes (acetate pathway via mevalonate): trichothecenes

4.1. Mycotoxicoses

Consumption of foodstuffs contaminated with mycotoxins may cause severe pathological manifestations in humans and animals, known as mycotoxicoses. Mycotoxicoses are considered foodborne diseases (food intoxications) that are neither infectious nor contagious.

They may be classified according to their cause (etiological classification) or according to their clinical syndromes (nosological classification). Hepatotoxicoses, nephrotoxicoses, neurotoxicoses, dermatotoxicoses, and others have been described. There are also toxins with estrogenic, photosensitizing, or abortifacient activity.

Symptoms depend on the nature of the mycotoxin involved, the dose and duration of exposure, the age, sex, and health status of the exposed individual, as well as other synergistic factors such as genetic predisposition, diet, and interactions with other toxic substances. Mycotoxicoses may cause acute intoxications (rapid onset) and/or chronic effects, which may result from cumulative exposure and include mutagenic, teratogenic, and carcinogenic effects. Numerous cases of mycotoxicoses have been reported worldwide, the oldest being ergotism, known since the Middle Ages and caused by *Claviceps purpurea*.

Mycotoxins enter the food chain as a result of crop contamination by molds, both before and after harvest. They can therefore be found in many plant-derived foodstuffs, particularly cereals, fruits, and processed foods derived from these products. They may also be detected in milk, eggs, meat, or offal if animals have been exposed to feed contaminated with

mycotoxins. Foods rich in carbohydrates constitute a more favorable environment for mold growth and mycotoxin production than protein-rich foods. Exposure to mycotoxins may be direct, through ingestion of contaminated foods, or indirect, through consumption of products of animal origin, such as milk.

Mycotoxins are small molecules with **low water solubility, difficult to degrade metabolically, and highly stable to acidity and heat**. They are particularly heat-resistant, making them especially dangerous to consumers, as they may remain in foods after cooking or even sterilization. Mycotoxins are considered natural food contaminants, in contrast to substances intentionally or accidentally introduced by humans, such as food additives or pesticide residues.

4.2. Major Families of Mycotoxins

Most toxic molds grow on food and release toxins that cause intoxications of varying severity. They are produced by six main fungal genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Byssoclamys*, *Claviceps*, and *Alternaria*. Several families of mycotoxins contaminate human food and are toxic, including aflatoxins, ochratoxin, trichothecenes, fumonisins, zearalenone, patulin, sterigmatocystin, citrinin, and penicillic acid. Some are carcinogenic (aflatoxins, fumonisins, ochratoxin), while others cause various organ damages affecting the liver (hepatotoxins), kidneys (nephrotoxins), skin, immune system, central nervous system (neurotoxins), or embryo.

Mycotoxins can affect some organs than others). E.g. liver (aflatoxins), kidneys (citrinin), nervous system (aspergillic acid), etc.

4.2.1. Aflatoxins – History and Structure

The discovery of most mycotoxins dates back to the 1960s. The discovery of **aflatoxin** in 1960 in England resulted from an investigation into the death of 100,000 turkeys that had consumed peanut meal imported from Africa. *Aspergillus flavus* was isolated from the suspected feed, and the toxin produced by this microorganism was named *A(spergillus) fla(vus) toxine*.

Aflatoxins are mycotoxins that persist in warm and humid environments. They are harmful to both humans and animals and exhibit strong carcinogenic activity. Aflatoxins comprise a group of 18 structurally related compounds, consisting of a coumarin nucleus fused with three furan rings. They are produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. Four major compounds (Bis-furanno-coumarin) have been identified: B₁, B₂, G₁, and G₂, which occur naturally in plant materials and differ in color and fluorescence (B toxins fluoresce blue and G toxins green at 450 nm).

Cows fed with feed contaminated by aflatoxins B₁ and B₂ excrete hydroxylated metabolites in their milk, known as M₁ and M₂ (M for milk). Among these, aflatoxin B₁ is the most abundant, followed by G₁ and M₁. Cooking or heat treatments such as pasteurization do not destroy aflatoxins. These molecules are resistant to boiling, and autoclaving for four hours at 121°C reduces but does not eliminate their toxicity.

Sodium hypochlorite at 5% and oxidizing agents may be used to decontaminate laboratory or industrial equipment. Alkaline treatments (e.g., ammonia under pressure) can inactivate these toxins.

5- Poisoning by Fishery Products

Intoxication or poisoning refers to all disorders resulting from the voluntary or involuntary introduction into the body of one or more toxic substances (poisons). Toxic substances enter the body through ingestion, inhalation, injection, or absorption through the skin or mucous membranes.

Food poisoning is a major public health concern, as its frequency continues to increase.

Poisoning caused by fishery products may have several origins:

5.1. Parasites

The presence of parasites in fish is extremely common and has consequences from both economic and public health perspectives. Parasitic organisms such as helminths have complex life cycles. They do not spread directly from one fish to another but must pass through several intermediate hosts during their development.

5.1.1. Nematodes (*Anisakis* spp.)

Ascarids or nematodes are common and occur in marine fish worldwide. The nematodes *Anisakis simplex* and *Pseudoterranova decipiens*, commonly known as herring worm and cod worm, are roundworms measuring 1–6 cm in length. When ingested alive by humans, they can penetrate the gastrointestinal wall and cause acute inflammation (herring worm disease).

Anisakis spp. has a complex life cycle and can infect individuals who consume raw or undercooked fish, causing anisakiasis. Following ingestion of improperly prepared fish, humans may become infested, triggering the release of specific IgE antibodies and allergic reactions, sometimes leading to anaphylactic shock upon subsequent exposure.

5.1.2. Cestodes (*Diphyllobothrium latum*)

Diphyllobothrium latum is a common human parasite that can reach up to 12 m in length in the human intestinal tract. Diphyllbothriasis occurs in regions where lakes and rivers coexist

with dietary habits involving the consumption of raw or undercooked freshwater fish. A related species, *D. pacificum*, is transmitted by marine fish and is common in coastal regions.

5.1.3. Trematodes (*Clonorchis sinensis*)

The life cycle involves two intermediate hosts: a snail (genus *Bithynia*) and a cyprinid fish (carp and goldfish), whose muscles harbor encysted infectious metacercariae. Dogs, cats, wild animals, and humans are the definitive hosts in which the fluke lives and develops in the bile ducts.

5.1.4. Control of Parasitic Diseases

Preventive control can be addressed at three levels (as outlined by the WHO for nematodes in 1989):

1. Avoid catching fish infected with helminths by selecting specific fishing areas.
2. Sort and discard fish infected with helminths or remove helminths manually (e.g., candling tables for nematodes).
3. Mandatory cooking or freezing at -20°C for 24 hours for all fish consumed raw, cold-smoked, marinated, or salted (EC Regulation 853/2004).

5.2. Chemical Contaminants

Clean aquatic environments naturally contain low concentrations of contaminants. Certain metals such as copper, selenium, iron, and zinc are essential nutrients for fish and crustaceans. However, at high concentrations, these substances may contaminate fish and seafood.

Chemical contaminants include:

- Inorganic substances: antimony, arsenic, cadmium, lead, mercury, selenium, sulfites.
- Organic compounds: polychlorinated biphenyls (PCBs), dioxins, insecticides (chlorinated hydrocarbons).

- Processing- or farming-related compounds (nitrosamines) and aquaculture contaminants (antibiotics, hormones).

5.2.1. Heavy Metals (Cadmium, Lead, Mercury, PCBs)

The risk posed by these chemical residues is particularly significant for fish and crustaceans caught by recreational fishers in coastal or heavily polluted waters. Heavy metals are not biodegradable through chemical or biological processes. They accumulate in the body (blood, liver, brain, kidneys) and are eliminated very slowly, with biological half-lives ranging from days to years.

Mercury-contaminated fish constitute a risk factor in Charcot disease. This has been confirmed by mercury levels found in toenails of patients (according to a study by the American Academy of Neurology in Boston). Exposure should be limited for vulnerable populations, including pregnant and breastfeeding women and children under 30 months of age.

5.2.2. Biotoxins

Biotoxins are toxic substances naturally produced by living organisms, such as bacteria, fungi, algae or certain animals. These are toxic compounds of biological origin capable of causing harmful, even serious, effects in living beings, even at very low doses.

They can contaminate food, water or the environment and lead to poisoning. For example, some marine biotoxins produced by microalgae can accumulate in fish or shellfish and become dangerous to humans.

Approximately 500 species of venomous fish worldwide pose a risk to humans upon consumption. Most are tropical or subtropical species. Poisoning by marine animals is caused by toxins present in the skin, flesh, and viscera of fish.

this table above (Tab.6) illustrates some examples of marine biotoxins and these effects:

Table 6.Marine biotoxins

Main toxin	Type of poisoning	Producing microorganism	Implicated foods	Main symptoms	Particularities
Saxitoxin	PSP (Paralytic Shellfish Poisoning)	<i>Alexandrium</i> , <i>Gymnodinium</i> , <i>Pyrodinium</i>	Shellfish (mussels, oysters)	Paralysis, numbness, respiratory distress	Can be rapidly fatal
Okadaic acid	DSP (Diarrhetic Shellfish Poisoning)	<i>Dinophysis</i> , <i>Prorocentrum</i>	Shellfish	Diarrhea, abdominal pain	Non-fatal but common
Brevetoxins	NSP (Neurotoxic Shellfish Poisoning)	<i>Karenia brevis</i>	Shellfish	Neurological symptoms, temperature reversal (hot/cold)	Can also occur via inhalation
Domoic acid	ASP (Amnesic Shellfish Poisoning)	<i>Pseudo-nitzschia</i>	Shellfish, fish	Digestive disorders + memory loss	May cause brain damage
Ciguatoxin	CFP (Ciguatera Fish Poisoning)	<i>Gambierdiscus</i>	Tropical fish	Digestive and neurological symptoms	Common in tropical regions
Palytoxin	Palytoxin poisoning	<i>Ostreopsis</i>	Fish, crustaceans, marine aerosols	Fever, muscle pain, cardiac disorders	Extremely potent toxin

These poisonings are **non-infectious** and caused by **marine biotoxins (phycotoxins)** accumulated in seafood.

Filter-feeding shellfish are the most commonly involved, as they accumulate toxins.

These are all **marine phycotoxicoses**, but they differ in:

- the type of toxin
- the symptoms
- the severity

Ichthyosarcotoxism refers to food poisoning caused by the ingestion of fish that have accumulated toxins in their flesh, skin, or viscera along the food chain. The term derives from Greek: *ichthys* (fish), *sarx* (flesh), and *toxicon* (poison).

The two most common forms are:

- **Ciguatera poisoning**, the most well-known and frequent, resulting from consumption of reef fish that have accumulated ciguatoxins produced by phytoplankton (algae). Symptoms appear after an incubation period of 2–20 hours and include digestive, cardiovascular, and neurological signs.



Reef fishes

- **Scombroid poisoning** (histamine ichthyosarcotoxism), following consumption of scombroid fish (red-fleshed fish such as tuna, mackerel, bonito, mahi-mahi, swordfish). This intoxication is due to histamine formation following bacterial degradation of histidine by bacteria such as *Clostridium*, *Escherichia*, *Salmonella*, and *Shigella*.

Clinical signs resemble **allergic reactions**, with sudden onset from minutes to three hours, digestive symptoms, hypotension, syncope, laryngeal edema, and bronchospasm.



Photo of scombrid

Other rarer intoxications include:

-tetrodotoxism is due to the consumption of tetrodons (Balloon fish, Fugu, pufferfish). This toxic fish can be made edible after a preparation that some Japanese cooks master.



Puffer fish

Tetrodotoxin is very powerful and acts by inhibiting synaptic transmission by blocking the release of acetylcholine, resulting in neuromuscular paralysis;

- **clupeotoxism** after ingesting tropical sardines,

- **palytoxicosis** after ingesting contaminated crabs,

- **carchatoxism** after ingesting large sharks,

- **chelonitoxism** after ingesting marine turtles,

- **ichthyalleinotoxism**, hallucinatory syndrome after ingestion of herbivorous fish.

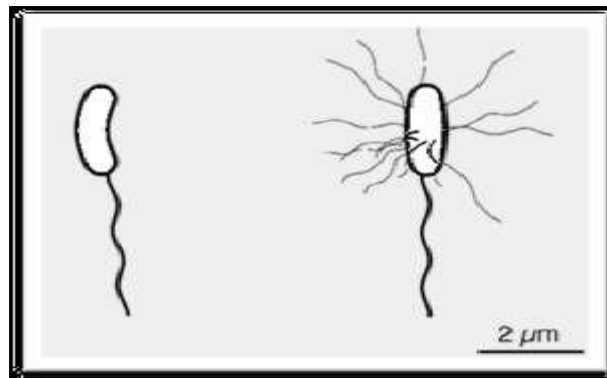
5.3. Microbiology and Viruses

The initial microflora of fish is highly diverse and often dominated by Gram-negative psychrotrophic bacteria. Fish captured in tropical regions may present higher levels of Gram positive and enteric bacteria.

5.3.1. Bacteria

a. *Vibrio parahaemolyticus*

A marine Gram-negative *vibrio*, facultative anaerobe, halophilic, highly motile, and rapidly multiplying between 10 and 45°C. It is found mainly in filter-feeding animals such as shellfish (mussels and oysters in particular) and certain fish. Some strains cause severe, sometimes pandemic, gastroenteritis in humans. Its toxins are not destroyed by cooking.



Vibrio cholera (left), *Vibrio parahaemolyticus* (right)

b. *Campylobacter jejuni*

Commonly found in the intestines of animals and in untreated surface water contaminated by fecal matter. Campylobacteriosis contracts through consumption of contaminated shellfish and crustaceans with *C. jejuni*. Particularly affecting children under 5 years and young adults aged 15–29 years.



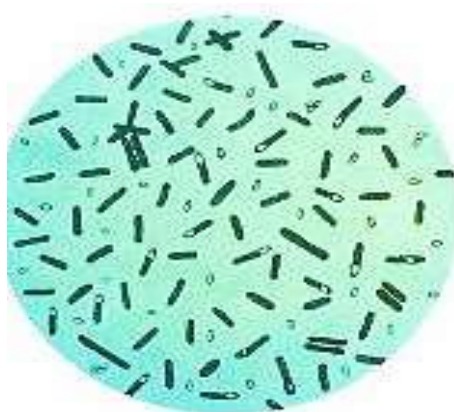
Photograph of *C. jejuni*

***c. Clostridium botulinum* type E**

Frequently found in improperly prepared artisanal or homemade fish preserves.

Three particularities are important depending on the epidemiology of botulism:

- The bacterium does not survive at pH below 4.6.
- Its spores are heat-resistant and require 120°C for 10 minutes for destruction.
- The toxin causes flaccid paralysis.



Microscope photograph of *C.botulinum* (vegetative bodies, endospores and spores)

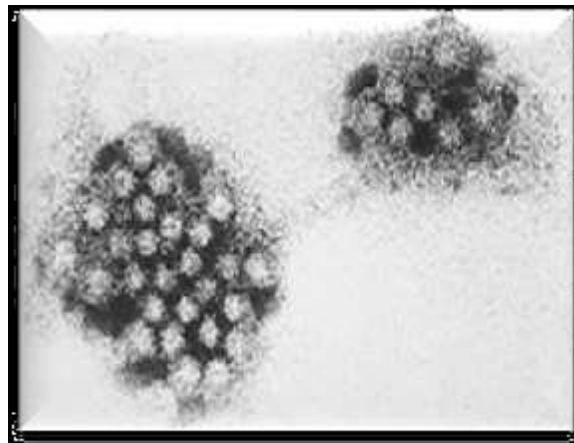
5.3.2. Viruses (*Norovirus*)

Oysters are the important secondary reservoirs for a majority of genotypes of this Virus. Contamination with this virus (norovirus) is a major cause of gastroenteritis occurring in coastal communities (nursing homes, hospitals, cruise ships...).

This contamination occurs after ingestion of raw or undercooked food (oysters retain viruses by filtering contaminated water) or during poor hygiene practices during the preparation of dishes.

Benign in most cases, this infection can become dangerous in elderly subjects or subjects with chronic pathologies or immunocompromised (AIDS...) with risks of renal failure that can lead to death.

Norovirus does not change the appearance of the oyster or its taste, screening it before ingesting it is impossible. And you should not eat oysters half-opened, they must be well closed and retract when a little lemon juice is put on them.



Norovirus sp.

(Viral particles from 27 to 32 nm)

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Annex: Canvas of the material

Annexe : Canevas de la matière

Etablissement : UFAS 1 LICENCE BIOTECHNOLOGIE ALIMENTAIRE

Année universitaire : 2025-2026

Intitulé de la licence : Biotechnologie Alimentaire

Semestre 5

Intitulé de l'UE : Unité fondamentale

Intitulé de la matière 1 : Microbiologie alimentaire

Crédits : 6

Coefficient : 3

Objectifs de l'enseignement

L'enseignement de la microbiologie alimentaire vise à fournir les informations nécessaires

concernant les principaux micro-organismes intéressant le domaine agro- alimentaire, à évaluer les qualités sanitaires et hygiéniques des aliments, à montrer que le développement de ces microorganismes peut avoir des incidents d'ordre sanitaire dans le cas des germes pathogènes. Sur le plan technologique, il s'agit de montrer aussi les propriétés et les capacités des micro-organismes à produire des substances utiles à l'industrie alimentaire et aux Bio-industries.

Connaissances préalables recommandées Microbiologie générale, Biochimie, Analyse

microbiologique, Chimie...etc.

Contenu de la matière :

Cours:45H00

Introduction (3H00)

I-Généralités

I.1. Définition de l'aliment

I.2. Définition des micro-organismes

Chapitre 1 : Les grands groupes microbiens intéressant la microbiologie alimentaire (14H00)

1.1. Généralités

1.2. Les entérobactéries

1.3. Les pseudomonas

1.4. Les bactéries acétiques

1.5. Les vibrions

1.6. Les brucella

1.7. Les microcoques

1.8. Les streptocoques

1.9. Les lactobacilles

1.10. Les actinobactéries

1.11. Les bactéries sporulées aérobies

- 1.12. Les bactéries sporulées anaérobies
- 2. Les champignons
- 2.1. Les moisissures
- 2.1.1. Caractères généraux
- 2.1.2. Applications dans l'industrie alimentaire
- 2.2. Les levures
- 2.2.1. Caractères généraux
- 2.2.2. Applications dans l'industrie alimentaire

Chapitre 2 : Influence des techniques de fabrication sur les microbes (6H00)

- 1. Facteurs influençant la destruction microbienne
- 2. Moyens de lutte contre les contaminations des aliments
- 2.1. Agents physiques de stabilisation ou de destruction des microorganismes
- 2.2. Agents chimiques
- 3. Utilisation industrielle des micro-organismes
- 3.1. Fermentation
- 3.1.1. Définition
- 3.1.2. Différent types de fermentation
- 4. Recherche des conditions de milieu optimal pour le développement de la flore

Chapitre 3 : Problèmes microbiologiques dans l'industrie alimentaire (6H00)

- 1. Types de contamination alimentaire
- 1.1. Contamination chimique
- 1.2. Contamination physique
- 1.3. Contamination microbienne
- 1.4. Contamination allergénique
- 2. Accidents de fabrication

Chapitre 4 : Procédés biotechnologiques (6H00)

- 1. Préparation des acides aminés,
- 2. Préparation des acides organiques,
- 3. Préparation des vitamines,
- 4. Préparation des protéines d'organismes unicellulaire

Chapitre 5 : Intoxications et toxi-infections (10H)

- 1. Botulisme
- 2. Salmonellose
- 3. intoxication alimentaire à staphylocoque
- 4. Mycotoxines
- 4.1. Définition
- 4.2. Principales familles de mycotoxines
- 4.3. Mycotoxicoses
- 5. Empoisonnement par les produits de pêche