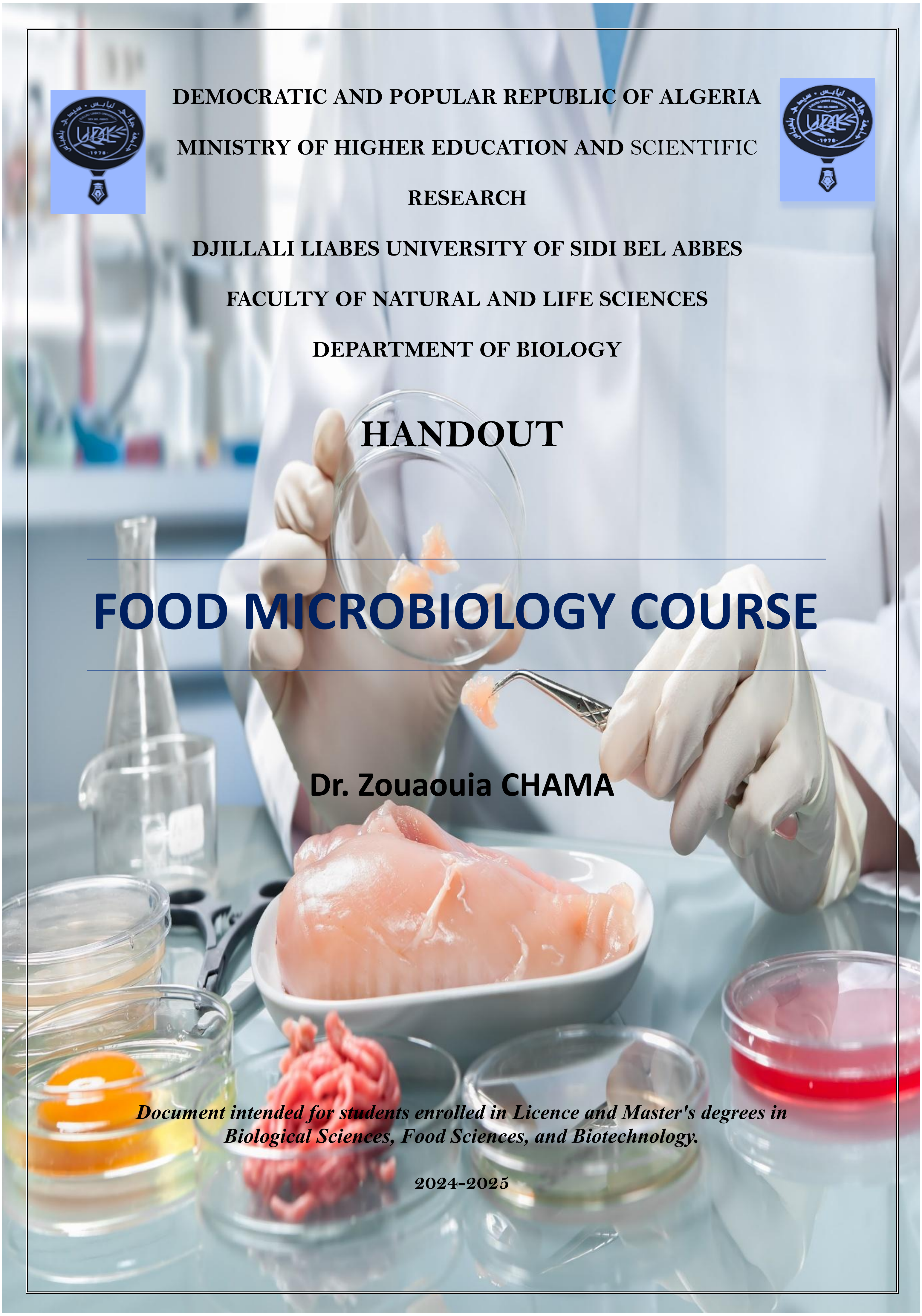


DEMOCRATIC AND POPULAR REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
DJILLALI LIABES UNIVERSITY OF SIDI BEL ABBES
FACULTY OF NATURAL AND LIFE SCIENCES
DEPARTMENT OF BIOLOGY

HANDOUT

FOOD MICROBIOLOGY COURSE

Dr. Zouaouia CHAMA

Document intended for students enrolled in Licence and Master's degrees in Biological Sciences, Food Sciences, and Biotechnology.

2024-2025


بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

بسم الله الرحمن الرحيم

(يَا أَيُّهَا الَّذِينَ آمَنُوا كُلُوا مِن طَيِّبَاتِ مَا رَزَقْنَاكُمْ وَاشْكُرُوا لِلَّهِ إِن كُنتُمْ إِيَّاهُ تَعْبُدُونَ)

[البقرة: 172]

(قُل لا أَجِدُ فى ما أوحِىَ إِلَىَّ مُحَرَّمًا عَلى طاعِمٍ يَطعَمُهُ إِلّا أَن يَكونَ مَيتَةً أَو دَمًا مَسفوحًا
أَو لَحمَ خِنزيرٍ فَإِنَّهُ رِجسٌ أَو فِسقًا أُهِلَّ لِغَيرِ اللَّـهِ بِهِ فَمَنِ اضطُرَّ غَيرَ باغٍ وَلا عادٍ فَإِنَّ
رَبَّكَ غَفورٌ رَحيمٌ) " الانعام۝١٤٥

عن ابن عباس رضى الله عنه

قال رسول الله صلى الله عليه وسلم:

"كلوا۝ واشربوا۝ والبسوا۝ وتصدقوا۝ فى غير إسراف ولا مخيلة"

قال الإمام على بن أبى طالب رضى الله عنه:

"العلم يبنى بيوتا لا عماد لها .. والجهل يهدم بيت العز والكرم"

"العلماء ورثة الأنبياء"

Preface

Food microbiology is one of the fundamental disciplines of modern food sciences. In a context where concerns about food safety are central to our society, understanding the interactions between microorganisms and food matrices becomes essential for any professional in the agri-food sector.

This document serves as a support for the detailed courses in food microbiology according to the curriculum of the Ministry of Higher Education and Scientific Research, enriched with a curated collection of works and studies from numerous researchers. It is primarily aimed at students in food science and technology, undergraduate microbiology programs, master's in microbial biotechnology, applied microbiology, as well as professionals wishing to deepen their knowledge in this constantly evolving field.

It has been designed to provide both theoretical and practical approaches to food microbiology, integrating recent scientific advancements and contemporary industrial challenges.

The objective of this work is to provide a solid foundation of knowledge enabling :

- The identification of the main characteristics of food groups and their microbial susceptibility.
- An understanding of the diversity and characteristics of microorganisms of interest in food microbiology.
- Mastery of the mechanisms of microbial spoilage of food and appropriate control measures.
- Analysis of intrinsic and extrinsic factors influencing microbial stability.
- Application of strategies for the prevention and management of microbiological risks.

This document is organized into three complementary chapters that offer a comprehensive and structured view of food microbiology. The first chapter provides a brief introduction to the main food groups, establishing the necessary foundations for understanding their microbiological behavior. The second chapter details the microorganisms of interest in food microbiology, their characteristics, and their impact on food matrices. Finally, the third chapter addresses microbial spoilage of food and control measures, crucial aspects for ensuring food quality and safety.

Each chapter is enriched with concrete examples and practical illustrations, facilitating the assimilation of theoretical concepts and their application in a professional context. This progressive pedagogical approach establishes logical links between the various aspects of food microbiology.

Microorganisms can be both formidable agents of spoilage and valuable technological aids in the food industry. This duality constitutes the guiding thread of our pedagogical approach, underscoring the importance of rigorous and balanced training in this field.

List of tables

List of figures

Liste of abreviations

ADH : Arginine dihydrolase.

CPS : Capsular polysaccharide

CT : Cholera Toxin

EMB : Eosin-methylene blue

GEIs : Genomic islands (GEIs)

HGCs : Homolog gene clusters
HPA : Health Protection Agency

LDC: Lysine decarboxylase

LPS : Lipopolysaccharides

MAMPs : Microbe-associated molecular patterns

NAG : Non-Agglutinating Vibrio

NCV : Non-Cholera Vibrio

NF-κB : Nuclear factor κB

ODC: Ornithine decarboxylase
ONPG : Ortho-Nitrophenyl-Galactopyranoside

ORFs : Open reading frames

PAMPs : Pathogen-associated molecular patterns.

TDA : Tryptophan deaminase
TDH : Thermostable Direct Hemolysin

TIAC : Foodborne toxi-infection

TRH : TDH-Related Hemolysin

VP : Voges-Proskauer

Table of contents

Chapiter1 : Brief Introduction to Major Food Groups

Chapter 2 : Microorganisms of Interest in Food Microbiology

Chapter 3 : Microbial Spoilage of Food and Control Measures

INTRODUCTION

INTRODUCTION

Food microbiology is the science that studies microorganisms (bacteria, viruses, fungi, protozoa) that have either beneficial or harmful effects on food quality and safety and/or are associated with foodborne intoxications.

It focuses on their growth, identification, and pathogenesis.

Knowledge of food microbiology and mastery of microbiological food control are essential to ensuring product quality, protecting consumer health, and complying with increasingly stringent regulations **(Guiraud, 1998).**

Microbial communities associated with food products are responsible for transformations that are sometimes beneficial. Indeed, the production of many food products relies on the metabolism of microorganisms, collectively referred to as positive microflora. These microorganisms contribute to the development of certain foods through fermentation and/or the aging process (eg., cheese or cured meats). These "beneficial" organisms play a crucial role in both product preservation and the acquisition of distinctive sensory characteristics. In addition to their direct use in the food industry, some microorganisms are employed in industrial biotechnology to produce chemical compounds such as antibiotics, polysaccharides, or ethanol **(Dorel et *al.*, 2020)**.

Alongside positive microflora, food microbiology also investigates spoilage flora, which includes microorganisms that degrade food products without necessarily posing a direct risk to consumer health. Each year, food spoilage due to microbial activity results in significant economic losses. Furthermore, some microorganisms transmitted through food can cause infectious diseases and lead to numerous cases of foodborne intoxications. Consequently, it is imperative, particularly in the food industry and food service sectors, to implement strict measures to minimize contamination and control microbial development in handled food products **(Lacasse, 2002 ; Dorel et *al.*, 2020).**

This course is divided into three chapters. The first section covers the major food groups that constitute a significant part of daily human consumption. The second section describes the main groups of microorganisms, both beneficial and contaminating, with a focus on their detrimental effects, including the degradation of organoleptic and nutritional quality, as well as their pathogenic impact on human health. This section also associates specific food products with each microbial category through a taxonomic approach and an analysis of contamination risk factors. Finally, the third section addresses the key physicochemical and nutritional factors influencing microbial establishment in food, along with the strategies implemented to control these contaminants.

Chapiter1 : Brief Introduction to Major Food Groups

1. Chapiter : Brief Introduction to Major Food Groups

1.1. Major Food Groups

There are seven primary food groups that provide a simplified classification of foods. This categorization is based on their nutrient composition, including macronutrients (proteins, lipids, carbohydrates), minerals (iron, calcium, magnesium, etc.), and vitamins (fat-soluble : A, D, E, K ; and water-soluble: B, C). The seven food groups considered are as follows:

- Meat, fish, and eggs
- Milk and dairy products
- Fats
- Vegetables and fruits
- Cereals and derivatives – legumes
- Sugars and sweetened products
- Water and beverages **(Desalme et *al.*, 2004).**

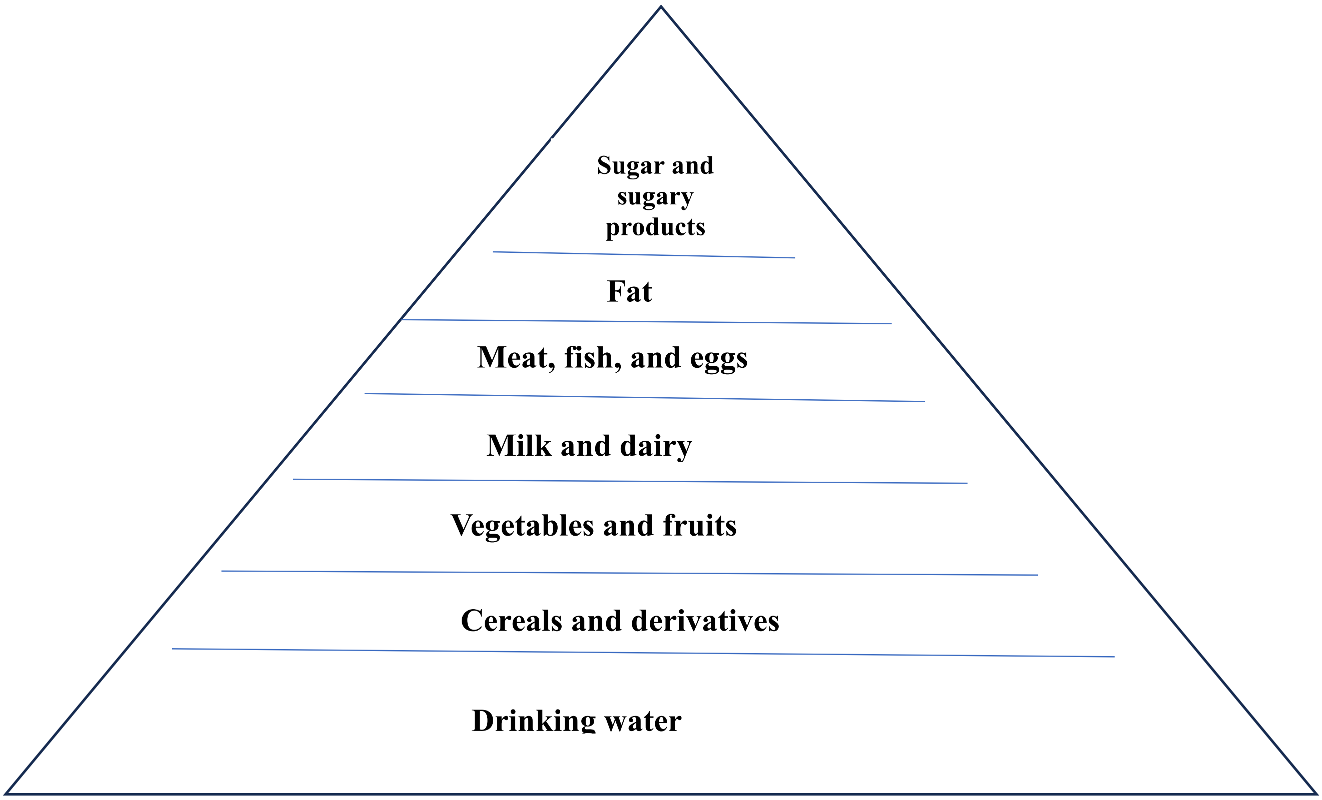


Figure 1 : Food Group Pyramid **(Desalme et *al.*, 2004).**

1.1.1. Meat, Fish, and Eggs

1.1.1.1. Meat and Meat Products

Meat contains an average of 20% protein. These proteins are primarily composed of myosin, myoalbumin, and collagen. Myosin and myoalbumin are high-quality proteins that contain all essential amino acids, giving meat a high protein efficiency ratio.

Collagen, on the other hand, is low in tryptophan and sulfur-containing amino acids, which reduces the biological value of meats that are rich in it. Additionally, meat provides a small amount of non-protein nitrogenous compounds, such as purines.

Meat does not contain fat-soluble vitamins but is rich in B-group vitamins. Organ meats, particularly the liver, are the richest sources of these vitamins and also provide significant amounts of vitamins A and D **(Fournaud, 1982 ; Favier et *al.*, 1995).**

Meat lipids primarily consist of saturated and monounsaturated fatty acids. Fat content varies depending on the species, the animal's fattening condition, and the specific cut of meat. Depending on these factors, meat can contain between 2% and 30% fat **(Favier et *al.*, 1995)**. All meats, even lean cuts, contain cholesterol, particularly organ meats. Based on fat content, meats are classified into three categories :

- Lean meats (<10% fat)
- Moderately fatty meats (10–30% fat)
- Very lean meats (~5% fat)

1.1.1.1.2. Processed Meats (cured meats)

Originally, processed meats were developed as a preservation method for meat. All processed meats undergo curing, using a mixture of salt and either potassium nitrate or sodium nitrite. These products contain 10–20% protein, with cooked and dry-cured hams being the richest sources.

Processed meats are characterized by their high lipid content, ranging from 20–35% in products like cooked sausages and salami to 35–40% in rillettes, dry sausages, and salamis. Cholesterol content varies :

- 100 mg/100 g in sausages and salami
- 150–260 mg/100 g in liver pâtés

- 60–70 mg/100 g in ham **(Favier et *al.*, 1995; Roberts, 1999)**

1.1.1.2. Fish

Fish and shellfish provide proteins of comparable quality to meat while containing little carbohydrate. They also have a higher proportion of non-protein nitrogenous compounds (such as ammonia and urea), which contribute to their characteristic odor. Fish is a good source of B vitamins (especially B12) and vitamin E. Fatty fish are also rich in vitamins A and D **(Dupin et *al.*,1992).**

Fish generally contain 18–19% protein, but levels can vary significantly :

- Tuna : 25–35%
- Oysters : 7–10% **(Dupin et *al* ., 1992)**

Lipid content varies from 0.5% to 15%, and cholesterol levels range from 50–70 mg per 100 g. Fish are categorized into three groups based on fat content (Favier et al, 1995) :

- Lean fish (0.5–5%): Whiting, sole, sea bream, cod
- Moderately fatty fish (5–10%) : Mackerel, sardines, salmon, tuna
- Fatty fish (>10%): Eel

Fish are an important source of phosphorus and selenium but contain relatively little calcium. Shellfish and crustaceans are richer in various minerals, including calcium, zinc, iron, and sodium (50–400 mg/100 g) **(Dupin et *al.*, 1992).**

1.1.1.3. Eggs

Egg proteins, including ovalbumin in the egg white and ovovitellin in the yolk, have excellent biological value. Whole eggs contain 14% protein, with 12–16% lipid content **(Dupin et *al.*, 1992).**

All egg lipids are found in the yolk (33.5 g per 100 g of yolk, or about 7 g of fat per yolk) and include a high proportion of phospholipids (Desalme et al., 2004). The yolk is also a significant source of cholesterol (1,500 mg per 100 g, or approximately 300 mg per yolk). Eggs are rich in phosphorus and iron.

Eggs provide B-group vitamins, while the yolk is particularly high in vitamins A and D. They contain negligible carbohydrates and 75% water **(Dupin et *al.*, 1992; Favier et *al.*, 1995)**.

1.1.2. Milk and Dairy Products

Milk is a complex source of nutrients, including proteins, carbohydrates, lipids, vitamins, and minerals. Its primary role is to nourish newborn mammals. Dairy products are among the most nutritionally diverse food sources.

The main proteins in milk are caseins and whey proteins, which include lactalbumins (β-lactoglobulin, serum albumin, and immunoglobulins) **(Gueroui, 2018)**.

Milk lipids are primarily triglycerides (98%), with smaller amounts of mono- and diglycerides, phospholipids, free fatty acids, cholesterol esters, and cholesterol itself (Gueroui, 2018). Milk triglycerides consist mainly of saturated (60–65%) and monounsaturated (32%) fatty acids. Essential fatty acids make up about 3%, while short- and medium-chain fatty acids (C4–C12) account for 11–15% **(Favier et *al.*, 1995).**

Lactose is the primary carbohydrate in milk, facilitating calcium absorption. While monosaccharides (mainly glucose and galactose), oligosaccharides, and glycosylated proteins are present, their concentrations are relatively low **(Gueroui, 2018).**

Milk is a significant source of minerals, especially calcium (1,200 mg/L), as well as phosphorus, sodium chloride, potassium chloride, and small amounts of sulfur, magnesium, and copper. However, it does not contain iron **(Enjalbert, 1993).** Because of its rich nutrient supply, nearly neutral pH (6.5–6.8), and high water activity, milk supports the growth of a wide range of microorganisms and is therefore highly perishable **(Gueroui, 2018).**

Whole milk provides substantial amounts of vitamins A and D. Nearly all B-group vitamins are present, particularly B12. However, skimmed milk lacks fat-soluble vitamins (A and D) **(Favier et *al.* , 1995; Martin, 2001; WHO, 2011)**.

1.1.3. Fats

Fats include butter, cream, oils, and margarines.

- Cream contains approximately 30–35% fat.
- Butter contains 82–84% fat, with saturated fatty acids making up more than 60% of total fatty acids.
- Butter also provides short- and medium-chain saturated fatty acids (about 13%).
- It is low in polyunsaturated fatty acids (2%) and contains 250 mg of cholesterol per 100 g.

- Butter is a rich source of vitamin A (with levels varying depending on its origin), contains small amounts of vitamin D, and lacks calcium **(Dupin et *al*., 1992; Martin, 2001).**

1.1.3.1. Animal Fats

Animal fats contain 90–100% lipids, with fatty acid composition varying depending on the animal's diet.

- Poultry fats (goose, duck) contain less saturated fat (30%), more monounsaturated fat (50–60%), and polyunsaturated fat (11–15%).
- All animal fats provide 100 mg of cholesterol per 100 g **(Dupin et *al*., 1992).**

1.1.4. Oils

Oils are liquid at room temperature, contain no cholesterol, and are composed of 100% lipids. Their fatty acid composition differs :

- Olive oil is particularly rich in monounsaturated fatty acids (70–75%) and has low levels of saturated and polyunsaturated fats **(Martin, 2001)**.

a. Rapeseed Oil (Canola Oil)

- High in monounsaturated fatty acids (60–65%).
- Contains 30% essential fatty acids, including 8% linolenic acid.
- Modern rapeseed varieties are virtually free of erucic acid.

b. Peanut Oil

- Contains 30–35% polyunsaturated fatty acids, with less than 1% linolenic acid.
- Rich in monounsaturated fatty acids (45–50%).
- Saturated fatty acids account for approximately 20%.

c. Polyunsaturated Oils

The best sources of polyunsaturated fatty acids (60–70% of total fatty acids) include :

- Corn oil
- Soybean oil
- Sunflower oil
- Grapeseed oil

- Walnut oil

Soybean and walnut oils are particularly rich in linolenic acid (7–15%) and are excellent sources of vitamin E **(Dupin et *al.*, 1992 ; Favier et *al.*, 1995 ; Martin, 2001).**

1.1.5. Fruits and Vegetables

The majority of fruits contain between 10% and 25% carbohydrates (fructose, but also sucrose or glucose, and more rarely starch), less than 1% protein, and less than 0.5% fat. The carbohydrate content in fruits includes cellulose, hemicellulose, lignin, and pectic substances, contributing to dietary fiber in human nutrition. Most fruits are relatively acidic ; acids can account for up to 2% to 3% of the total weight of fruits such as lemons. The major acids present in many fruits include malic acid and citric acid, while tartaric acid is abundant in most grape cultivars, and quinic acid is significant in certain fruits such as plums, cherries, and kiwis **(Gueroui, 2018).**

The average water content of vegetables is approximately 88%, with an average carbohydrate content of 8.6%, 1.9% protein, 0.3% fat, and 0.84% ash. The total percentage composition of vitamins, nucleic acids, and other constituents is generally less than 1% **(Gueroui, 2018).** Vegetables provide an important intake of potassium and calcium (especially in cabbages), magnesium, iron, and copper (leafy vegetables such as spinach), sulfur (cabbages, onions, garlic, leeks, turnips, radishes), and numerous other minerals. Vegetables are rich in water-soluble vitamins, including vitamin C (cabbages, leafy vegetables, tomatoes), provitamin A or beta-carotene (colored parts of plants such as green leafy vegetables and carrots), and B-group vitamins **(Favier et *al.*, 1995).**

Plant fibers mainly consist of cellulose, hemicellulose, and pectic substances. Potatoes are distinguished by a higher starch content (20%) and relatively low vitamin C content, especially after a few months of storage **(Favier et *al.*, 1995).**

A fruit is the result of the transformation of a flower following its fertilization. It contains the seeds that enable the plant to reproduce **(Xenou et *al.*, 2022).** Fruits are classified into six families :

- Stone fruits (plum, peach, cherry, apricot);
- Pome fruits (apple, pear, grape, quince);
- Berries and red fruits (blueberry, currant, blackcurrant);
- Citrus fruits (lemon, grapefruit, orange, clementine);

- Nuts (walnut, almond, chestnut, hazelnut);
- Exotic fruits (lychee, pineapple, mango, papaya)

1.1.5.1. Vegetables

Vegetables are known as the segments of plants that serve as food for humans and other animals. Collectively, the term "vegetable" refers to any edible part of a plant, fungus, or protist (including several algae) that is not sweet in taste **(Ebabhi & Adebayo, 2022)**. Depending on the species, fresh vegetables can come from all parts of the plant : roots (carrots, turnips), tubers (potatoes), stems (celery), leaves (spinach), flowers (cauliflower), fruits (tomato, zucchini), etc. **(Amiot-Carlin & Georgé, 2017)**. Thus, all edible fruits are considered vegetables.

- Bulb vegetables are those where only the bulbs are consumed, such as garlic, onion, shallot, green onion, and leek.
- Flower vegetables are those where the inflorescences or flower buds are consumed, such as artichoke, broccoli, or cauliflower.
- Stem vegetables are those from which parts of the transformed stem are consumed, such as celery **(Tirilly & Bourgeois, 1999 ; Vierling, 2003).**

Table 1 : Different Types of Vegetables **(Dess ,1999).**

Vegetables	**Categories.**
Leaf vegetables	-Whole leaf :whole bases - Petioles,Beet, cabbage, chives, watercress, spinach, lettuce, mâche, sorrel, parsley, dandelion, leek, celery, fennel, rhubarb.
Stem vegetables	Asparagus, kohlrabi
Root vegetables	Beet, carrot, celeriac, cassava, turnip, parsnip, sweet potato, radish, horseradish, rutabaga, salsify.
Bulb vegetables	Garlic, shallot, onion
Fruit vegetables	- Fleshy- Immature dry- Ripe dry Eggplant, cucumber, gherkin, squash, zucchini, pepper,

	plantain, bell pepper, pumpkin, tomato, melon; okra, green bean; chestnut, walnut, hazelnut.
Whole seedlings	Soybean sprouts, radish sprouts, alfalfa sprouts
Buds	Brussels sprouts, endive, bamboo shoots
Inflorescences	Artichoke, broccoli, cauliflower
Seeds	Broad bean, bean, lentil, pea
Tubers	Potato, taro, Jerusalem artichoke, yam, Japanese knotweed
Rhizomes	Ginger, lotus
Fruiting bodies	Mushroom

1.1.5. 2.Cereals and Derivatives - Legumes

Cereals, the fruits of cultivated grasses belonging to the monocotyledon family *Gramineae*, are arguably the most important agricultural crops worldwide. The eight major cereal crops include wheat, rice, maize, barley, sorghum, millet, oats, and rye.

Cereal grains contain 10% to 15% water, 70% to 76% carbohydrates, primarily in the form of starch, and 8% to 12% protein (14% in durum wheat). The lipid content is low, ranging from 2% to 4% (mainly present in the germ). Whole cereals and flours also provide fiber. Cereals and their derivatives are low in calcium but contain a significant amount of phosphorus in the form of phytic acid, especially in whole flour products. They lack vitamins A, C, and D but have higher levels of B vitamins, particularly vitamin B2 (riboflavin) and vitamin B1 (thiamine) **(Dupin et *al.*, 1992 ; Favier et *al.*, 1995).**

The legumes category includes pulses (lentils, beans, peas, chickpeas, etc.), soybeans, and peanuts.

- Pulse : These foods are rich in protein (24%), minerals (phosphorus, iron 85% to 90%), and B-group vitamins. They are also high in fiber (12% to 25% of dry weight), which can sometimes hinder digestibility, and contain 45% lipids.
- Soybeans and peanuts **:** These foods are comparable to pulses in terms of protein, vitamin, and mineral content. Additionally, they contain 18% lipids **(Favier et *al.*, 1995 ; Martin, 2001)**.

1.1.5.2.1. Nutritional and Therapeutic Benefits of Fruits and Vegetables.

Consumed raw or cooked, fruits and vegetables play a significant role in human nutrition. They are characterized by their low content of fats and carbohydrates, as well as their richness in vitamins, minerals, and dietary fibers **(Abosede & Raimot, 2022).** Furthermore, due to their abundance of phytochemical compounds, particularly antioxidants, they are regarded as essential foods considered in dietary recommendations **(Slavin & Lloyd, 2012).**

Nowadays, several studies have linked a low consumption of fruits and vegetables to chronic diseases such as cardiovascular diseases, hypertension, hypercholesterolemia, osteoporosis, various cancers, chronic obstructive pulmonary diseases, respiratory issues, and mental health problems **(Adriouch et *al.*, 2017)**. The secret to these benefits lies in their nutritional composition.

a. Fiber Content

Fruits and vegetables are foods that are very high in fiber, with contents ranging from 1 to 5.2 g per 100 g of fresh material. These compounds help regulate transit and nutrient absorption, making them beneficial for physiological parameters such as satiety, fecal excretion, and intestinal motility **(Dreher, 2018)**. The consumption of fruits and vegetables is also highly advantageous for metabolic parameters, particularly concerning postprandial lipemic response and, in the long term, baseline lipemia and characteristics of colonic flora, attributed to the prebiotic effects of certain fibers **(Arcusa et *al.*, 2021).**

b. Vitamin Content

Fruits and vegetables significantly contribute to the recommended dietary intake of certain vitamins, such as vitamin C, folates (vitamin B9), and vitamin A provided by provitamin A carotenoids **(Amiot-Carlin & Georgé, 2017)**. These vitamins play essential roles in various cellular processes.

Vitamin C possesses reducing properties fundamental to its biological activity. It has antioxidant activity and functions as a cofactor in oxygen-catalyzed reactions. Additionally, it is recognized for its ability to inhibit the synthesis of nitrosamines, which are carcinogenic compounds **(Titchenal & Dobbs, 2005).**

Vitamin A, generated from provitamin A carotenoids (α- and β-carotenes), is crucial for the physiology of nerve cells in the retina. As for vitamin B9 in fruits and vegetables, it is represented by the group of folates or polyglutamates. Folates are involved in the metabolism of amino acids and nucleic acids. A deficiency of folate in women during the time of conception is associated with an increased risk of neural tube defects in the fetus **(Eichholzer et *al.*, 2001).**

Vitamin K is an essential cofactor for the carboxylation of specific proteins involved in blood coagulation and the activation of osteocalcin, necessary for bone mineralization **(Weber, 2001 ; Luo & Xu, 2003 ; Lanham-New, 2008)**. Green vegetables and aromatic herbs are the primary sources of vitamin K, with parsley and kale being notable. For example, they contain 1220 µg and 817 µg per 100 g of dry matter, respectively **(Lefsrud et *al.*, 2008 ; Slimestad et *al.*, 2020).**

c. Mineral Content

Vegetables are a significant source of potassium. They also provide calcium (especially in cabbages), magnesium, iron, and copper (found in leafy vegetables like spinach), sulfur (in cabbages, onions, garlic, leeks, turnips, and radishes), and many other mineral substances. Many of these minerals are essential for the proper functioning of the body.

Potassium works closely with sodium to maintain the body's acid-base balance and fluid levels. It is necessary for the transmission of nerve impulses and muscle contraction, including that of the heart muscle **(Amiot-Carlin & Georgé, 2017).** When the body's potassium needs are not met, it negatively impacts blood pressure. The protective effect of a high dietary intake of potassium on the development of hypertension is well documented, and an intake of 4.5 g/day is recommended for the primary prevention of hypertension **(Lelong, 2017).** The usual potassium consumption ranges from 2340 to 5850 mg per 24 hours **(Mizehoun, 2015).** Dietary potassium primarily exists in the form of phosphate, citrate, and bicarbonate.

As for magnesium, it is a very important element in human biology as it is the second most abundant intracellular cation. Most metabolic pathways are magnesium-dependent, and this element plays a key role in the ionic balance of membranes **(Amiot-Carlin & Georgé, 2017).**

The proper functioning of the vascular system. A magnesium deficiency can also be exacerbated by factors that disrupt the homeostatic mechanisms of magnesium, such as stress and diabetes.

Epidemiological studies, as well as experimental research, show an inverse correlation between serum magnesium levels and blood pressure **(Kouassi et *al.*, 2013).** Moreover, a higher intake of magnesium through food, including fruits and vegetables, or even supplementation, could also be a means of preventing type 2 diabetes in obese children **(Huerta et *al.*, 2005).**

1.1.5.2.2. Bioactive Compounds in Fruits and Vegetables

A bioactive molecule is one that possesses biological properties or biologically active substances for therapeutic or preventive purposes **(Grigoraş, 2012).** Fruits and vegetables contain a variety of bioactive compounds .

a. Carotenoids

Carotenoids protect cells against free radicals and specifically against oxidative damage related to light. They can be converted into vitamin A according to the body's needs. In cases of deficiency, the body will draw from dietary sources to produce vitamin A **(Brat & Brillouet, 2003)**. Several carotenoids are provitamin A, and some also exhibit anticancer and antioxidant activities **(Amiot-Carlin & Georgé, 2017).** Additionally, they stimulate the synthesis of antibodies. Carotenoids are primarily found in spinach and carrots, which contain 7240 µg and 12,800 µg per 100 g, respectively **(Xu et *al.*, 2017).**

b. Polyphenols

Several epidemiological studies have suggested that a high consumption of fruits and vegetables can reduce the incidence of cardiovascular diseases **(Wang, 2011).** Indeed, fruits and vegetables are recognized as significant sources of polyphenols. Notable examples include black grapes (91.23 mg/100 g), apples (56.35 mg/100 g), guavas (126.4 mg/100 g), strawberries (97.56 mg/100 g), broccoli sprouts (73.78 mg/100 g), and artichokes (260.3 mg/100 g).

These polyphenols are largely represented by flavonoids, phenolic acids, stilbenes, and lignans **(Duchêne-Massias, 2015 ; Gollucke et *al.*, 2013 ; Amiot-Carlin & Georgé, 2017).** These compounds play an essential role in the body as they possess antioxidant and free radical-scavenging activities.

1.1.5.2.3. Production of Fruits and Vegetables

According to the latest statistics from the FAO, global production of fruits and vegetables increased by approximately 50% from 2000 to 2018. The global consumption of fruits and vegetables per person per day rose from 306 g in 2000 to 390 g in 2017 **(FAO, 2020).** In 2018, the quantities of fruits and vegetables produced were 868 million and 1089 million tonnes, respectively.

The main fruits produced, in order of quantity, were bananas, citrus fruits, melons, apples, and grapes. The main vegetables produced in 2018 included tomatoes, certain alliaceous vegetables (onions, garlic, shallots, leeks), brassicas (cabbage, cauliflower, broccoli), and cucumbers **(FAO, 2021).**

East Asia is by far the leading production area, followed by South Asia. Other major production regions include South America, Southeast Asia, and Southern Europe (for fruits).

These statistics only account for major producers, and a significant portion of what small-scale farmers cultivate is not included **(FAO, 2021)**. Notably, in East Africa and West Africa, vegetable production has doubled between 2000 and 2018 **(FAO, 2020).** While the world is producing more fruits and vegetables, this increase is still insufficient. Furthermore, a large portion of the produced quantities is lost before reaching the consumer's plate.

Managing these foods is challenging because fresh fruits and vegetables are seasonal and perishable products **(Desbordes, 2017).** Additionally, production areas tend to be quite specialized and sometimes far from major consumption centers.

1.1.5.2.4. Different Ranges of Fruits and Vegetables

Due to various constraints, the sectors involved in this field use multiple preservation techniques. Fresh fruits and vegetables, along with their preparations, are sometimes classified into different "ranges" based on preservation methods, elaboration techniques, and conservation processes. Food products, particularly fruits and vegetables, are categorized into six ranges :

- ✓ 1st Range : Fresh fruits and vegetables in their raw state, with no transformation.
- ✓ 2nd Range : Canned or semi-preserved products, generally stored at room temperature.
- ✓ 3rd Range : Frozen products, which include raw or prepared items stored below -18 °C.
  - ✓ 4th Range : Raw, fresh fruits and vegetables that are ready-to-use. These products are packaged under vacuum or in a modified atmosphere and preserved by refrigeration. Examples include bagged salads and pre-peeled vegetables.

- ✓ 5th Range : Products cooked under vacuum, offering consumers pasteurized or sterilized fruits and vegetables, primarily kept in a cold chain. The losses during transformation are similar to those in the 4th range.
- ✓ 6th Range : Dehydrated or Freeze-Dried Products

This range includes dry foods, typically ionized, that have a long shelf life at room temperature **(Jeannequin et al., 2015)**. These products undergo processes that remove moisture, making them lightweight and convenient for storage and transportation.

1.1.5.2.4.1. Definition of Fourth Range Fruits and Vegetables

-Fourth range fruits and vegetables are plant-based food products that have been cut, washed, peeled, diced, frozen, mashed, or pressed without prior pasteurization or mixing **(Jeannequin et *al.*, 2015).** They are packaged in trays and/or films that may or may not be in a gas atmosphere.

-The challenge is to keep the product fresh while preserving its nutritional qualities and ensuring a sufficiently long shelf life for distribution. These products offer several advantages to consumers, including :

- ✓ Convenience and time savings in meal preparation.
- ✓ Retention of fresh taste and nutrients.
- ✓ Reduced food waste due to longer shelf life.
- ✓ Easy storage and handling.

1.1.5.2.4.2. Advantages and Challenges of Fourth Range Fruits and Vegetables

-Fourth range fruits and vegetables reduce preparation time and ensure consistent quality and uniformity in prepared dishes. They allow consumers to enjoy fruits and vegetables of high nutritional quality, require less storage space, and generate less waste. However, a major challenge is microbial instability and various alterations that the product may undergo during storage. Their shelf life is generally between 5 to 9 days under optimal storage conditions at 4 °C, without breaking the cold chain **(Tirilly & Bourgeois, 1999 ; Varoquaux, 2002 ; Jeantet et *al.*, 2007)**.

1.1.5.2.4.3. Overview of the Fourth Range Products Sector

-The French market for processed fresh fruits and vegetables was estimated at €1.1 billion in 2006 (factory gate price), across all distribution channels. The value growth of 8.4% in 2006 outpaced that of the overall food sector. However, growth varied by segment, with fourth range fruits experiencing more moderate growth. This sector must adapt to a constantly changing environment. Significant modifications in the global fruit landscape have led to a reorganization in the Southern Hemisphere around major international operators. The roles of wholesalers and importers are evolving. Large retailers hold the key to many markets. At the end of the supply chain, consumers have new demands regarding convenience, health, and quality.

-Fresh fruits and vegetables are competing with processed products (ranging from fourth range items to ultra-fresh products, canned goods, and juices) in an effort to maintain their market share. The key challenges facing the market today include :

- **Countering Devaluation** : Addressing the devaluation caused by the development of innovation policies that create added value and "economic ranges."
- **Offering a Diverse Range** : Proposing a diversified product range by positioning on promising market segments such as fourth range fruits, exotic fruits, and health-focused products.
- **Targeting International Development** : Focusing on international growth, particularly in Europe, while considering constraints related to shelf life (DLC) and logistical costs **(Ctil F, 2016).**

1.1.6. Sugars and Sweetened Products

This category includes all foods with a pronounced sweet taste, such as chocolate, honey, jam, pastries, and spreads. However, these foods also contain so-called "hidden" fats.

The primary nutritional contribution of this group is carbohydrates (sucrose, glucose, or fructose), with no other significant nutrients except in chocolate. The term "sugar" is reserved for mono- and disaccharides, excluding polyols, according to national and European regulations on food labeling and presentation.

- ✓ Confectionery **:** A food preparation in which sugar is the dominant component, excluding jams, jellies, and marmalades.
- ✓ Honey : Honey consists of 3% to 6% sucrose, 35% glucose, and 35% fructose. Vitamins and minerals are present only in trace amounts.

- ✓ Chocolate **:** Chocolate is obtained by mixing sugar and cocoa paste. On average, chocolate contains 50% to 65% sucrose, 20% to 30% lipids (mainly cocoa butter), 6% protein, minerals, and small amounts of vitamins **(Dupin et *al.*, 1992 ; Martin, 2001).**

1.1.7. Water and Beverages

1.1.7.1. Water

1.1.7.1.1. Importance of Water for Public Health

Water is an exceptional raw material as it is essential for all forms of life, whether human, animal, or plant. It has always been regarded as a widely available and renewable resource.

Water holds critical biological and economic significance ; the hydrosphere is the foundation of life and ecological balance **(Uwamungu, J., Jiang, Y, 2010)**. Water serves multiple purposes : it is both a food source and potentially a medicine, as well as an industrial, energy, and agricultural raw material, and a means of transport. Its uses are therefore diverse, but concerning human health, they are primarily dominated by agriculture and aquaculture, industry and craftsmanship, aquatic recreation including swimming, and especially the collective or individual provision of drinking water, which can be used for food purposes (drinking water, cooking) as well as for domestic and hygiene needs **(Festy, B., et *al.*,2003).**

1.1.7.1.2. Other Roles of Water

a. Structural Role.

Water is a fundamental component of the human body. It is present in all cells, tissues, and intra- and extracellular compartments.

b. Chemical Role.

Water functions as a solvent, a medium for reactions, a reactant, and a product of chemical reactions. It is also a product of oxidative metabolism.

c. Transport Role.

Water is the major component of blood. It transports nutrients to cells and helps eliminate waste from the body.

d. Thermoregulation Role.

Water helps maintain the body at the appropriate temperature during exposure to high heat or extreme cold **(Marie, G, 2012).**

1.1.7.1.3. Water Resources.

Water resources, linked to the hydrological, geographical, and demographic situation of countries, represent multiple and interdependent challenges : public health and food autonomy, economic competitiveness and the arbitration of various uses, geopolitical and financial parameters, and finally, environmental preservation **(Roignant, F, 2007).**

Water supply for the population can come from two sources with distinctly different characteristics :

a. Surface Water.

Surface water (lakes, rivers, reservoirs, etc.) is widely used today, as it is capable of providing substantial quantities for various consumption needs. However, it is inevitably subject to contamination from runoff and wastewater. It can carry microorganisms and chemical pollutants, necessitating appropriate treatment before use **(Festy, B et *al.*, 2003).**

b.Groundwater

Groundwater, including recognized sources such as spring water, natural mineral water, and thermal water,it is exclusively of underground origin, sourced either from a spring (a natural outlet of groundwater) or through drilling (a well, generally vertical, allowing access to the rock containing the groundwater - the aquifer - and enabling pumping) **(Lachassagne, P, 2019).** Groundwater is typically more mineralized, with this mineralization depending on the nature of the rocks traversed, the solubility of mineral salts, the contact time of the water with the minerals, and the varying recharge of the aquifers. Among these groundwater sources, shallow aquifers and deep aquifers are distinguished. Breathing, drinking, and eating are imperative necessities for humans **(Viland, M et *al.*, 2001).** Groundwater and surface water resources are subject to controls of their physical, chemical, biological, and bacteriological characteristics.

1.1.7.1.4. Water Intended for Human Consumption (WIHC).

The main types of water intended for human consumption are those supplied by a distribution network and bottled water **(ORE, 2016).**

a. Tap Water.

Tap water is potable water produced from water extracted from an underground aquifer (wells, boreholes, springs) or from a surface resource (rivers, lakes, reservoirs, streams). It is distributed directly to consumers. Depending on the quality of the extracted water, after various treatments, it is kept potable through the addition of chlorine, which makes the water safe to drink. The quality of tap water is highly regulated and subject to numerous health controls, making its consumption safe **(ARSA, 2015).**

b. Bottled Water.

There is a wide variety of water qualities marketed in bottles. However, from a regulatory standpoint, there are only two categories.

- Spring water falls into this category.
- Natural mineral water (NMW) : This is spring water that has therapeutic efficacy recognized by the National Academy of Medicine **(Chocat, B et *al.*, 2015).**

b.1. Spring Water (SW).

Spring water is exclusively of underground origin, suitable for human consumption, microbiologically safe, and protected against pollution risks, without treatment or additives other than those permitted for this water **(Journal officiel de la république Algérienne, 2004).** It meets the same physico-chemical and radiological quality requirements as tap water. The only treatments allowed by regulation are the separation of naturally present constituents ; disinfection of the water is prohibited **(OMS, 2015).** Spring water differs from natural mineral water in that it must comply with drinking water standards, does not have to have a constant and characteristic mineral composition, and does not claim to have health benefits (**Gerard, G., Philippe, H. 2014).**

b.2. Natural Mineral Water (NMW).

Natural mineral waters are naturally occurring underground waters that are microbiologically safe. They are distinctly different from other waters intended for human consumption due to their purity and specific content of mineral salts, trace elements, or other constituents, as well as their stable physico-chemical compositions. These characteristics are assessed from geological, hydrogeological, physical, chemical, physico-chemical, microbiological, and pharmacological perspectives **(Labadi, A.S., Hammache, H, 2016).** Natural mineral waters constitute a special case as their therapeutic qualities favorable to human health have been recognized by the National Academy of Medicine ; they are permitted regarding sometimes high mineral salt content **(Chocat, B et *al*., 2015).**

Natural mineral water is not subject to any treatment except in cases of interventions authorized by regulation (separation of naturally occurring ingredients ; purification of the water is prohibited). Indeed, water is distinguished by its presence at varying concentrations depending on the regions, which is why some waters have high mineral value compared to others ; these properties are recognized as genuine medicines to be consumed in moderation by the National Academy of Medicine **(OMS, 2015).**

Table 2 : Comparison of Different Types of Water **(CSEM, 2008).**

	Tap Water	**Spring Water**	**Natural Mineral Water**
Origin	Multiple : lakes, rivers, aquifers,….. etc	Underground	Underground
Natural Protection	/	Mandatory	Mandatory
Chemical Treatments	Potabilization Treatments (including chemical disinfection for transport)	No potabilization treatment	No potabilization treatment
Mineral Composition	Variable	Not necessarily stable	Must be stable
Recognized Effect on Health	/	/	Favorable effect on health, recognized by the Academy of Medicine."

b.2.1. Classification of Natural Mineral Waters

a. Non-carbonated Natural Mineral Water.

Non-carbonated natural mineral water is a type of natural mineral water that, in its natural state and after any permitted treatment and packaging, does not contain free carbon dioxide in a proportion greater than that necessary to keep the bicarbonate salts dissolved in the water **(Commission du Codex Alimentarius, 2007).**

b. Naturally Carbonated Natural Mineral Water.

Naturally carbonated natural mineral water is a type of natural mineral water whose gas content, after any permitted treatment, is the same as at the source, considering usual technical tolerances **(Journal officielle de la république Algérienne septembre, 2005).**

c. De-carbonated Natural Mineral Water.

De-carbonated natural mineral water is a type of natural mineral water whose carbon dioxide content, after any permitted treatment and packaging, is not the same as at the source **(Journal officielle de la république Algérienne, 2005).**

d. Carbonated Natural Mineral Water Reinforced with Source Carbon Dioxide.

Carbonated natural mineral water reinforced with source carbon dioxide is a type of natural mineral water whose carbon dioxide content, after any permitted treatment and packaging, is not the same as at the source and has had additional carbon dioxide from the source added **(Journal officielle de la république Algérienne, 2005).**

Carbonated Natural Mineral Water.

Carbonated natural mineral water is a type of natural mineral water that has been made carbonated, after any permitted treatment and packaging, by the addition of carbon dioxide from another **source (Commission du Codex Alimentarius, 2007).**

1.1.7.1.5. Drinking Water Standards

Water must meet pre-established standards that set "threshold" concentrations for a number of hazardous substances that may be present. However, while drinking water may comply with these standards, it does not mean it is free from these substances ; rather, their levels are low enough not to endanger consumer health.

The World Health Organization (WHO) regularly publishes guidelines for drinking water quality, which many countries use to develop their own national standards. These guidelines represent a scientific assessment of the health risks associated with biological and chemical substances in drinking water and the effectiveness of measures taken to address them.

The WHO recommends that national authorities consider social, economic, and environmental aspects when conducting a comparative risk and benefit assessment while adapting these guidelines to national standards **(Taleb. S, 2014).**

Table 3 : Standards issued by the World Health Organization (WHO 2006) for drinking water safety.

Element/Substance	Symbol/Formula	Normally Found Concentration in Surface	WaterGuidelines Set by WHO
Aluminium	Al		0,2 mg/l
Ammonium	NH_4^+	< 0.2 mg/l (can go up to 0.3 mg/l in anaerobic water)	No constraints
Antimoine	Sb	< 4 µg/l	0.02 mg/l
Arsenic	As		0,01 mg/l
Amiante			No guideline value
Baryum	Ba	< 1 µg/l	0,7 mg/l
Béryllium	Be	< 1 mg/l	No guideline value
Bore	B	< 1 µg/l	0.5mg/l
Cadmium	Cd		0,003 mg/l
Chlore	Cl		No value but a taste can be noted starting from 250 mg/l
Chrome	Cr+3, Cr+6	< 2 µg/l	Total chromium: 0,05 mg/l
Color:			No guideline value
Cuivre	Cu2+		2 mg/l
Cyanure	CN-		0,07 mg/l
Dissolved oxygen	O2		No guideline value

Fluorure	F-	< 1,5 mg/l (up to 10)	1,5 mg/l
Hardness	mg/l CaCO3		200 ppm (TH (f)=20°)
Sulfure d'hydrogène	H2S		0.05 à 1 mg/L
Fer	Fe	0,5 - 50 mg/l	No guideline value
Plomb	Pb		0,01 mg/l
Manganese	Mn		0,4 mg/l
Mercure	Hg	< 0,5 µg/l	Inorganic : 0.006 mg/l
Molybdenum	Mb	< 0,01 mg/l	0,07 mg/l
Nickel	Ni	< 0,02 mg/l	0,07 mg/l
Nitrate and nitrite			50 and 3 mg/l (short-term exposure) 0.2 mg/l (long-term exposure)
Turbidity	NO_3, NO_2		Not mentioned
pH			No guideline value but an optimum between 6.5 and 9.5
Selenium	Se	< < 0,01 mg/l	0,01 mg/l
Argent	Ag	5 – 50 µg/l	No guideline value
Sodium	Na	< 20 mg/l	No guideline value
Sulfate	SO4		500 mg/l
Inorganic tin	Sn		No guideline value: low toxicity

TDS			No guideline value but optimum below 1000 mg/
Uranium	U		0.015 mg/l
Zinc	Zn		3 mg/l

1.1.7.1.6. Minerals in Water

a. Cations

- **Calcium**

  Calcium is one of the predominant constituents of carbonate rocks. The concentration of calcium in groundwater is controlled by the solubility of certain minerals. It is the major component of water hardness. Concentrations exceeding 200 mg/L diminish the water's usability due to the formation of calcium deposits **(Collin, 2004).**

- **Magnesium**

  Magnesian minerals are less soluble than calcium-containing minerals **(Collin, 2004)**. High concentrations of magnesium can affect the taste of water and may have a laxative effect **(Samake, 2002).**

- **Sodium**

  Elevated sodium levels can originate from geological sources or contamination. Concentrations exceeding 200 mg/L lead to noticeable changes in taste (SAMAKE, 2002). Excessive sodium intake can cause health issues, particularly increasing blood pressure and exacerbating symptoms of certain diseases such as nephritis **(Court, 1986 ; Desjarains, 1990).**

- **Potassium**

  Potassium is primarily found in igneous rocks and clays. Despite its abundance, potassium is generally present in low concentrations in natural waters. In clay minerals, this is attributed to the difficulty in mobilizing the K ion. In groundwater, potassium concentrations typically do not exceed 10 mg/L **(Chery, 2006).**

b. Anions

- **Chlorides**

  Chlorides are widely distributed in nature, occurring in rocks as sodium chloride (NaCl) and potassium chloride (KCl). In water, chlorides are always in ionic form.

Chlorides present in water can have several origins :

- ✓ Percolation of water through saline soils ;
- ✓ Infiltration of seawater into the groundwater ;
- ✓ Human discharges ;
- ✓ Extractive industries, such as potash mining or saltworks.

In coastal regions, rain brings oceanic chlorides that can accumulate on the surface through evaporation and subsequently be transported into aquifers **(Rejsek, 2002).**

- **Sulfates**

  Sulfates primarily originate from gypsum present in the soil and can also result from the leaching of atmospheric sulfates by precipitation. The presence of sulfates imparts a bitter taste to water, and since sulfates are virtually non-assimilable, water with high sulfate content can produce laxative effects in humans **(Mabillot, 1986).** They penetrate cell membranes and are rapidly excreted by the kidneys. High sulfate concentrations may contribute to the corrosion of distribution systems**.**

- **Bicarbonates**

  Most natural waters have a pH ranging from 4.5 to 8.3, and their alkalinity is primarily attributed to bicarbonates**.** Bicarbonates originate from the dissolution of calcium and magnesium carbonates according to the following chemical reactions :

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO^-_3$$

$$CaCO^-_3 + H^+ \leftrightarrows Ca^{+2} + HCO^-_3$$

Ou:

$$MgCO^-_3 + H^+ \leftrightarrows Mg^{+2+}\ HCO^-_3$$

These reactions require the presence of CO_2 in the water, which comes from the atmosphere and the intense activity of bacteria in the soil.

c. Elements Considered Undesirable

- **Iron**

The presence of iron in groundwater has multiple origins : iron, in the form of pyrite (FeS_2), is commonly associated with sedimentary rocks deposited in reducing environments (marls, clays) and metamorphic rocks. Depending on the concentration of divalent iron in reducing groundwater, turbidity, a yellow coloration, and over time, the precipitation of iron hydroxide may occur upon contact with atmospheric oxygen. The development of iron-dependent bacteria is also possible. Consequently, the taste may be adversely affected, and the appearance of brown or black residues may occur **(Rodier et *al.*, 2005).**

- **Copper**

  In groundwater, normal copper concentrations are below 0.005 mg/L **(Collin, 2004).** Elevated copper levels may arise from copper installations, primarily observed in stagnant water **(C.I. Eau, 2005).**

- **Aluminum**

  Aluminum may enter water through flocculating agents or aluminum anodes (cathodic protection against corrosion).

d. Elements Considered Toxic

According to Degrement (2005), elements considered toxic in water intended for human consumption include :

- **Antimony**

Antimony is suspected of influencing blood composition and is limited to 5 µg/L in drinking water by the WHO.

- **Barium**

  Barium is typically of natural origin ; this metal could cause cardiovascular problems, and its presence in drinking water is regulated to 0.7 µg/L.

- **Selenium**

  Selenium is widely distributed in nature and is toxic to humans at the liver, angles, and hair levels. The WHO limits selenium in drinking water to a concentration of 10 µg/L.

- **Fluor**

  The main sources of fluoride in groundwater are sedimentary rocks, as well as magmatic rocks and certain veins. Thermal areas are also affected. Fluoride is recognized as an essential element for the prevention of dental caries (fluoride toothpaste). However, regular ingestion of water with a fluoride concentration greater than 2 mg/l (WHO) can lead to issues with skeletal and dental fluorosis (brown discoloration of teeth that can progress to loss). Children are particularly vulnerable to this condition **(Hillel, 1988).**

Microorganisms of Interest in Food Microbiology

2. Chapter : Microorganisms of Interest in Food Microbiology

2.1. Foodborne Intoxications

Food can serve as a vector or an actual culture medium for microorganisms. As a result, they can potentially cause various illnesses in consumers, the severity of which depends primarily on the type and quantity of microorganisms and/or the toxicity of their excreted products. In developing countries, foodborne intoxications are facilitated by :

- The predominantly warm climate in many of these regions.
- The lack of developed hygiene services, making effective control impossible.
- Food shortages, which compel consumers to accept spoiled food **(Ait Abdelouahab, 2001).**

The pathogenicity of bacteria can depend on several factors, leading to different classifications :

- **Infection :** Some species possess infectious capabilities and cause illness by invading the host. The most common cases result from ingestion of microorganisms belonging to the genera *Salmonella, Shigella, Listeria, Brucella, Mycobacterium, Escherichia, Campylobacter, Clostridium, Yersinia, Vibrio*, and certain viruses. These microorganisms behave as parasites within the host, utilizing host nutrients for growth and proliferation **(Cuq, 2007).**
- **Intoxination :** Some species produce specific toxins *in vivo* (e.g., diphtheria, tetanus) or within food. The symptoms are entirely due to the toxin's effects without the need for pathogen proliferation within the host. Examples include botulinum toxin (*Clostridium botulinum*), staphylococcal enterotoxin (*Staphylococcus aureus*), and mycotoxins from molds **(Cuq, 2007).**
- **Foodborne Toxi-infection (TIA) :** Some bacterial species exhibit a mixed mode of pathogenicity, causing disease through both endotoxins (released upon cell lysis as a defense response) and exotoxins (released during bacterial multiplication). Examples include *Vibrio cholerae* (cholera), *Vibrio parahaemolyticus* (seafood-related infections), *Salmonella typhimurium* (salmonellosis), *Shigella sonnei* (shigellosis), and *Campylobacter jejuni* (gastroenteritis, commonly from poultry) **(Meyer et *al.*, 1984 ; Guiraud, 1998).**

A collective foodborne toxi-infection (TIAC) is defined by the occurrence of at least two similar cases presenting symptoms linked to the same foodborne source.

Intoxications : Other bacterial species act by transforming substrates into toxic compounds, leading to foodborne intoxications. This is usually due to contamination from poor hygiene practices, followed by prolonged storage at ambient temperatures. Examples include histamine intoxication caused by *Pseudomonas, Clostridium perfringens, Proteus morganii, Klebsiella pneumoniae*, and even *Escherichia coli* **(Ait Abdelouahab, 2001).**

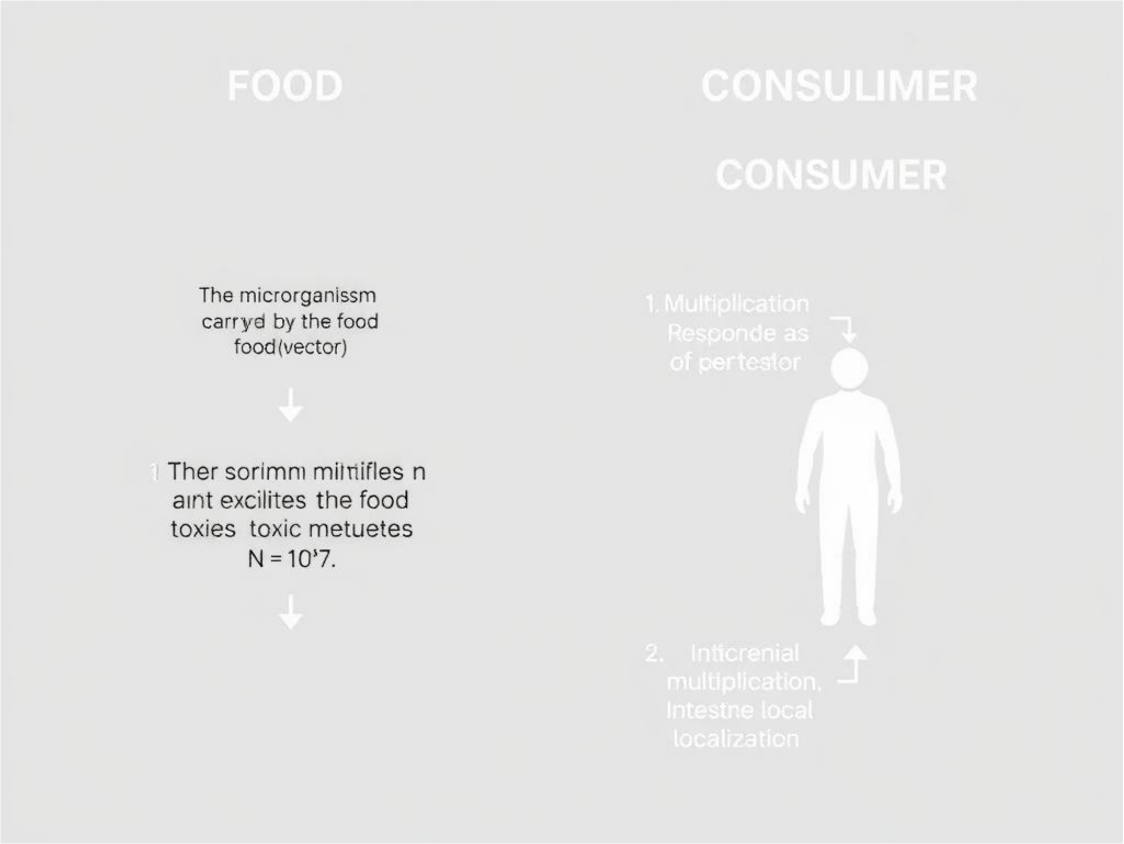


Figure 2. Modes of Toxic Action **(Cuq, 2007)**

2.2. Major Microorganisms Involved in Food Microbiology

Microorganisms contaminate a wide range of food products and can pose significant risks to their quality and preservation. Some species present serious health hazards, while others play essential roles in beneficial fermentation processes, contributing to various industries **(Guiraud, 1998).**

Microorganisms relevant to food microbiology are heterotrophic. Some species are saprophytes, living freely in nature ; others are commensals of humans and animals ; and some are parasites with pathogenic potential.

2.2.1. General Aspects of Lactic Acid Bacteria

Lactic acid bacteria belong to a group of beneficial bacteria that are widely distributed in nature, including the human digestive system. For millennia, they have been used in human food production. Today, in the food industry, lactic acid bacteria play a crucial role as processing aids. While they are primarily recognized for their role in the dairy sector **(Dortu & Thonart, 2009 ; Moraes et *al*., 2010)**, they are also used in vegetable fermentation, meat and fish curing, baking, and winemaking. Generally, they hold the GRAS (Generally Recognized As Safe) status **(Vescovo et *al*., 1996).**

2.2.1.1. Definition

The group of lactic acid bacteria was defined by ORLA-JENSEN (1919) and includes several genera characterized by their ability to ferment carbohydrates, producing lactic acid as a major end product. Lactic acid bacteria are defined as living, prokaryotic, heterotrophic, and chemo-organotrophic cells **(De roissart, 1986).** They form a heterogeneous group composed of cocci and bacilli, with their main characteristic being lactic acid production through sugar fermentation **(Badis et *al*., 2005).**

Lactic acid bacteria are Gram-positive bacteria. They are generally immobile, non-sporulating, and microaerophilic. They lack catalase, nitrate reductase, and cytochrome oxidase **(Köning & Förhlich, 2009).**

Lactic acid bacteria comprise 13 genera, including *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Bifidobacterium, Carnobacterium, Oenococcus, Weissella, Aerococcus, Tetragenococcus,* and *Vagococcus* **(Dortu, 2009).** These bacteria have the ability to ferment sugars such as glucose, fructose, mannose, galactose, sucrose, and lactose into lactic acid **(Kandler & Weiss, 1986).**

Lactic acid bacteria used in food production are generally considered non-pathogenic. However, some species within the *Streptococcus* and *Enterococcus* genera are classified as opportunistic pathogens **(Aguirre & Collins, 1993)**.

Lactic acid bacteria have extensive nutritional requirements, including amino acids, peptides, salts, fatty acids, and carbohydrates **(Holzapfel et *al*., 2001 ; Gevers, 2002).**

2.2.1.2. Habitat

Lactic acid bacteria are ubiquitous and can be found in various ecological niches, including milk and dairy products, plants, meat, fish, human and animal mucous membranes, and the digestive tract. This widespread distribution explains their heterogeneous growth temperature range **(Mayo et *al*., 2010 ; Klein et *al*., 1998).**

Species of the *Lactococcus* genus are primarily isolated from milk or plants, which serve as their natural reservoirs. *Lactococcus lactis* subsp. *lactis* was first isolated from fermented milk by Lister in 1873 and was later recognized as the primary agent responsible for milk curd acidification **(Sandine, 1988).**

- Species of the *Leuconostoc* genus are found in milk, dairy products, fruits, vegetables (particularly beets), fermented plant materials (such as sauerkraut), bakery products, and viscous sugar solutions in sugar refineries **(Devoyod and Poullain, 1988).**
- Species of the *Pediococcus* genus are mainly present in decaying plant material, sometimes in alcoholic beverages, milk, various cheeses (such as Parmesan and other Italian cheeses), and culinary preparations (including sausages, salted anchovies, and soy sauce) **(Chapman and Sharpe, 1981 ; Dellaglio et *al.*, 1994)**.

Species of the *Lactobacillus* genus are found in various environments :

- In milk and cheeses (*Lactobacillus casei* subsp. *casei*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, and *Lactobacillus brevis*).
- In fermented milk (*Lactobacillus kefir*, *Lactobacillus brevis*, and *Lactobacillus fermentum*).
- In fermented plant products, marinades, silage, wine, and fresh or fermented meats (*Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus buchneri*, and *Lactobacillus sanfranciscensis*) **(Demazeaud, 1996).**

2.2.1.3. Historical Background and Classification

Lactic acid bacteria are among the oldest microorganisms, discovered in sediments dating back 2.75 billion years—before oxygen appeared in the atmosphere—which may explain their anaerobic nature **(Quiberoni et *al.*, 2001)**. They emerged before photosynthetic cyanobacteria **(Quiberoni et *al.*, 2001 ; Drider and Prévost, 2009).**

The phenotypic classification of lactic acid bacteria is based on several criteria, including :

- Morphology (cocci or bacilli).
- Growth at different temperatures to determine optimal and tolerable ranges.
- Sugar fermentation patterns (homofermentative or heterofermentative pathways).
- Ability to grow at different salt concentrations to assess osmotolerance.
- Tolerance to acidic and alkaline pH conditions.
- Lactic acid configuration (D- or L-lactic acid production).
- Arginine hydrolysis as a metabolic trait.
- Acetoin formation (important in flavor production).

Additionally, a classification based on cell wall composition has been proposed **(Deambrosini et *al*., 1996),** which includes the nature of the fatty acids that constitute the bacterial membrane **(Gilarova et *al*., 1994).**

Another classification system, based on the nature of metabolic products derived from carbohydrate fermentation, divides lactic acid bacteria into three groups **(Mcleod et *al*., 2008):**

- ✓ **Group I:** Contains mainly homofermentative Lactobacillus species, which primarily convert glucose into lactic acid.
- ✓ **Group II:** Includes heterofermentative bacteria, such as species from the genera *Leuconostoc, Oenococcus*, and *Weissella*, as well as some *Lactobacillus* species. These bacteria produce lactic acid along with other by-products like ethanol, CO_2, or acetic acid.
- ✓ **Group III:** Contains certain *Lactobacillus* species and the majority of *Enterococcus, Lactococcus,* and *Streptococcus* species. This group is considered **intermediate**, as its members can switch between homofermentative and heterofermentative metabolism depending on environmental conditions.

This metabolic classification provides insights into the functional diversity of lactic acid bacteria and their applications in fermentation and food industries.

According to **Ludwig et al. (2008)**, the phylum Firmicutes consists of three classes : *Bacilli, Clostridia*, and *Erysipelotrichi. Lactic* acid bacteria (LAB) belong to the class Bacilli and are further divided into three families :

- **Family Lactobacillaceae** : Includes the genera *Lactobacillus, Paralactobacillus,* and *Pediococcus*.
- **Family Leuconostocaceae** : Contains *Leuconostoc, Oenococcus,* and *Weissella*.
- **Family Streptococcaceae** : Comprises *Streptococcus, Lactococcus,* and *Lactovum*.

Historically, the genus Bifidobacterium was also considered part of the LAB group due to physiological and biochemical similarities and its presence in similar ecological habitats, particularly in the gastrointestinal tract **(Klein et *al*., 1998).** However, it is now classified within the phylum Actinobacteria rather than Firmicutes.

2.2.1.4. Metabolic Pathways of Lactic Acid Bacteria (LAB)

Depending on the bacterial species and culture conditions, glucose catabolism follows two main pathways :

1. Homofermentative Pathway (Hexose Diphosphate Pathway / Embden-Meyerhof-Parnas Glycolysis)
    - o The primary product is lactic acid, which is excreted in large amounts.

- Reaction : 1 Glucose→2 Lactic Acid+2ATP
- This pathway is mostly found in Lactococcus, Streptococcus, and some Lactobacillus species.

2. Heterofermentative Pathway (Pentose Phosphate Pathway / 6P-Gluconate Pathway)
   - Produces lactic acid along with other byproducts such as CO_2, ethanol, and acetic acid.
   - Reaction : 1 Glucose→1 Lactic Acid+1 Ethanol (or Acetate) +1 CO_2+1ATP1
   - Found in Leuconostoc, Weissella, Oenococcus, and some Lactobacillus species.
   - The production of carbon dioxide (CO_2) is a key characteristic of heterofermentative LAB and can be used as a diagnostic test in laboratory settings.
   - Homofermentative LAB, in contrast, do not produce gas (or produce it in negligible amounts).

These metabolic differences have practical implications in food fermentation, probiotic development, and industrial applications **(Mensah et *al*., 1990; Kimmons et *al*., 1999).**

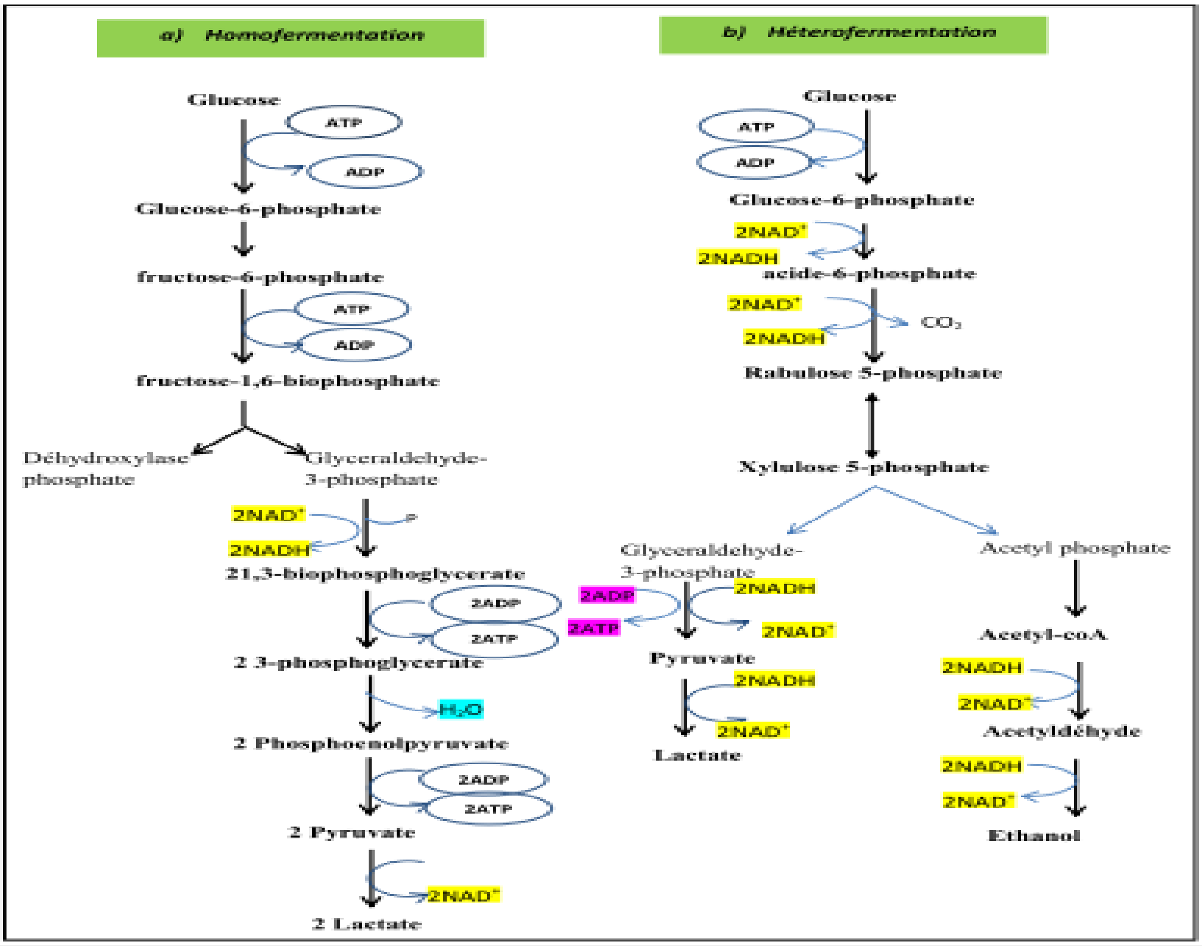


Figure 3. Types of Lactic Fermentation : Homofermentation by Lactic Bacteria (a) and Heterofermentation (b) **(Prescotte et *al.*, 2010).**

There is a new classification of lactic bacteria into three groups based on their enzymatic equipment :

- Strict homofermenters, which possess a fructose-1,6-bisphosphate aldolase but lack glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, following the hexose diphosphate pathway ; for example, *Lactobacillus delbrueckii* (Figure 3).
- Strict heterofermenters, which contain both dehydrogenases but lack fructose-1,6-bisphosphate aldolase, following the pentose phosphate pathway ; for example, *Lactobacillus brevis* (Figure 3).
- Facultative heterofermenters, which possess all three enzymes and are capable of following either metabolic pathway ; for example, *Lactobacillus casei* (Figure 3) **(De Roissart, 1994).**

2.2.1.5. Classification and Taxonomy

-The group of lactic bacteria includes fermentation agents that produce lactic acid : bacilli (Lactobacillaceae) and cocci (Streptococcaceae).

Bacterial species belonging to the lactic group that exhibit these general properties are classified within the genera Lactococcus, Streptococcus, Enterococcus, Leuconostoc, Pediococcus, and Lactobacillus. Species within the genera Streptococcus, Enterococcus, Lactococcus, Leuconostoc, and Pediococcus show very similar guanine-cytosine content (% G+C) in their DNA. Furthermore, the variations among strains are also minimal **(Prescott et *al.*, 2010).**

2.2.1.5.1. Streptococcus, Lactococcus, and Enterococcus

The genera Streptococcus, Lactococcus, and Enterococcus were previously grouped into a single genus, Streptococcus. The separation of the genera Streptococcus and Lactococcus occurred only in 1985 with the establishment of the genus Lactococcus **(Schleifer and Ludwig, 1995),** and they are phylogenetically close. Lactococcus lactis and Lactococcus cremoris are widely used in cheese fermentation. The first available genome of lactic acid bacteria was that of a strain of Lactococcus lactis *ssp. lactis* in 2001 **(Bolotin et *al.*, 2001)**. Within the genus Streptococcus, the only species used as a ferment in the food industry is Streptococcus thermophilus, particularly in yogurt production with Lactobacillus bulgaricus.

For the genus Enterococcus, the most commonly encountered strains in food are primarily S. faecalis, S. durans, and S. bovis. These are indicator organisms for fecal contamination and are opportunistic pathogens with high infectious doses (10^8 to 10^{10} cells).

2.2.1.5.2. Oenococcus, Leuconostoc, and Weissella

The group comprising the genera Oenococcus, Leuconostoc, and Weissella belongs to the family Leuconostocaceae. This group includes bacteria that are widely used in the agri-food industry, such as Oenococcus oeni (formerly Leuconostoc oenos), which plays a crucial role in wine production for malolactic fermentation, and *Leuconostoc citreum*, utilized for the fermentation of certain vegetables. Additionally, *L. mesenteroides ssp. mesenteroides* is employed in the production of sauerkraut and dextran.

2.2.1.5.3. Pediococcus

Currently, the genus Pediococcus includes 11 species **(Zhang and Cai, 2014)**. Following the reclassification of P. urinae-equi to the genus Aerococcus and P. halophilus to the genus Tetragenococcus, Pediococcus can be described as the only acidophilic and homofermentative lactic cocci. These bacteria are significant in the agri-food sector, both negatively and positively. P. damnosus are the primary microorganisms responsible for beer spoilage due to the production of diacetyl/acetoins, which impart a buttery flavor. Conversely, P. acidilactici and P. pentosaceus have demonstrated their utility in the development of various naturally fermented meat products. Pediococci are also indigenous lactic bacteria that facilitate cheese maturation **(Thu, 2008).**

2.2.1.5.4. Lactobacillus

The genus Lactobacillus is the principal genus within the family Lactobacillaceae. It encompasses numerous species that serve as agents of lactic fermentation, playing roles in various industries or appearing as contaminants. They can be found on vegetation, in the natural microflora of humans, and in various fermented food products. The G+C content is approximately 32 to 53%. Typically, they are catalase-negative (some possess a pseudocatalase). Some are obligate homofermenters (e.g., Lactobacillus delbrueckii), while others are heterofermenters (e.g., *Lb. brevis*) and facultative heterofermenters (e.g., *Lb. casei*) **(Guiraud, 1998 ; Singleton, 1999)**. The genus Lactobacillus was subdivided by Orla Jensen (1919) into three groups, and this classification is still utilized in industrial settings.

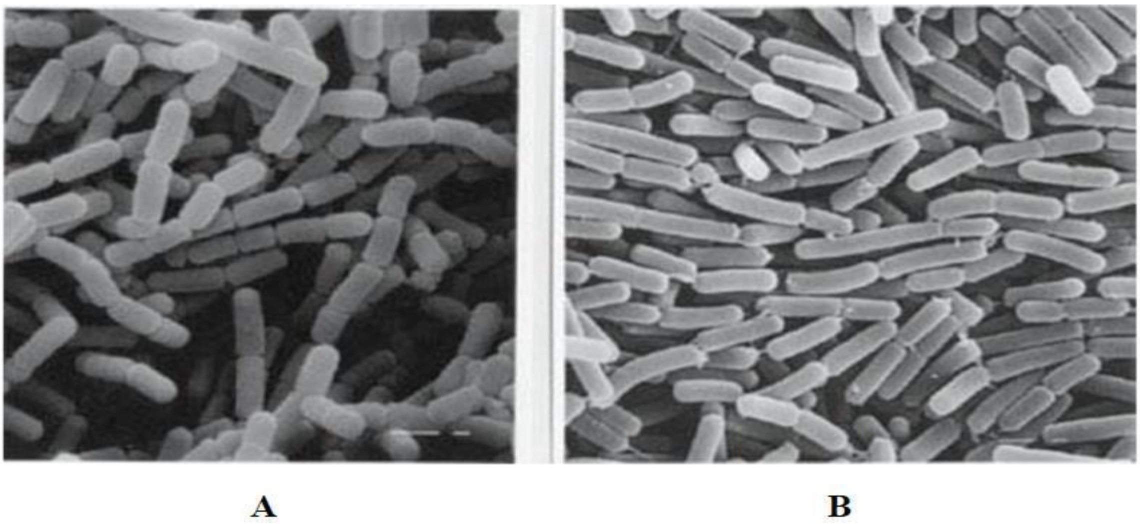


Figure 4 : Morphology of A : *Lactobacillus casei* and B : *Lactobacillus acidophilus* (Examined by electron microscopy, ×7000) **(Photo Bottazzi, 1988)**

- **Group I or “Thermobacterium”**

This group includes strict homofermentative lactobacilli, most of which are thermophilic, thriving at 45 °C but not at 15 °C. The most common species found in food (such as milk, yogurt, and cheese) are *L. helveticus*, *L. jugurti*, *L. bulgaricus*, *L. lactis*, *L. acidophilus*, *L. leichmanii*, *L. delbrueckii*, *L. kefirofaciens*, and *L. mali*.

- **Group II or “Streptobacterium”**

This group encompasses facultative heterofermentative mesophilic lactobacilli that can grow at 15 °C. These bacteria ferment pentoses via the heterofermentative pathway and hexoses via the homofermentative pathway. It includes *L. casei*, which are the predominant lactobacilli in milk, and *L. plantarum*, found in sauerkraut, along with *L. curvatus*, *L. sake*, *L. acetotolerans*, *L. graminis*, and *L. rhamnosus*, which are present in various plant and animal food matrices.

- **Group III or “Betabacterium”**

- **Group III or “Betabacterium”**

This group comprises strict heterofermentative lactobacilli. The most common species found in food include *L. fermentum*, *L. buchneri*, *L. brevis*, *L. fructivorans*, *L. hilgardii*, and *L. sanfrancisco* **(Thu, 2008).**

2.2.1.5.5. Bifidobacterium

The genus *Bifidobacterium* (formerly known as *Lactobacillus bifidus*) consists of bacilli found in the intestinal flora of newborns. Although classified among lactic acid bacteria, the *Bifidobacterium* genus is phylogenetically unrelated to them **(Patrignani et *al.*, 2006)**. They belong to a group of Gram-positive bacilli rich in G+C content (57.2-64.5%) and are phylogenetically close to actinomycetes, while other lactic acid bacteria are closer to clostridia **(Holzapfel et *al.*, 2001; Thu, 2008)**. In humans, *Bifidobacterium* are commensals found in the mouth, esophagus, stomach, intestines, respiratory tract, and vagina. In animals, they are primarily found in the intestinal flora. The presence of α-galactosidase quickly differentiates *Bifidobacterium* from *Lactobacillus*. The optimal growth temperature does not exceed 39 °C for human-derived species, while animal-derived species prefer 43-45 °C. *Bifidobacterium bifidus* is killed at 60 °C. Growth of *Bifidobacterium* is not possible at pH 4.5-5 and pH 8-8.5. These bacteria are glycolytic, producing acetic and lactic acids, along with small amounts of formic acid, ethanol, and succinic acid **(Bourgeois et *al.*, 1996).** Both *Bifidobacterium* and species of *Lactobacillus* may have a preventive role against colonization by enteropathogens. *Bifidobacterium* is used in certain "probiotic" yogurts, and its presence may exert an anti-infectious effect in the intestines due to a bifidogenic factor **(Guiraud, 1998)**. An interesting application of these bacteria is their use as a biocontrol agent in meat fermentation. The incorporation of *Bifidobacterium* into Hungarian salami has been used to inhibit the growth of *Listeria monocytogenes* and *E. coli* O111 **(Thu, 2008).**

Table 2 Contains the main species of lactic acid bacteria with these characteristics.

Table 4. Main Species of Lactic Acid Bacteria **(Federighi, 2005).**

Genus	Morphology	Type of Fermentation	Species	Growth Temperature
Streptococcus	Cocci in tetrads	Homofermentative	*S. thermophilus*	40-45 °C
Lactococcus	Cocci in pairs	Homofermentative	*L. lactis*, *L. cremoris*	10-40 °C
Leuconostoc	Cocci in chains	Homofermentative	*Ln. mesenteroides*	10-30 °C
Pediococcus	Cocci in pairs	Homofermentative	*P. acidilactici*	25-35 °C
Lactobacillus	Rods in chains	Homo/heterofermentative	*Lb. acidophilus*, *Lb. bulgaricus*, *Lb. delbrueckii*, *Lb. lactis*, *Lb. casei*, *Lb. plantarum*	45-50 °C

2.2.1.6. Role of Lactic Acid Bacteria

Lactic acid bacteria are widely used in the food industry as starters in industrial fermentation processes to meet the increasing consumer demand for food products.

2.2.1.6.1. Impact on Structure, Texture, and Organoleptic Characteristics

Lactic fermentation is employed in the production of various fermented products (cheeses, yogurts, numerous fermented plant products, and charcuterie), developing specific organoleptic characteristics without altering taste or odor, while also extending shelf life. Their beneficial contributions improve the quality of fermented products.

The action of lactic acid bacteria during fermentation is primarily associated with the development of aroma and texture in the final product, as well as maintaining food safety due to the organic acids produced.

In fermented milks, acidification causes milk coagulation (facilitating rennet action) and increases curd formation. Depending on the product, the desired texture can be firm (set yogurt) or creamy (stirred yogurt ; kefir) **(Satura and Federighi, 1998)**.

In addition to lactic acid, other products such as acetone, diacetyl, acetaldehyde, and ethanol are responsible for characteristic flavors **(Boudjemaa, 2016).**

Lipolysis hydrolyzes free fatty acids, forming ketonic acids and methyl ketone, while proteolysis yields peptides, amino acids, and ammonia ; this series of enzymatic operations contributes to cheese ripening **(De Roissart, 1994 ; Bourgeois et *al.*, 1996).**

The viscosity of milk can be modified by the mucilaginous capsules of some bacteria, such as *Leuconostoc* spp.

In the technology of blue-veined cheeses, such as Roquefort, the CO2 produced causes the formation of cavities in the curd, which are later colonized by *Penicillium roqueforti* **(Bourel et *al.*, 2001).**

The CO2 produced also gives the slightly effervescent and creamy aspect to butter **(Kihal, 1996).**

2.2.1.6.2. Role in Preservation

-Lactic Acid Production : Lactic acid bacteria play an important role in inhibiting non-lactic flora.

-They produce various metabolites with antimicrobial activity, such as organic acids, hydrogen peroxide, carbon dioxide, reuterin, diacetyl, and bacteriocins **(Bazinet and Castaigne, 2011).**

-Bacteriocin Production : Some bacteriocins may be useful in limiting food contamination by pathogens such as *Listeria monocytogenes* or *Clostridium botulinum* **(Ross et *al.*, 2002).**

2.2.1.6.3. Health Domain

The probiotic properties of lactic acid bacteria and their inhibition of pathogenic bacteria are particularly important and are also used in the treatment of certain conditions such as diarrhea, food allergies, lactose intolerance, and hypercholesterolemia. The bacteria most frequently used as probiotics are *Lactobacillus* and *Bifidobacterium* (e.g., *Bifidobacterium lactis* in yogurt) **(Boudjemaa, 2016).**

2.2.1.6.4. In the Food Industry

a. Streptococcus

- Used in agricultural silage production.
- Dairy products and other fermented foods (*Streptococcus thermophilus* in yogurt).

b. Lactobacillus

- Fermented products (*L. delbrueckii* sp).
- Yogurt (*L. bulgaricus*).
- Production of fermented milks associated with products like silage for animal feed (*L. acidophilus*), sauerkraut, and pickled products.
- Acidification and flavoring of meat products : salami and sausages (*L. sakei*, *L. curvatus*, *L. plantarum*).
- Baking starters (*L. plantarum*, *L. brevis*).
- Acidification and flavoring in sauerkraut and fermented vegetables (*L. brevis*, *L. plantarum*).
- Lactic acid bacteria are responsible for the final stages of lactic fermentation.

c. Pediococcus

- Degradation in brewing (*P. cerevisiae*), which makes beer viscous due to extracellular polysaccharide accumulation. They are sometimes used as lactic starters for charcuterie.
- Lactic acid and acetic acid production (*P. damnosus*).
- Acidification and flavoring in sauerkraut and fermented vegetables (*P. pentosaceus*).

d. Leuconostoc

- Malolactic fermentation of wines (*L. oenos* = *Oenococcus oeni*). Production of certain cheeses (blue cheese) with CO_2 production.

  - Silages (*L. mesenteroides*) and fermented plant products, olives, sauerkraut, as well as cocoa and coffee **(Prescott et *al.*, 2010).**

e. Enterobacteria

e.1. General Characteristics of the Group

Enterobacteria are a highly heterogeneous family in terms of pathogenicity and ecology. The species in this family can be parasitic (*Shigella*, *Yersinia pestis*), commensal (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella* sp.) in humans and other animals, or saprophytic (*Serratia* sp., *Enterobacter* sp.) in soil and water. They are widespread in nature due to environmental contamination from animal feces and sewage. They are frequent food contaminants, and some can be dangerous, leading to food poisoning **(Singleton, 1999 ; Guiraud, 1998).**

e.2. Enterobacteriaceae Family Characteristics

- Gram-negative bacilli (2 to 4 microns long and 0.4 to 0.6 microns wide) and non-spore forming.
- Motile with peritrichous flagella or non-motile.
- Grow on standard culture media.
- Facultative anaerobes.
- Ferment glucose with or without gas production.
- Reduce nitrates to nitrites.
- Oxidase-negative and catalase-positive, with some exceptions.

The presence of Enterobacteriaceae, coliforms, or thermotolerant coliforms indicates poor hygienic quality of the finished product or during production, suggesting fecal or environmental contamination, inadequate treatment processes, poor hygiene of equipment used, or cross-contamination from other origins (e.g., plant sources). For ready-to-eat products stored under refrigeration, Enterobacteriaceae may also indicate storage at excessively high temperatures or for too long.

e.3.Classification and Identification

The distinction between genera is based on the study of biochemical characteristics, the most important of which are the IMViC tests (Indole, Methyl Red, Voges-Proskauer, Inositol, and Citrate), urease tests, and decarboxylase tests. Typically, *Escherichia coli*, *Klebsiella pneumoniae*, and certain strains of *Citrobacter* can ferment lactose, whereas *Salmonella*, *Shigella*, *Proteus*, and *Yersinia* cannot. Other genera in this family include *Enterobacter*, *Erwinia*, and *Serratia* **(Singleton, 1999).**

Within each genus, species are distinguished by studying biochemical or antigenic characteristics. Enterobacteriaceae possess somatic wall antigens (O antigens). Mobile

Enterobacteriaceae also have flagellar antigens (H antigens), while some possess a capsule or K antigen.

e.4. Coliforms and *E. coli*

Among Enterobacteriaceae, which are Gram-negative bacteria primarily found in the intestines of humans and animals, coliform bacteria are characterized by their ability to ferment lactose at varying rates. Fecal coliforms have two additional characteristics related to their habitat: the ability to multiply at 44 °C and in the presence of bile salts. Additionally, *E. coli* typically produces indole in peptone water **(Gueroui, 2018).**

In food microbiology, coliforms serve as indicators of fecal contamination and are good markers of the overall hygienic quality of food **(Sutra et *al.*, 1998 ; Singleton, 1999)**. The sensitivity of *E. coli* to detecting fecal pollution is notable as they are numerous in intestinal contents. *Klebsiella* also shows good specificity, while *Citrobacter* and *Enterobacter* are nearly devoid of it **(Gueroui, 2018).**

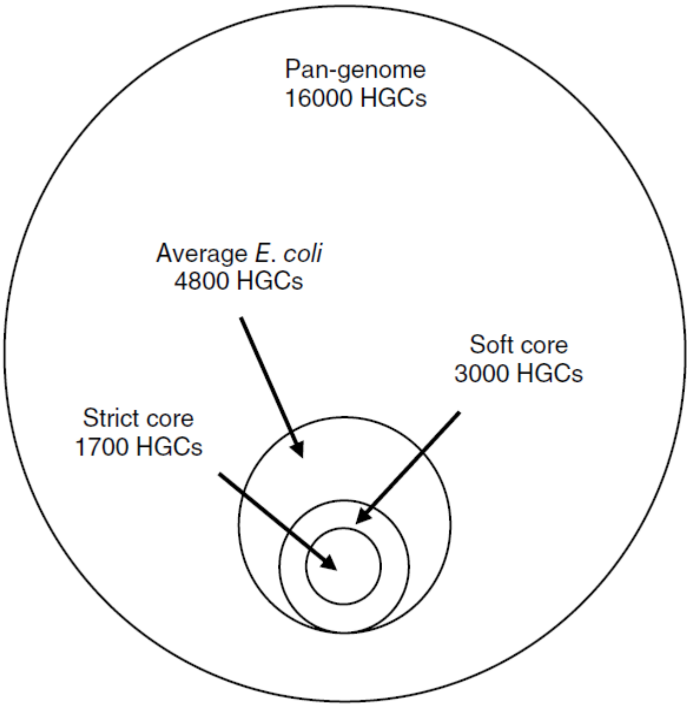
Certain serotypes of *E. coli* can be pathogenic and cause specific digestive disorders, possessing one or more toxins (hemolysin, cytotoxin, and enterotoxin). *E. coli* GEI is responsible for Gastroenteritis in infants (headache, fever, vomiting, diarrhea), and certain biotype are encountered in urinary infections : ECUP (Uropathogenic *E. coli*). Animal-derived products (meats and some dairy products), and sometimes water, are implicated in *E. coli* contamination. Infections are most often caused by the consumption of contaminated and undercooked beef, but can also arise from consuming contaminated water, raw milk, fruits, and vegetables **(Guiraud, 1998 ; Sutra et *al.*, 1998).**

e.4.1 . Organization of the *E. coli* Genome

The bacterial genome is a dynamic molecule subject to variations through the loss or acquisition of genetic material. The *E. coli* genome can be regarded as comprising a core genome, which encompasses a set of genes that form the basis of the genetic information necessary for essential cellular functions, and a flexible genetic pool that is not common to all strains **(Grasselli et *al.*, 2008).**

A study based on the analysis of 186 *E. coli* genome sequences revealed that the typical E. coli genome contains approximately 4,800 gene groups, of which only about 1,700 gene groups are shared by 100% of E. coli strains, and 3,000 gene groups are present in at least 95% of the strains. In total, over 16,000 gene groups constitute the pangenome of *E. coli* **(Balikó et *al.*, 2018 ; Kaas et *al.*, 2018).**

The differences in genome size among E. coli strains reflect the variation in the size of the flexible genetic pool, with most pathogenic strains possessing larger genomes compared to commensal strains **(Mainil, 2003 ; Ge et *al*., 2014)**. This flexible pool is a collection of strain-specific genetic information that can provide additional properties, allowing these strains to adapt to particular environmental conditions. A significant proportion of the flexible genetic pool consists of open reading frames (ORFs) with no obvious function, as well as a group of accessory genetic elements such as plasmids, transposons, insertion sequences, prophages, and genomic islands (GEIs). These elements can be integrated into the chromosome or replicate independently as extrachromosomal elements **(Grasselli et *al*., 2008)**.



HGCs : Homolog gene clusters

e.4.2 . Cultural and Biochemical Characteristics of *E. coli*

Escherichia coli can grow aerobically or anaerobically on ordinary nutrient media at temperatures ranging from 18 ºC to 44 ºC. Colonies are small, convex, smooth, and colorless on nutrient agar. They appear bright pink on MacConkey agar and dark green-black on eosin-methylene blue (EMB) agar. Rough colonies are larger with irregular edges. Mucoid colonies are voluminous, appear moist, and are sticky when handled **(Nolan et *al*., 2013).**

Excluding the previously described non-motile and gas-producing strains classified as *Alkalescens dispar*, identifying *E. coli* is not problematic. *E. coli* can ferment lactose due to the activity of β-galactosidase (ONPG positive). It produces indole from the amino acid tryptophan through the action of the enzyme tryptophanase. *E. coli* is incapable of hydrolyzing urea and does not produce gelatinase. Hydrogen sulfide production is generally absent. *E. coli* does not grow on Simmons citrate agar, which contains citrate as the sole carbon source. Tests for inositol, arginine dihydrolase (ADH), tryptophan deaminase (TDA), and Voges-Proskauer (VP) are also negative **(Percival and Williams, 2014).**

The biochemical characteristics of *Escherichia coli* are presented in the table below.

Table 5 : Biochemical Characteristics of *Escherichia coli* **(after Denis et *al.*, 2011)**

Characteristics	**Reaction**
Mobility	+1
Indole	+
H2S	-2
Simmons Citrate	-
Urea	-
Ortho-Nitrophenyl-Galactopyranoside (ONPG)	+1
Lysine decarboxylase (LDC)	-
Ornithine decarboxylase (ODC)	-
Arginine Dihydrolase (ADH)	-
Tryptophan deaminase (TDA)	-
Voges-Proskauer (VP)	-
Lactose	1
L-arabinose	d

*Note: d indicates variability among strains. 1 Except for E. coli biovar alkalescens dispar. 2 Except for metabolic plasmids***.**

e.4.3. Different Groups of *Escherichia coli*

The species includes two main groups : pathogenic *E. coli* and commensal *E. coli*. The figure below illustrates the various groups and subgroups of *E. coli*.

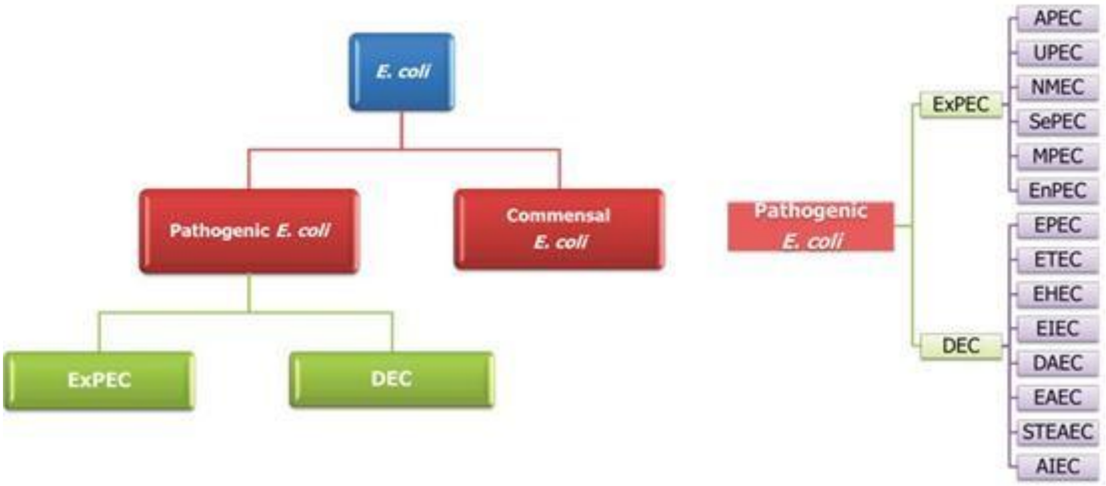


Figure 5 : Representation of the Different Groups of *E. coli* **(after Kunert Filho et *al.*, 2015)**

e.1.4.4. Commensal Strains

The vast majority of *E. coli* strains are commensal or non-pathogenic. These bacteria primarily reside in the mucus covering the epithelial cells of the cecum and colon. Commensal strains contribute to digestive mechanisms and the biosynthesis of certain vitamins (such as vitamin K, vitamin B12, folic acid, etc.). They also play a defensive role against enteric pathogens by competing with harmful pathogens for colonization of the gastrointestinal tract **(Mainil, 2003; Tenaillon et *al.*, 2010; Kunert Filho et *al.*, 2015).**

Commensal enteric *E. coli* may serve as a natural reservoir for pathogenic strains. Several studies have shown that commensal *E. coli* strains have become pathogenic through the acquisition of virulence genes of chromosomal or extrachromosomal origin. A beneficial commensal strain can thus become a harmful pathogen **(Duriez et *al.*, 2001).**

The intestinal commensal microbiota also seems to play an important role in the emergence of antibiotic resistance. The high density of commensal bacteria in the intestine promotes the emergence of potentially resistant strains within this population after antibiotic treatment **(Tenaillon et *al.*, 2010).**

e.1.4.5. Pathogenic Strains

There are numerous pathogenic groups of *E. coli* responsible for diseases in humans and animals, including enterotoxigenic *E. coli* (DEC), which are intestinal tropic strains, and extraintestinal pathogenic *E. coli* (ExPEC), which are associated with diseases outside the gastrointestinal tract. Each of these groups contains different subgroups causing various infections (**Kunert Filho et *al.*, 2015).**

The diversity of *E. coli* pathotypes is due to the acquisition of various virulence-associated genes. These virulence genes are generally transferred horizontally and belong to the flexible genome of *E. coli*, such as pathogenicity islands, bacteriophages, plasmids, and transposons **(Hejnova et *al.*, 2005).**

Table 6 : Definition and Classification of Different Pathogenic *E. coli* in Humans and Domestic Animals **(after Mainil, 2003)**

Class	**Nom**	**Acronyme Anglophone**	**Definition**	**Target species**
Diarrheagenic: Enteritis Entérocolites	**Enteroinvasive**	**EIEC**	**Invasion of enterocytes**	**Humans, primates**
	Enterotoxigenic	**ETEC**	**Production of enterotoxins with fluid accumulation in the intestine, fimbriae F2 to F6, F41**	**Ruminants, pigs, humans (dogs)**
	Enteropathogenic	**EPEC**	**Production of attaching and effacing (A/E) lesions**	**Animals, Human**
	Verotoxigenic	**VTEC (STEC)**	**Production of toxins active on Vero cells in culture**	**Ruminants Humans**
	Enterohemorrhagic	**EAEC**	**Responsible for often hemorrhagic enterocolitis, production of A/E lesions and verotoxins**	**Humans, ruminants**
	Enteroadherent		**Aggregative adhesion on cultured cells: AAF/I adhesins**	**Humans**
	Diffuse adherent	**DAEC**	**Diffuse adhesion on cultured cells: AIDA-I or Afa adhesins**	**Humans animals**

	Necrotoxicogenic	**NTEC**	**Production of cytotoxic necrotizing factors 1 (CNF1), cytotoxic necrotizing factors 2 (CNF2), fimbriae P, S and/or F17, Afa adhesins, hemolysin (enterotoxins, aerobactin, complement resistance)**	**Animals and humans (NTEC1), ruminants (NTEC2)**
Enterotoxemic: Edema Disease	**Verotoxigenic**	**VTEC (STEC)**	**Production of toxins active on Vero cells in culture**	**Piglets**
Uropathogenic (UPEC): **Cystitis, Pyelonephritis**	**Necrotoxigenic**	**NTEC**	**Production of CNF1 or CNF2, Distending Cytotoxins (CDT), fimbriae P, S and/or F17, Afa adhesins, hemolysin α (aerobactin, complement resistance)**	**Human, dogs, cats**
	Others	**?**	**Production of fimbriae P and/or S, Afa adhesin, hemolysin α (aerobactin, complement resistance)**	**Humans, animals**

Mammopathogenic: Mastitis		**?**	**No specific virulence factors: fecal origin**	**Animals, especially ruminants**
Invasive: Septicemia Bacteremia Systemic Infections	**Necrotoxicogenic**	**NTEC**	**Production of CNF1 or CNF2, Distending Cytotoxins (CDT), fimbriae P, S and/or F17, Afa adhesins, hemolysin α (aerobactin, complement resistance)**	**Animals, humans**
	Neonatal Meningitis *E. coli*	**NMEC**	**Production of aerobactin, complement resistance, fimbriae P, S and/or F17, Afa adhesins, K1 capsular antigen (hemolysin α)**	**Humans**
	« *Avian Pathogenic E. coli* »	**APEC**		**Birds**
	Others	**?**	**Production of aerobactin, complement resistance (hemolysin α)**	**Animals, human**

e.1.4.6. Diarrheagenic *E. coli*

Among a wide range of microbial agents, including viruses, bacteria, and parasites, diarrheagenic *E. coli* (DEC) is one of the most common causes of diarrhea. This group of pathogens is subdivided into eight major pathovars based on their virulence factors and the type of disease they cause, namely enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic E. coli (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffuse adherent *E. coli* (DAEC), Shiga toxin-producing enteroaggregative *E. coli* (STEAEC), and adherent-invasive *E. coli* (AIEC) **(Kunert Filho et *al.*, 2015).**

e.1.4.7. Extraintestinal *E. coli*

The intestinal microbiota is a powerful reservoir for extraintestinal pathogenic *E. coli* (ExPEC). These strains reside as harmless commensals in the intestines of healthy individuals ; however, they can cause infections in immunocompromised hosts if they reach an extraintestinal site, such as the urinary tract in humans or the respiratory tract in poultry. This group includes uropathogenic *E. coli* (UPEC) responsible for urinary infections (cystitis, pyelonephritis), *E. coli* associated with meningitis and septicemia (neonatal meningitis *E. coli* or NMEC), sepsis-associated pathogenic E. coli (SePEC), mammary pathogenic *E. coli* (MPEC), endometrial pathogenic *E. coli* (EnPEC), as well as the avian pathovar, avian pathogenic *E. coli* (APEC) **(Kunert Filho et *al.*, 2015).**

Although there are several subgroups of ExPEC, I will focus more in-depth on APEC, which are particularly relevant to this work.

e.1.4.8. Avian Pathogenic *E. coli* (APEC)

APEC are responsible for extraintestinal infections in chickens, turkeys, ducks, and other avian species, causing a range of localized and systemic infections known as colibacillosis **(Dho-Moulin and Fairbrother, 1999; Guabiraba and Schouler, 2015; Nolan et *al.*, 2015).**

Infections associated with APEC are highly variable: omphalitis and yolk sac infections, chronic respiratory disease, colisepticemia, salpingitis, peritonitis, necrotic dermatitis, chronic skin disease ("swollen-head disease"), and granulomas ("Hjarres's disease") **(Stordeur and Mainil, 2002; Kunert Filho et *al.*, 2015; Nolan et *al.*, 2015).**

The main serogroups involved in APEC are O1, O2, and O78, and their prevalence varies by region and country **(Paixão et *al.*, 2016).** Several APEC strains show phylogenetic similarity to human ExPEC strains in terms of phylogeny, serogroup, and virulence genes. Genomic studies have demonstrated that APEC strains are very similar to UPEC and NMEC **(Cunha et *al.*, 2017)**

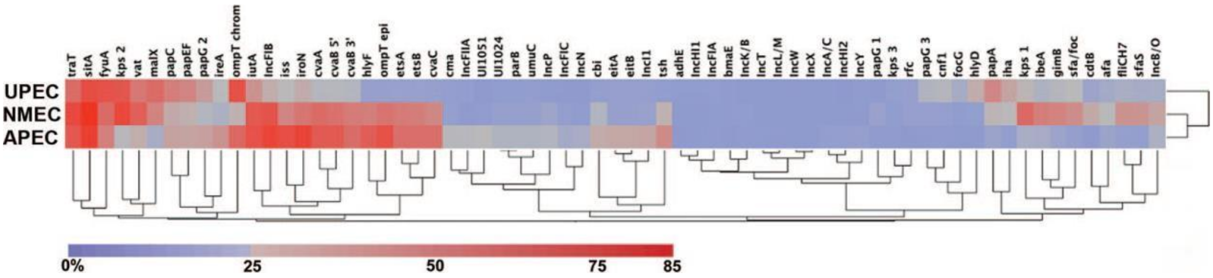


Figure 6 : Prevalence of Genes in Three Pathotypes of ExPEC **(after Johnson et *al.*, 2008)**

-Zoonotic Risk of APEC

The genetic similarity between APEC primarily isolated from chickens and UPEC that cause urinary infections in humans may indicate that meat, particularly chicken, can be a reservoir for ExPEC strains causing urinary infections in humans **(Sarowska et *al*., 2019).**

The zoonotic risk of ExPEC appears to be primarily linked to their large conjugative plasmids. Increasing evidence shows that APEC plasmids could be a source of virulence genes for other ExPEC strains. For example, the ColV plasmids of APEC and NMEC share common virulence genes for these two *E. coli* species **(Mellata, 2013).** However, according to interspecies studies, all ExPEC strains have zoonotic potential ; APEC has caused disease in rats, and human ExPEC strains were virulent for chicks **(LeStrange, 2017)**

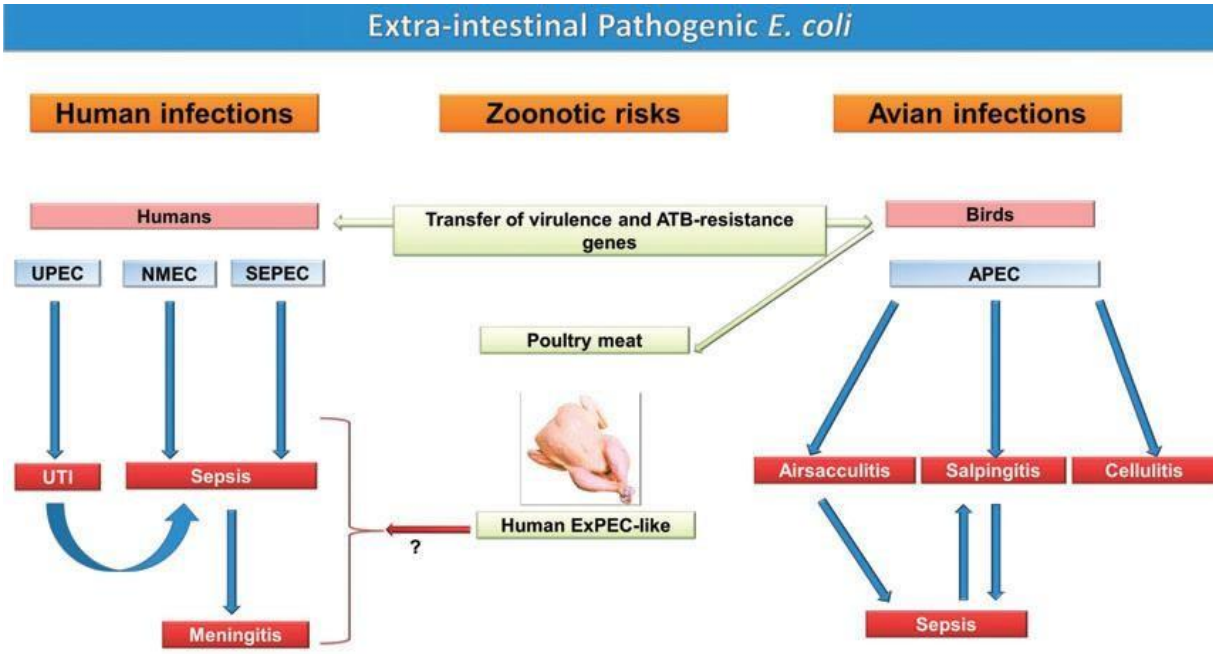


Figure 7 : Zoonotic Potential of ExPEC **(after Mellata, 2013)**

-Virulence Factors

APEC, like other pathogenic *E. coli*, have acquired genes through horizontal transfer that code for virulence factors. These virulence genes can be grouped into chromosomal or plasmid pathogenicity islands **(Nolan et *al*., 2013).**

There is no single virulence factor that distinguishes an APEC strain from a commensal strain ; however, the assembly of a significant number of genes that code for virulence properties increases the likelihood that the strain is virulent **(Nolan et *al*., 2015).**

According to some authors, strains with a minimum of five virulence factors should be classified as pathogenic APEC, while those with fewer than five are considered commensal strains. Conversely, another study showed that only five genes (iutA, iss, ompT, iroN, and hlyF) were more associated with pathogenesis and could be used to differentiate APEC pathogens from commensal *E. coli* strains **(Robineau and Moalic, 2010; De Carli et *al*., 2015; Guabiraba and Schouler, 2015).** An embryonic lethality test can also be used to differentiate APEC from commensal strains ; in contrast, thermos-table toxins, metabolic activity, motility, R plasmids, and resistance to phages are generally not correlated with virulence **(Nolan et *al*., 2015).**

Table 7 : Virulence Factors of ExPEC **(after Köhler and Dobrindt, 2013)**

Functional category	**Virulence factor**
Adhesin	**Type 1 fimbriae (Fim) P fimbriae (Pap/Prf)** **S/F1C fimbriae (Sfa/Foc)** **N-acetyl d-glucosamine-specific fimbriae (Gaf) M-agglutinin (Bma)** **Bifunctional enterobactin receptor/adhesin (Iha) Afimbrial adhesin (Afa)** **Temperature sensitive hemagglutinin (Tsh)**
Invasin	**Invasion of brain endothelium (IbeA)**
Iron acquisition	**Siderophore receptor IreA Aerobactin (Iuc) Yersiniabactin (Ybt) Salmochelin (Iro)** **Periplasmic iron binding protein (SitA)**
Toxins	**alpha-Hemolysin (HlyA)** **Cytolethal distending toxin IV (CDT 1) Cytotoxic necrotizing factor 1 (CNF-1) Putative hemolysin (HlyF)** **Colibactin (Clb)** **Serine protease autotransporters Sat, Pic**
Protectins	**Group II capsule incl. K1 capsule** **Conjugal transfer surface exclusion protein (TraT) Outer membrane protease T (OmpT)** **Increased serum survival (Iss) Colicin V (Cva)**
Others	**d-Serine deaminase (DsdA)** **Maltose and glucose-specific PTS transporter subunit IICB (MalX)** **Flagella**

-Colisepticemia in Broiler Chickens

Colisepticemia in broiler chickens leads to high morbidity and mortality rates. It is the most widespread form of colibacillosis and occurs in stressed and immunocompromised birds following viral or mycoplasmic infections or due to deteriorating environmental conditions in poultry houses, such as exposure to high levels of dust and ammonia. These factors promote the colonization of the upper and lower respiratory tracts by APEC strains present in the environment. Fimbrial adhesins are necessary for the adherence of bacteria to the trachea and lungs. This adhesion allows the bacteria to multiply locally, thereby causing a respiratory infection **(Guabiraba and Schouler, 2015).**

Hemolysin and necrotizing cytotoxin (CNF) are two types of toxins that may be involved in the crossing of the epithelial barrier by *E. coli*. When *E. coli* penetrate the mucosa, they enter the bloodstream. Resistance to the bactericidal activity of complement, linked to the iss gene (increased survival in serum), is essential for the survival of bacteria in this compartment. The passage into the bloodstream is a critical step for the dissemination of bacteria, allowing further multiplication in the blood and colonization of internal organs (liver, heart, spleen, etc.). In this context, iron acquisition systems are useful for bacterial growth in the host's biological fluids, where iron is present in low concentrations **(Robineau and Moalic, 2010; Guabiraba and Schouler, 2015).**

Inflammation of various tissues leads to increased vascular permeability, resulting in the infiltration of protein-rich fluid outside the blood compartment and the appearance of edema in serous membranes. Fluid begins to accumulate in body cavities and initially attracts heterophils. After a few hours, macrophages replace the heterophils and become predominant. Plasma fibrinogen is converted to fibrin by thrombin when it is outside the blood vessels. The exudate continues to accumulate and eventually undergoes caseation, forming lesions resembling cheese **(Nolan et *al.*, 2015).**

The most affected organs include the air sacs (air sacculitis), with the formation of fibrinous masses resembling omelets ; the liver, which exhibits perihepatic inflammation due to irritation of the peritoneum covering the organ; and the pericardium, which becomes caseous and takes on a yellowish to whitish color **(Stordeur and Mainil, 2002).**

e.5. Salmonella Genus

Salmonella are Enterobacteriaceae that do not ferment lactose and do not produce urease. They grow well on standard media, can ferment glucose, reduce nitrates to nitrites, and are oxidase-negative. Their differential identification relies on their ability to produce hydrogen sulfide

(H2S) (with some exceptions), the presence of lysine decarboxylase, and the absence of tryptophan deaminase **(Gueroui, 2018).**

Salmonella are parasites of humans, mammals (rodents), birds (poultry), and cold-blooded animals (reptiles). They are responsible for various infections (salmonellosis), including typhoid and paratyphoid fevers, gastroenteritis, and collective foodborne infections. The primary mode of human contamination is ingestion from water (especially *S. typhi*), food (e.g., dairy products, eggs, meat, poultry, fish, and other seafood), or carrier pets (turtles).

Recent taxonomic studies have recognized two species within the *Salmonella* genus. The first is *Salmonella enterica*, which is divided into six subspecies : *S. enterica* subsp. *enterica* (subspecies I), *S. enterica* subsp.*salamae* (subspecies II), *S. enterica* subsp. *arizonae* (subspecies IIIa), *S. enterica* subsp.*diarizonae* (subspecies IIIb), *S. enterica* subsp. *houtenae* (subspecies IV), and *S. enterica* subsp.*indica* (subspecies V). The second species is *Salmonella bongori*. Identification of species and subspecies is based on biochemical characteristics. Within these species and subspecies, strains can be differentiated by serotyping (somatic O antigens, flagellar H antigens, and Vi capsular antigens) defining serovars or serotypes. Subspecies I represents over 99.5% of clinical isolates, further divided into several serovars (currently 2541 serovars). Some serovars have adapted to hosts and are specific to animal species. The most notable are: *Salmonella typhi* in humans, *Salmonella dublin* in cattle, *Salmonella gallinarum* and *pullorum* in poultry, *Salmonella abortusequi* in horses, *Salmonella abortusovis* in sheep, and *Salmonella arizona* in reptiles. *S. Enteritidis* and *S. Typhimurium* are predominant in the food domain, but their relative importance varies with time and regions (countries). All serotypes should therefore be considered potentially pathogenic **(Bergeron, 2009 ; Aubry and Gaüzère, 2020).**

Salmonellosis encompasses two main types of infections : typhoid fever (*S. enteritica* serovar *S. typhi*) and paratyphoid fevers (*S. paratyphi* A, B, and C), during which two invasion mechanisms overlap : an enteroinvasive process and the action of endotoxin (LPS). Non-typhoidal salmonellosis (the bacteria most often involved in foodborne infections due to *S. Typhimurium*, *S. Enteritidis*) may involve either an enteroinvasive process or an endotoxin mechanism **(Pennec and Garré, 2003 ; Aubry and Gaüzère, 2020).**

f. Micrococci

f.1. Staphylococcus Genus

Members of this genus are Gram-positive cocci, often in clusters. Some species contain orange or yellow carotenoid pigments. They are non-motile, facultative anaerobes, and chemioorganotrophic. Their carbon sources include various sugars, and they are commonly halotolerant and catalase-positive. Staphylococci are divided into coagulase-positive and coagulase-negative strains. Among the former are *S. aureus* and *S. intermedius* ; among the latter is *S. epidermidis*. They are commensals and pathogens in humans and other animals. The G+C content is approximately 30 to 39%. Commonly used culture media include Chapman and Baird-Parker media **(Catteau, 1996 ; Singleton, 1999).** Coagulase-positive *S. aureus* is pathogenic ; it produces thermostable enterotoxins released into food during growth, acting on intestinal and gastric receptors (vagus nerve, hypothalamus). Food poisoning is characterized by a short incubation period (1 to 6 hours, average 3 hours), followed by various symptoms : nausea, vomiting, abdominal pain, diarrhea, headache, drop in blood pressure, intestinal hypermotility, and occasionally fever. The minimal infectious dose (MID) is on the order of 10^5 to 10^6 CFU/g, and the amount of toxin dangerous to humans is 0.1 to 10 µg/kg. Numerous foods are dangerous : meats, prepared dishes, dairy or egg products, pastries, ice creams, etc. Enterotoxins are not hydrolyzable by digestive proteases (pepsin, trypsin), are highly resistant to thermal treatments, withstand pasteurization (60°C/30 min), and resist up to 30 min at 100°C. Food poisoning usually occurs from contaminated food via infected hand injuries, the normal flora of skin or respiratory tract of handlers. Foods with high concentrations of salts and sugars provide a good growth medium for *S. aureus* **(Catteau, 1996 ; Guiraud, 1998).**

g. Spore-Forming Bacteria

g.1. Aerobic Spore-Forming Bacteria : Bacillus Genus

These are Gram-positive rod-shaped bacteria, typically motile and spore-forming, with spore morphology being an important criterion for classification. Depending on the species, they can be aerobic or facultative anaerobic, catalase-positive. Their metabolism is respiratory or facultatively fermentative. Most species are chemoorganotrophic. Many can grow on nutrient agar. They are found as saprophytes in soil and water. Some species can be pathogenic to humans and other animals (including insects), with G+C content ranging from 30 to 70. They contaminate many food products and are often proteolytic. Due to their ability to form spores, they withstand adverse conditions and can degrade food preserves. *B. stearothermophilus*, *B. coagulans*, and *B. cereus* are common species found in soil, on plants, and particularly in cereals and animal skin. They are often involved in toxic infections (10^4 to 10^7 CFU/g) **(Catteau, 1996; Guiraud, 1998; Singleton, 1999).**

B. cereus produces five toxins : hemolysin, phospholipase C, murine toxin, cytolysin, and an emetic toxin. The implicated foods include meats, prepared dishes, milk, pastries, and certain plant products.

g.2.Anaerobic Spore-Forming Bacteria : Clostridium Genus

These bacteria belong to the Bacillaceae family. They are commonly found in soil, sewage, and intestines. They can contaminate and degrade food products under anaerobic conditions (e.g., canned goods). *Clostridium* are Gram-positive rods, often large, isolated or in chains. The shape and position of the spores are important in taxonomy (spores can withstand several minutes at 100°C), and they are generally non-motile. They are catalase-negative and strict anaerobes, with oxygen being lethal to these species. They multiply easily on standard media. Under anaerobic conditions, they are proteolytic or saccharolytic depending on the species and are frequently gas-producing. They contaminate many products : water, milk, fish, fermented or frozen foods, and especially canned foods. They degrade sugars and proteins, releasing butyric acid or H2S. Some species are responsible for intoxications or gastroenteritis (*Cl. perfringens*) or severe, often fatal, intoxications (*Cl. botulinum*). *Clostridium* is classified based on its metabolism and action on substrates, distinguishing :

- **Saccharolytic Clostridia** are gas-producing (CO_2+, H_2+, H_2S-), often producing butyric acid, acidifying and coagulating milk, and do not liquefy gelatin, including mesophilic species like *Cl. butyricum* and *Cl. tyrobutyricum*.
- **Proteolytic or putrefactive Clostridia** are very active in protein degradation and can be fermentative and gas-producing (H_2S^+), liquefy gelatin, and digest casein in milk with or without coagulation: *Cl. botulinum* and *Cl. putrefaciens*.
- **Clostridia that are both proteolytic and saccharolytic** liquefy gelatin but also possess intense fermentative activity (H2S+), often referred to as "sulfite-reducing" *Clostridia* **(Guiraud, 1998 ; Singleton, 1999).**

g.2.1.*Clostridium difficile*

-Clostridium difficile was first identified in 1935 during a study by Hall and O'Toole on the intestinal bacterial flora of newborns **(Hall, 1935).** For a long time, it was considered a commensal of the intestinal microbiota in healthy adult humans. Clostridium difficile was named for its difficulty in isolation and culture **(Sabri, 2018)**. In 1978, Bartlett et al. discovered its pathogenic potential related to pseudomembranous colitis (PMC) **(Bartlett et *al*., 1978)**. Toxin-producing strains of this bacterium have since been implicated in nosocomial bacterial diarrhea, accounting for 15 to 25% of post-antibiotic diarrhea and in nearly all cases of pseudomembranous colitis **(Barbut and Petit, 2001).**

- Infections caused by Clostridium difficile spread on a scale that ranges from asymptomatic digestive colonization to simple watery diarrhea without colitis, up to more significant and severe symptoms such as pseudomembranous colitis (PMC) and fulminant colitis. This is often accompanied by complications such as toxic megacolon, paralytic ileus, intestinal perforation, septic shock, and even patient death **(Sabri, 2019)**.

The rate of C. difficile infections has been steadily increasing over the past two decades. These infections are becoming increasingly severe worldwide, particularly in industrialized countries, due to the excessive use of antibiotics and the spread of hypervirulent clones.

Clostridium difficile is a Gram-positive, rod-shaped, strictly anaerobic, spore-forming bacterium. The spores are oval, subterminal, and deformable. Their size ranges from 0.5 to 2 µm n width and 3 to 15 µm in length. They can cluster in chains of 2 to 6 cells and are occasionally motile due to peritrichous flagella **(Denchiche, 2014)**.

It is cultured anaerobically on heart-brain agar supplemented with 5% horse blood, 0.1% taurocholate, 250 mg/l cycloserine, and 10 mg/l cefoxitin **(Denchiche, 2014)**. The colonies range in size from 2 to 4 mm and can reach up to 8 mm after 48 hours ; they appear gray, opaque, with a shattered glass appearance, dull, and have a characteristic odor of horse manure. They are fluorescent under ultraviolet light, and their edges are irregular (Detail, 2018). In taxonomy, Clostridium difficile belongs to the phylum Firmicutes, class Clostridia, and family Clostridiaceae **(Sandhu et *al.*, 2018)**. It presents two types of strains : one classified as toxinogenic, which produces toxins A and B (an enterotoxin and a cytotoxin), and a non-pathogenic strain**.**

The carriage of this bacterium can be symptomatic or asymptomatic, with approximately 3% of the adult population being asymptomatic carriers **(Eckert, 2014)**.

Cells and spores of Clostridium difficile are found in feces, as it is inherently part of the intestinal flora. Its mode of transmission occurs either via the fecal-oral route, by manual handling, or from the environment. Vegetative cells cannot withstand exposure to air or gastric acidity, which is why the bacterium protects itself by sporulating. The spores are the primary factor for contamination, as they can persist for months (Sabri, 2019 ; Denchiche, 2014).

g.2.1.1. Epidemiology of *Clostridium difficile* Infections

In recent years, the incidence of Clostridium difficile infections has been steadily increasing, ranging from 1 to 10 cases per 10,000 patients per day. This rise is influenced by the global dissemination of the hypervirulent strain (027/NAP1/BI), which drives the increased severity of Clostridium difficile infections and mortality rates **(Detail, 2018)**

Numerous epidemiological studies **(Barbut et *al*., 2012)** have been conducted to understand and effectively manage this major public health issue. In North America, the incidence of Clostridium difficile infections increased 2 to 3 times among individuals over 65 years old between 1996 and 2003. The severity of complicated forms rose from 7.1% to 18.2% between 1991-1992 and 2003, while the recurrence rate increased from 28.9% to 58.4%, and the response to treatment with metronidazole significantly declined. The percentage of patients who died within 30 days of diagnosis ranged from 5% to 13%, leading to a mortality rate of 47%.

The emergence and spread of the hypervirulent clone 027 was first observed in North America before spreading to Europe, where it was responsible for several epidemics, including those in Great Britain, Belgium, and the Netherlands in 2005 **(Sabri, 2019)**

The European study conducted in 2009 across more than 100 healthcare facilities in 34 countries showed that the rate of Clostridium difficile infections increased to 4.1 cases per 10,000 people per day. This study revealed significant disparities within the same facility, between different facilities, and across various countries **(Bauer et *al*., 2009).**

Another European epidemiological study involving over 37 hospitals in 14 different countries reported an infection rate ranging from 0.6 to 18.5 cases per 10,000 patients per year **(Freeman et *al*., 2010).**

The Health Protection Agency (HPA) located in Great Britain indicated that in Europe, the average rate of Clostridium difficile infections in 2012 was 7.9 per 10,000 patients per day **(Chikhoune, 2019).**

The national ICD-RAISIN study (alert, investigation, and surveillance network for nosocomial infections) in France estimated that the incidence of CDI was 2.28 cases per 10,000 patient-days for short-stay hospitals and 1.14 cases for medium- or long-stay hospitals **(Eckert et *al*., 2013).** The European network EUCLID **(Davies et *al*., 2014)** showed an increase of 157% for France

In the Nord-Pas-de-Calais region **(Kuijper et *al*., 2006)**, an outbreak emerged between 2006 and 2007 due to the clone 027, which is characterized by its persistent establishment and rapid dissemination. This clone represents 18% of strains in Europe **(Bouza et *al*., 2017).**

Clostridium difficile is a pathogen that emerges not only in healthcare settings but also in the community. This germ particularly spreads to nursing homes and among populations that do not present typical risk factors **(Chitnis et *al*., 2013).** Clone 078 is primarily involved in this type of infection and apparently originates from environmental reservoirs **(Goorhuis, 2008).**

g.2.1.2. Mechanism of Action and Pathophysiological Effects

The manifestation of *Clostridium difficile* infections is primarily influenced by the colonization of the gastrointestinal tract by spores. This colonization is facilitated by dysbiosis of the intestinal microbiota, which loses its bacterial diversity. This loss of diversity is caused by unhealthy diets, antibiotic therapy, or anticancer chemotherapy, leading to the disruption of the protective barrier and a decreased resistance to the colonization by toxin-producing Clostridium difficile **(Eckert and Barbut, 2010).**

The pathophysiology of CDI primarily involves the alteration of the intestinal microbiota (dysbiosis) and the ingestion of C. difficile spores. These spores can withstand gastric acidity, allowing them to germinate and release vegetative cells under the influence of bile salts.

The second step involves adhesion and digestive colonization. This type of implantation of the toxinogenic strain of *C. difficile* is mediated by various colonization factors responsible for adherence to the host's intestinal cells. Key factors include adhesins such as S-layer proteins, the fibronectin-binding protein Fbp68, the Cwp66 protein, the heat shock protein GroEL, and the flagellar proteins FliC and FliD **(Borriello, 1998).**

Subsequently, the toxinogenic strain of C. difficile produces toxins A (TcdA) and B (TcdB) in the intestinal lumen **(Kuehne et *al.*, 2011)**, which have enterotoxic and cytotoxic activities, respectively **(Voth and Ballard, 2005)**. These two toxins, which are glucosyltransferases that inactive the Rho, Rac, and Cdc42 GTPases in epithelial cells, disrupt the tight junctions of enterocytes by depolymerizing the actin filaments of the cytoskeleton. This leads to an intense inflammatory response, resulting in necrosis of the enterocytes and watery diarrhea

h. Vibrio

The study of Vibrio affecting aquaculture species and their virulence mechanisms has primarily benefited from knowledge gained from numerous research efforts on pathogenic bacteria of the genus Vibrio that affect humans. The most studied species is V. cholerae, the etiological agent of cholera, which still poses a serious threat in countries where access to safe drinking water remains a problem. Described in 1854 by the Italian PACINI, V. cholerae was the first bacterium of the Vibrio genus to be studied. PACINI's work was later expanded by KOCH a few years later (1883-1884), and research continues on its origins, evolution, and virulence.

V. vulnificus and V. parahaemolyticus, responsible for foodborne infections, are also extensively studied due to their significant public health impact. **(Sakazaki R, 1963).**

In the mid-20th century, marine microbiologists observed the predominance of the Vibrio genus in bacterial cultures derived from seawater samples or associated with fish and shellfish (**Travers et *al.*, 2014** ; **Reichelt J.L et *al.*, 1976).** Subsequently, cases of vibriosis leading to substantial economic losses emerged in aquaculture farms, and Vibrio became a growing concern for global aquaculture.

h.1. Historical Background

Historically, the role of Vibrio in human pathology has been recognized due to one of its members, *Vibrio cholerae*, being responsible for one of humanity's plagues since ancient times : cholera, which remains a disease of global importance. It was first recognized in 1817 when it spread from the Indian subcontinent to the Middle East and East Africa by 1823. The second pandemic affected Asia, the Middle East, Europe, Africa, and North America from 1829 to 1851. The third pandemic, occurring from 1852 to 1859, reached Latin America in addition to the regions already affected. The faster progression of this third pandemic is linked to the advent of steam propulsion used in trains and boats.

The fourth pandemic, from 1863 to 1879, benefited from the opening of the Suez Canal, which facilitated its spread. The fifth pandemic, which took place from 1881 to 1896, affected all continents except Australia and was marked by the discovery of the cholera agent, the cholera vibrio, by Robert Koch in 1883 and 1884. The sixth pandemic spread through Asia, the Middle East, and Eastern Europe between 1899 and 1923 but did not reach Western Europe and America, where hygiene standards had begun to improve. *Vibrio cholerae* was the first species of the Vibrio genus to be described by Pacini in 1854, but it was Koch who demonstrated in 1884 that this germ is indeed responsible for cholera **(Fournier et Quilici, 2002)**

In 1951, a new pathogenic species of Vibrio for humans was identified. This organism was isolated from the stools of patients suffering from food poisoning in Osaka, Japan, as well as from the suspected food: a partially dried sardine called "Shirasu." Initially placed in the genus *Pasteurella*, its current name, *Vibrio parahaemolyticus*, was established by Sakazaki et al.in 1963.

The species *Vibrio alginolyticus* was described in 1968 ; it is abundant in marine environments but rarely isolated from humans.

Starting in the early 1970s, cases of extra-intestinal infections involving tissue necrosis and edema, as well as septicemic infections with occasional sudden mortality, emerged in the United States. The isolated agent was initially confused with *Vibrio parahaemolyticus* or referred to as a non-choleric vibrio. Recognized as a new species by Maurin in 1976, microbiologists proposed placing this organism in the genus *Beneckea* and named it *vulnificus*, meaning "wound" in Latin. In 1979, Farmer suggested that this bacterium be classified in the genus

Vibrio, as many microbiologists contested the previous classification. By 1980, the organism was officially named *Vibrio vulnificus*.

Later, less severe forms of cholera associated with vibrio strains very similar to the cholera bacterium were recognized. These organisms, lacking the characteristic O1 antigen of *V. cholerae*, are now identified as *V. cholerae* non-O1 or NAG (Non-Agglutinating Vibrio) or NCV (Non-Cholera Vibrio) **(ICMSF, 1996).**

In 1992, a new cholera strain that could not agglutinate with O1 antiserum but produced cholera toxin was discovered in Bangladesh and India and recognized as the causative agent in a typical cholera case. This serotype was described as belonging to the new serogroup O139 in 1993. Today, within the species *Vibrio cholerae*, we distinguish between the strains known as "cholera vibrios," namely serogroups O1 and O139 responsible for cholera, and the non-O1 and non-O139 strains isolated in cases of gastroenteritis, as well as soft tissue infections and septicemia in immunocompromised individuals **(Fournier et Quilici, 2002).**

Other species are also considered pathogenic to humans : *V. fluvialis*, *V. furnissii*, *V. hollisae*, *V. mimicus*, and *V. metschnikovii* have been isolated from gastroenteritis cases; *V. carchariae*, *V. cincinnatiensis*, and *V. damsela* have been implicated in extra-intestinal infections only **(FAO/WHO, 2002).**

h.2.Taxonomic, Phenotypic, and Ecological Characteristics

h.2.1. Taxonomy

The taxonomic characterization of *Vibrio* was primarily based until the mid-twentieth century on a few phenotypic criteria, such as the morphology of strains (presence of flagella, cell curvature, appearance of cultures) and sugar metabolism. These criteria led to several classification errors regarding new species and even genera.

By the late 1980s, the identification tools for bacteria in the genus *Vibrio* significantly improved with the use of more suitable phenotypic test batteries and digital taxonomy analyses. These analyses relied on various compounds as sources of carbon and energy, the study of enzymatic activities (arginine dihydrolase, gelatinase, chitinase, oxidase, catalase, lysine, and ornithine decarboxylases), salt tolerance, luminescence, temperature preferences, antibiograms, and the GC content of the genome **(Alsina, M., Blanch, A.R, 1994).**

However, due to genotypic variability within the same species and the phenotypic versatility of environmental strains, a classification based solely on phenotypic criteria can lead to problems and result in taxonomic classification errors. For example, in the polyphyletic group *V. splendidus*, phenotypic tools such as API galleries do not allow for the distinction among the four species in the group: *V. kanaloe*, *V. lentus*, *V. chagasii*, and *V. pomeroyi* **(Le Roux F,2004 ; Le Roux F. et Austin B, 2006).**

Similarly, the API 20E and 20NTF galleries do not allow for the distinction of *V. harveyi* from *V. alginolyticus*, *V. parahaemolyticus*, and *V. vulnificus* **(Owens L. et Busico-Salcedo N, 2006).**

Over the past two decades, the development of molecular genotyping tools has significantly enhanced prokaryotic taxonomy studies. Initially, the sequencing of ribosomal genes (5S, 23S, and particularly 16S rRNA) facilitated the reorganization of *Vibrio* classification **(Kita-Tsukamoto K et *al.*, 1993).** Furthermore, the close relationship between the genera *Vibrio* and *Photobacterium* has been validated using this method **(Ruimy et *al.*, 1994).**

However, the analysis of 16S rRNA sequences has its limitations in distinguishing certain strains, such as within the *V. splendidus* group **(Le Roux F et *al.*, 2004),** as well as differentiating *V. brasiliensis* from *V. tubiashii*, *V. coralliilyticus* from *V. neptunius*, and *V. anguillarum* from *V. ordalii* **(Thompson F.L et *al.*, 2004)**

Quantitative DNA/DNA hybridization remains the reference tool for the specific determination of a new bacterial strain compared to the closest phylogenetic reference strain based on 16S rRNA. The accepted threshold is 70% for DNA/DNA hybridizations **(Stackebrandt E et *al.*, 2002).** Today, numerous molecular typing techniques, varying in their discriminative power, are available for taxonomic studies, including DNA/DNA hybridization, 16S rRNA sequencing, multilocus sequence typing (MLST), and phenotyping.

The combination of these tools has highlighted the polyphyletic nature of vibrios **(Thompson J.R et *al.*, 2004)** In a recent study, Sawabe et al. (2013) differentiated new groups based on multilocus sequence analysis (MLSA). The percentage of similarity among sequences of eight genomic markers (*gapA*, *gyrB*, *ftsZ*, *mreB*, *pyrH*, *recA*, *rpoA*, and *topA*) allowed for the distinction of 23 groups within 96 identified species.

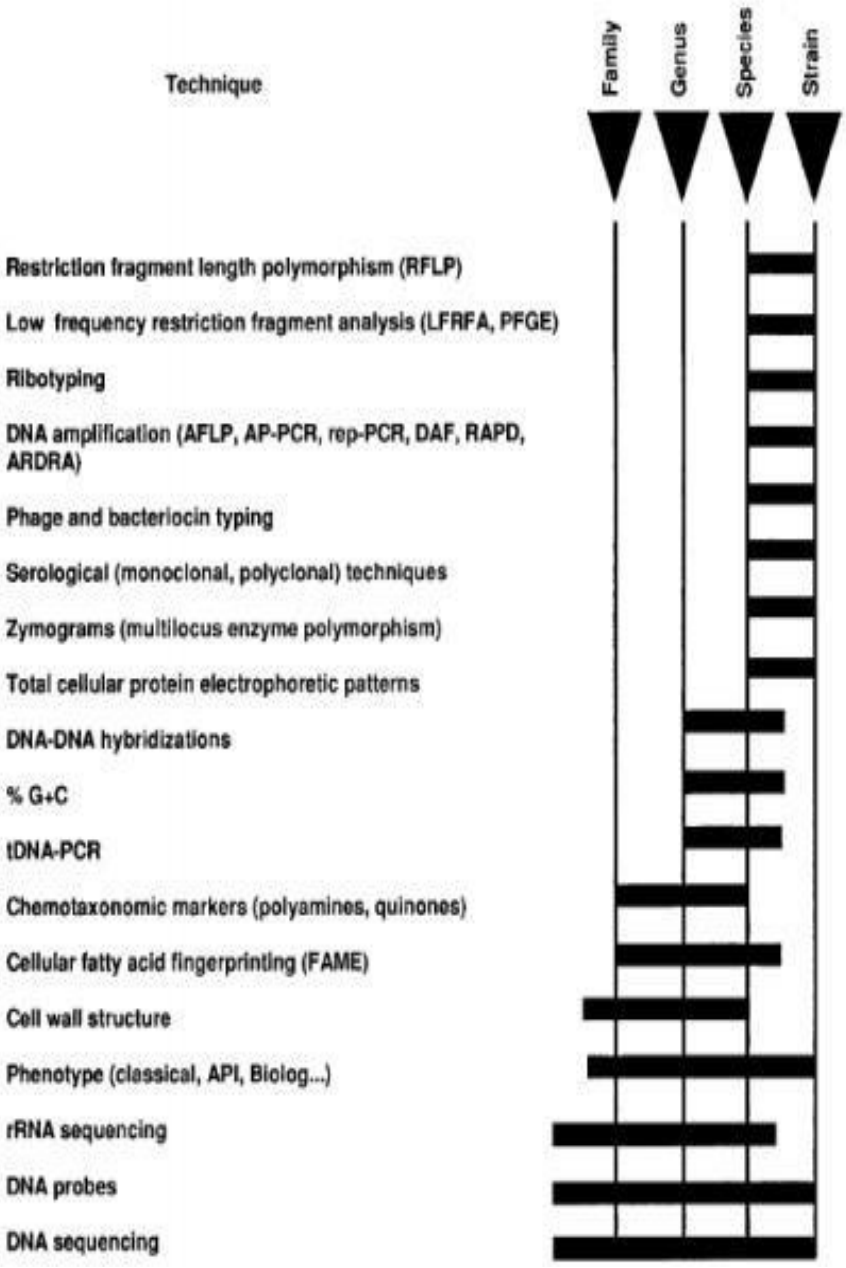


Figure 8 : Taxonomic resolution of the most commonly used typing tools **(Vandamme P et *al.*, 1996).**

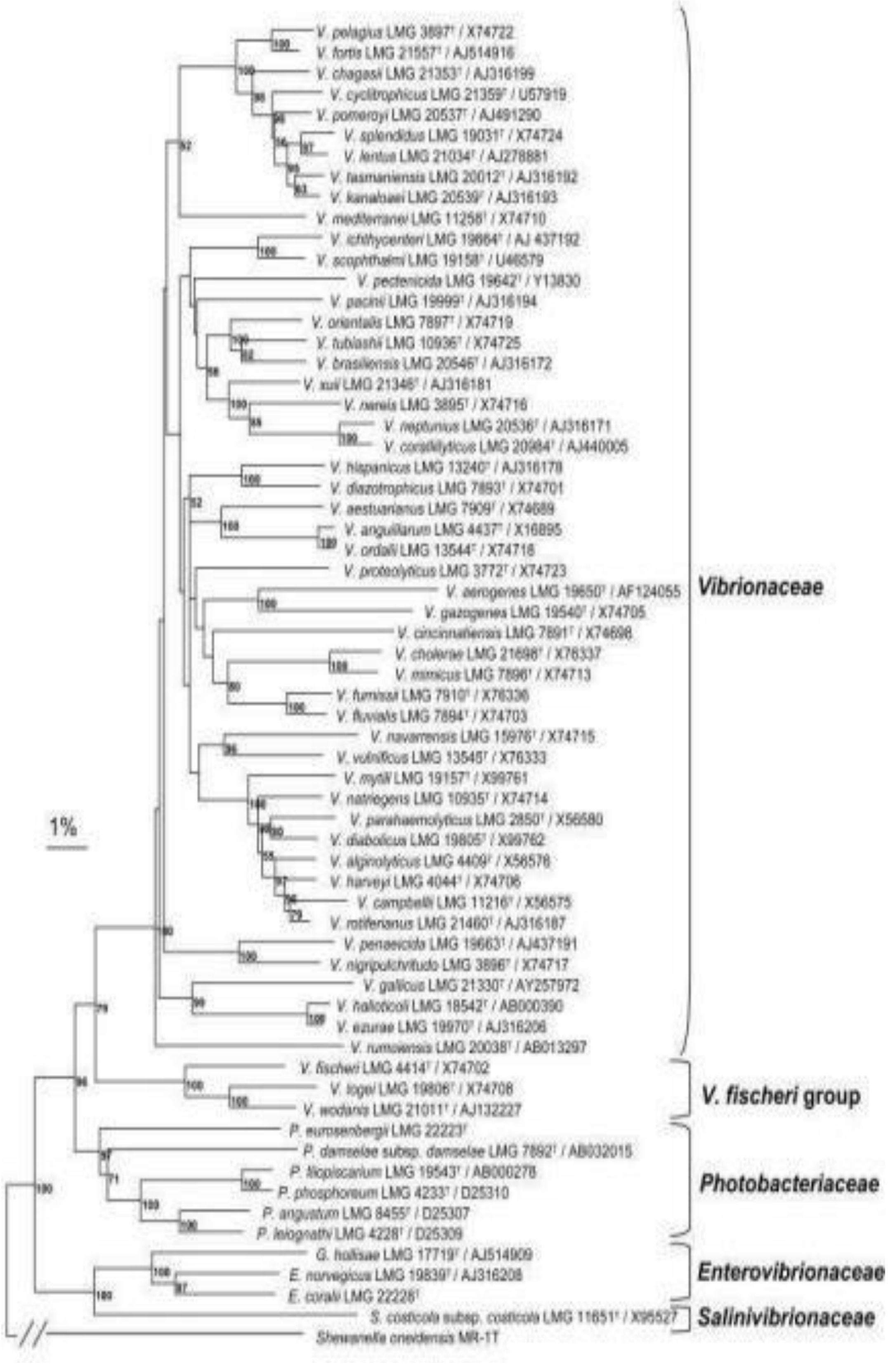


Figure 9 : Phylogenetic tree using the neighbor-joining method, based on the sequences : 16S rRNA, recA, and rpoA (2,898 bp), highlighting different groups of *Vibrio* ***(Sawabe et al., 2013).***

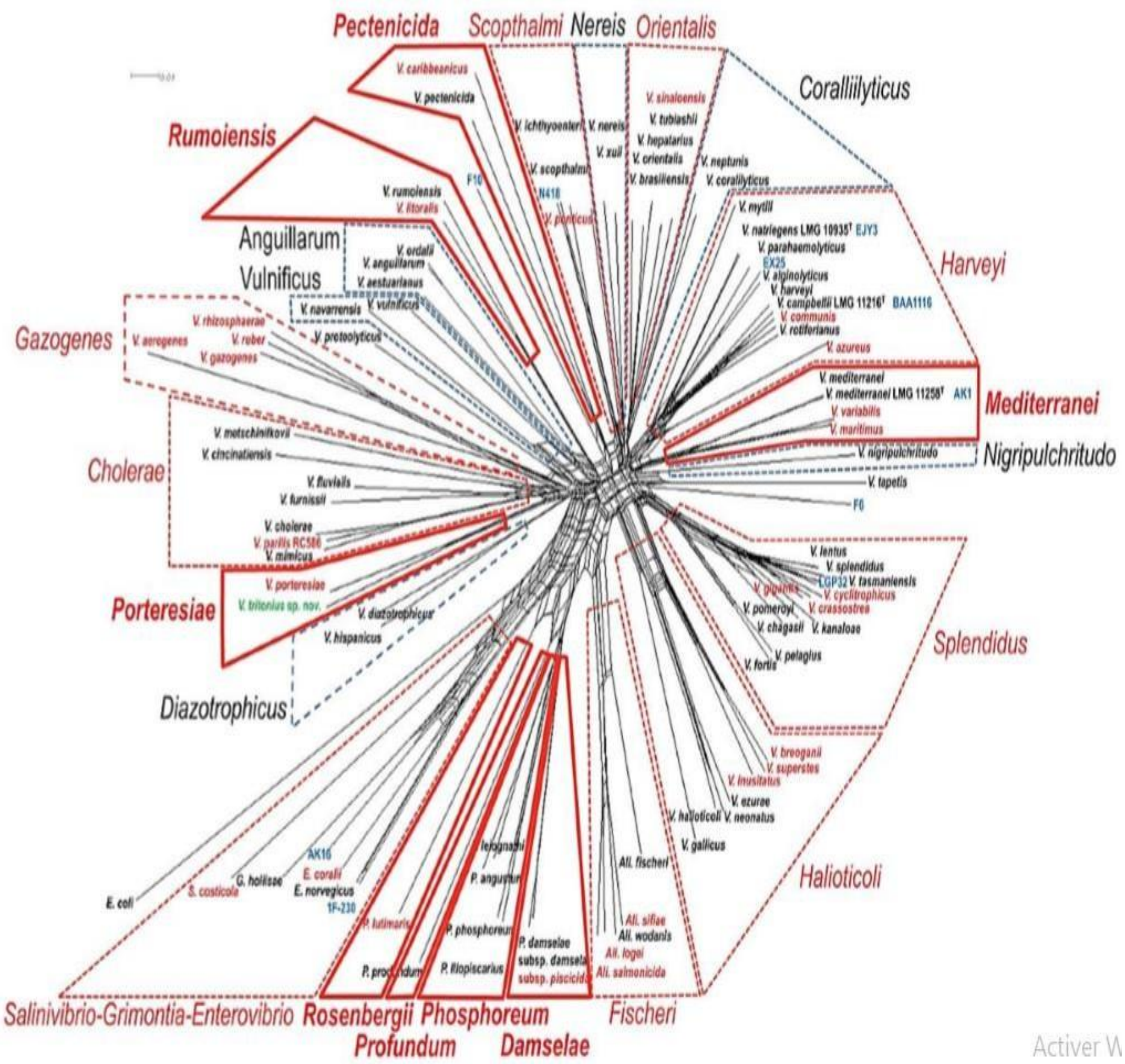


Figure 10 : Phylogenetic tree based on the sequencing of eight reference genes (gapA, gyrB, ftsZ, mreB, pyrH, recA, rpoA, and topA) from 96 species of *Vibrio*. The new groups are indicated with solid red lines, and the dashed lines in red or black indicate changed or unchanged groups, respectively **(Sawabe et *al*., 2013).**

h.2.2. Phenotypic Characteristics

The genus *Vibrio* is primarily aquatic and mostly marine. It includes Gram-negative bacteria that are straight or curved, measuring 0.5 to 1 µm in diameter and 1.2 to 3.5 µm in length. These bacteria are non-spore-forming, facultative anaerobes, with both oxidative and fermentative metabolism. They are most often oxidase positive, nitrate reductase positive, and frequently produce acetoin. They are chemo-organotrophic, fermenting (most often without gas), and utilize D-glucose, often capable of growing in a mineral medium.

containing glucose as the sole source of carbon, energy, and NH_4^+ ions as the sole source of nitrogen **(Travers, M.A et *al.*, 2015).**

Vibrios are characterized by high motility (Figure 1.4). They move in liquid media by "swimming" using their polar flagella and in solid media by "swarming" through their lateral flagella **(McCarter, L, 1999).** They can also exhibit "twitching" motility generated by the anchoring and retraction of pili on a solid surface **(Wall, D et *al.*, 1993 ;Watnick, P.I., Kolter, R ,1999).** However, some species lack flagella and are immobile.

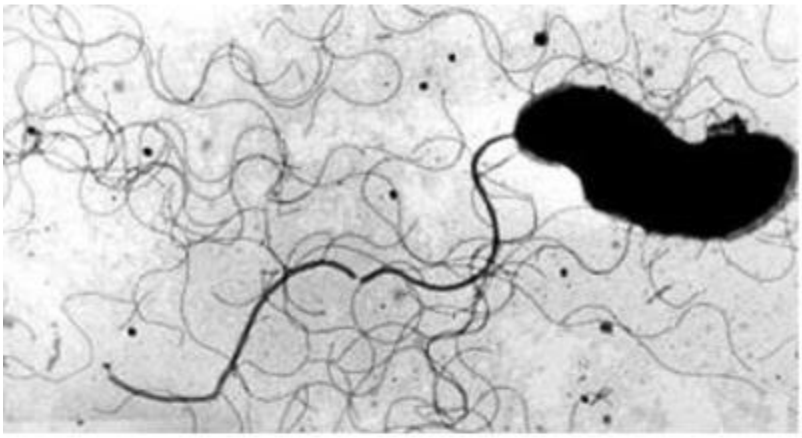


Figure 11 : Micrograph of *Vibrio pectenicida* strain A365T on solid medium showing polar and lateral flagella (×14,000) obtained by transmission electron microscopy **(Lambert, C, 2002).**

Vibrio generally grow on "marine agar." A TCBS agar (Thiosulfate Citrate Bile Salt Sucrose Agar) has been developed for the selective isolation of *V. cholerae*, and many other *Vibrio* species can also be cultivated on it.

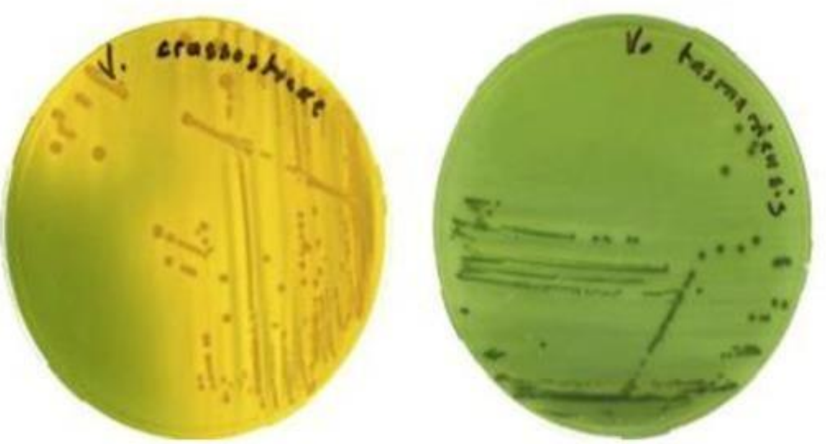


Figure 12 : Phenotypic diversity of two strains belonging to the *Vibrio* group on TCBS medium **(Travers, M.A et *al.*, 2015).**

h.2.3. Pathogenicity

Bacteria can be classified according to their pathogenicity, which represents their potential ability to cause disease **(Casadevall, A., Pirofski, L, 1999).** These microorganisms include different categories, such as avirulent commensals, opportunists that can cause harm when the host is weakened, and virulent pathogens that can cause disease under any circumstances. The *Vibrio* group encompasses both symbiotic bacteria and pathogens affecting humans as well as marine organisms like fish, crustaceans, and mollusks.

h.2.3.1. Pathogenic *Vibrio* Species in Humans

More than 12 *Vibrio* species have been described as involved in human infections, ranging from simple skin wound infections and external ear infections to often fatal septicemias and severe gastrointestinal syndromes (gastroenteritis, ileitis, etc.). Thus, a variety of clinical presentations corresponding to different pathologies can be caused by *Vibrio* infections. However, certain diseases are more frequently encountered, better understood, and responsible for well-identified pathological complexes. The three main *Vibrio* species associated with significant mortality in humans and public health concerns are *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*. These bacteria can infect their host either orally after ingestion of food or water contaminated with human feces or wastewater, or raw seafood and fish, or through the skin by exposing cuts or open wounds to aquatic environments and marine animals **(Lee, R.J., Younger, A.D,2002 ; Toti, L., 1996).**

a. *Vibrio cholerae*

V. cholerae is the etiological agent of cholera, a severe intestinal infection characterized by acute diarrhea that can lead to fatal dehydration within days if adequate rehydration therapy is not provided. Since 2000, the incidence of cholera has steadily increased. Between 2003 and 2004, the World Health Organization (WHO) reported 15,000 new cases of cholera in Africa, primarily in Mali, Mozambique, and Zambia. In October 2010, cholera cases were reported in Haiti for the first time in over a century. Research revealed that it was *V. cholerae* serogroup O1, biotype Ogawa. By July 2011, more than 220,000 cases had been recorded, resulting in 5,968 deaths **(Ceccarelli, D et *al.*, 2011 ; Pfrimmer, D. M et *al.*, 2010, Tappero, J. & Tauxe, R, 2011).**

The *V. cholerae* strains described exhibit variable virulence statuses, with the existence of non-pathogenic strains and others that are highly pathogenic. The classification of *V. cholerae* strains is based on the characterization of the O antigen (a component of the lipopolysaccharides, LPS, of the outer membrane), which shows significant serological diversity allowing for the definition of over 200 serogroups. Some serogroups have been subdivided into biotypes, such as serogroup O1, divided into classic biotype and El Tor biotype. Since 1992, another serogroup, O139, has emerged in Bengal and Bangladesh, causing fatalities and potentially leading to the eighth cholera pandemic **(Sack D.A et *al.*, 2004).**

The primary route of contamination is through the ingestion of seafood and water when sanitary conditions are inadequate (particularly water treatment). Among the reservoirs of *V. cholerae* are copepods, aquatic plants, chironomid insects, and cyanobacteria **(Lipp, E. K et *al.*, 2002, Islam, M at *al.*, 2004, Halpern, M., et *al.*, 2006).**

b. *Vibrio vulnificus*

V. vulnificus is an infectious agent for humans, transmitted through the consumption of contaminated seafood or by contact of a wound with seawater, which can lead to severe injuries or fatal septicemia in 65-75% of cases, sometimes within 24 hours. Some cases of gastroenteritis have also been associated with this pathogen. Several hundred deaths occur each year worldwide due to *V. vulnificus*. Three biotypes have been described based on LPS, capsule characteristics, and more recently, genetic analyses. Biotype I is the main pathogen for humans ; biotype II (reclassified into serogroup E since only one type of LPS has been identified for these strains) primarily concerns infections in eels, although some cases of infections associated with this biotype have been reported in humans; finally, biotype III has been identified in a few cases of infectious disease in humans **(Gulig, P. A, 2005).** Within each characterized biotype, there are both highly pathogenic and non-pathogenic strains. Typing of biotype I was mainly based on five types of LPS, but it was found that a significant number of strains (39-49%) could not be typed using this approach due to considerable antigenic heterogeneity among these strains. Since then, other approaches based on antigenic typing of the capsule, ribotyping, PFGE profiles, 16S rRNA analysis, and PCR-RFLP have led to the same conclusions, namely the extremely polymorphic nature of *V. vulnificus* strains and the impossibility of systematically and accurately associating a given virulence status with a specific phylogenetic group or biotype. Among these virulence factors, the capsular polysaccharide (CPS) appears to play an important role in inflammation and in resistance to immune responses **(Horseman, M.A., Surani, S, 2010).**

c. *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is the primary cause of gastroenteritis caused by consuming raw or undercooked seafood. Its pathogenicity is linked to the production of hemolysins TDH (Thermostable Direct Hemolysin) and TRH (TDH-Related Hemolysin), which are related to the TDH toxin **(Raghunath, P, 2015, Suthienkul, O et *al.*, 1995)**. These enzymes have lytic, cytotoxic, and enterotoxic activities. They lyse erythrocytes and disrupt the organization of the cytoskeleton as well as calcium homeostasis **(Raimondi, F., et *al* ., 2000)**. In the natural environment, most strains of *V. parahaemolyticus* are non-pathogenic **(Velazquez-Roman, J et al., 2012)**. In contrast, bacteria isolated from infected patients possess genes coding for at least one of the two hemolysins **(Kaufman, G.E et *al.*, 2002).**

The classification of *V. parahaemolyticus* is based on serotyping, which reveals 13 O-type serogroups and 71 K-type serogroups (related to the antigenic properties of the capsule). Several serogroups are known to induce infections. However, the correlation between serotype and pathogenicity of the bacteria was not established until the emergence in 1996 of a strain responsible for diseases belonging to serotype O3:K6. Since then, this bacterium has been detected in various locations around the world where the consumption of raw fish and seafood is significant, such as in Chile, France, Japan, Korea, Spain, Taiwan, the United States, and particularly in East Asian countries like India, Bangladesh, and Thailand **(Toyofuku, H, 2014).**

h.2.3.2. Pathogenic Vibrios in Marine Organisms

Bacteria of the genus *Vibrio* are dominant in marine ecosystems. Apart from a few species pathogenic to humans, the majority of pathogenic species affect marine animals. The genus *Vibrio*, along with other groups such as *Achromobacter*, *Flavobacterium*, and *Pseudomonas*, constitutes a significant portion of the bacterial flora in aquatic environments **(Vandenberghe, J et *al.*, 2003).** However, certain species are classified as etiological agents responsible for significant economic losses in various aquaculture sectors.

a. In Fish

Today, 17 species of *Vibrio* are potentially involved in mortality episodes in fish **(Buller, N. B, 2004)**, with the most studied species posing significant problems in aquaculture being *V. anguillarum* and *V. salmonicida*. Table 1.2 summarizes all the vibrios described as pathogenic in fish.

a.1. *Vibrio anguillarum*

V. anguillarum is an infectious agent first described in eels in 1909 in the Baltic Sea and has since been identified in around fifty species of fish from the Atlantic and Pacific Oceans. It is currently responsible for the highest mortality rates attributed to a *Vibrio* species in fish, particularly in salmonids.

This pathogen poses significant challenges in aquaculture. The main diseases caused by *V. anguillarum* include hemorrhagic septicemia, necrosis of the heart, kidneys, and spleen, as well as ocular and intestinal lesions. In severe cases, the speed of infection can be so rapid that no symptoms are observed before the animal's death **(Frans, I et *al.*, 2011).**

The classification of *V. anguillarum* is based on serotyping, which identifies 23 O-type serogroups (O1 to O23). Strains belonging to serogroup O1 are the most pathogenic for fish in general, while strains classified in serogroup O2 are more frequently associated with eel mortality. Finally, although O3-type strains are commonly isolated from the marine environment, they are rarely linked to mortality episodes **(Pedersen, K et *al.*, 1999).**

a.2. Vibrio salmonicida

Vibrio salmonicida is responsible for Hitra disease, also known as "cold water vibriosis." It affects farmed salmon and cod in Canada and Nordic countries. This bacterium operates at low temperatures below 15°C and causes severe anemia and significant hemorrhaging **(Toranzo, A.E et *al.*, 2005).**

b. In Mollusks

Vibrio species affect marine mollusks regardless of their family, genus, or species. In cephalopods, *V. alginolyticus* infects cuttlefish such as *Sepia officinalis*, *S. apama*, and *S. pharaonis* , while *V. lentus* causes skin lesions in the wild octopus *Octopus vulgaris* **(Farto, R et *al.*, 2003)**

In gastropods, Li et al. (1998) reproduced the white spot disease in the abalone *Haliotis discus* following experimental infection with *V. fluvialis* II strains. Likewise, NICOLAS et al. demonstrated the pathogenicity of *V. carchariae*, which infects both wild and farmed populations of *Haliotis tuberculata*.

Bivalves are the most affected class by vibriosis. Following mortality episodes observed in oyster farms, numerous studies have emerged, identifying various bacterial strains **(Beaz-Hidalgo, R et al., 2010 ; Romalde, J.L et *al.*, 2014).** Table 3 summarizes the main species or groups described.

A particular emphasis is placed on the *V. splendidus* group, which is often linked to several mortality episodes. This polyphyletic clade currently includes 16 species, some of which are pathogenic to bivalves (*V. celticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. tasmaniensis*, and *V. splendidus*), while others are not **(Vanhove, A.S et *al.*, 2015)**.

However, these bacteria with contrasting pathotypes can collaborate in polymicrobial infections. Indeed, virulent and avirulent strains can interact and facilitate the pathology induced in oysters by *V. crassostreae* or *V. tasmaniensis* **(Gay, M.et *al.*, 2004).**

c. In Crustaceans

Vibriosis affects several species of crustaceans. In marine crabs, the infection leads to bacteremia or shell disease . *Vibrio* bacteria have been isolated from the hemolymph of blue crabs (*Callinectes sapidus*), *Callinectes bocourti* , and rock crabs (*Cancer irroratus*). Moreover, the pathogenicity of these isolates has been demonstrated under experimental conditions **(Wang, Z et *al.*, 2010).**

Similarly, *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus* have been identified as causative agents of shell disease in lobsters. The luminescent strain *V. harveyi* has also caused significant mortality in larval cultures of *Sagmariasus verreauxi* in New Zealand . Infection by *V. harveyi* results in anorexia, stunted growth, and reduced swimming activity in affected shrimp. The animals become opaque and may exhibit luminescence (hence the term "luminescent bacterial disease"), along with tissue degeneration of the hepatopancreas **(Porter, L., et *al.*, 2001).**

These same species (*V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus*), along with *V. anguillarum* and *V. splendidus*, have been associated with pathologies observed in *Penaeus monodon* shrimp farms in India. Strains *V. nigripulchritudo* and *V. penaeicida* have also caused significant mortality in *Litopenaeus stylirostris* shrimp cultivated in New Zealand **(Jayasree, L et *al.*, 2006).**

d. In Corals

Bleaching is one of the most severe and widespread diseases affecting corals . It manifests as discoloration due to the disruption of the symbiosis between corals and microalgae (zooxanthellae). This phenomenon is generally correlated with specific environmental conditions such as elevated temperatures or reduced salinity, but also with the presence of pathogenic *Vibrio* bacteria **(Rubio-Portillo, E et *al.*, 2014).**

Vibrio shiloi is the etiological agent of bleaching in *Oculina patagonica*. Its virulence is strictly linked to elevated temperatures, ranging from 24 to 29°C . Under these conditions, the bacterium penetrates the host's tissues and transforms into a viable but non-culturable (VBNC) form, allowing it to multiply. Additionally, its internalization is accompanied by the production of a P toxin capable of inhibiting the photosynthesis of zooxanthellae **(Rosenberg, E., Ben-Haim, Y, 2002).**

Vibrio coralliilyticus is also a temperature-dependent pathogen affecting *Pocillopora damicornis*. It can induce bleaching at 25°C and lyse tissues between 27 and 29°C **(Ben-Haim, Y et *al.*, 2003).** Recently, Rubio-portillo et al. 2014 demonstrated that *V. coralliilyticus* can act synergistically with *V. shiloi*, inducing disease at lower temperatures (20°C).

V. alginolyticus and various unidentified *Vibrio* spp. have been isolated from corals affected by yellow band disease in *Monastraea spp.* in the Caribbean Sea . The implicated *Vibrio* species target the zooxanthellae rather than the coral polyps, and the infectious mechanism is closely linked to temperature, similar to the other coral diseases caused by *V. coralliilyticus* and *V. shiloi* **(rubio-portillo et *al*., 2014).**

h.3. The Virulence Factors of the *Vibrio* Genus

h.3.1. The Concept of Virulence in Bacteria

Before describing the virulence factors associated with the *Vibrio* genus, it is essential to distinguish the concepts of pathogenicity and virulence, as well as to understand their relationship. Traditionally, a pathogen is defined as a microorganism capable of causing disease or multiplying in living tissues. Pathogenicity refers to a microorganism's ability to induce disease **(Cervino, J. M et al., 2004).**

This definition implies that pathogenicity is an intrinsic characteristic of the microorganism, independent of the host. However, this interpretation is insufficient, as a microorganism can colonize its host without causing harm. In 1999, Casadevall and Pirofski proposed a reformulation of these definitions, stating that a pathogen is a microorganism capable of causing damage to the host, and pathogenicity is the ability of a microorganism to cause such damage. Thus, pathogenicity becomes the result of an interaction between the host and the microorganism **(Casadevall, A., Pirofski, L., 1999).**

Regarding virulence, several definitions have been proposed **(Casadevall, A., Pirofski, L., 1999):**

- The degree of pathogenicity.
- The strength of a pathogen's activity.
- A synonym for pathogenicity.
- The percentage of mortality from infection.
- The relative ability to overcome the host's defenses.
- A measure of a microorganism's ability to infect or damage a host.
- The relative capacity to enter and multiply within a given host.

-Later, a more stringent definition of virulence was established, which similarly associates the host and the infectious agent : it is the relative ability of a microorganism to cause damage to the host. Some definitions vary between and within disciplines, which can lead to confusion. Thus, most pathologists choose to use a broad definition that links the concept of virulence to that of pathogenicity **(Shapiro-Ilan, D. I, 2005).**

-Similarly, the terminology used to describe virulence factors has evolved from "microbial products that allow a pathogen to cause disease" to a broader definition: "any component of a microorganism that is necessary or enhances its ability to cause disease" . This absolute definition implicitly includes non-toxic substances for the host that can still be considered virulence factors if their absence makes the microorganism less virulent **(Thomas, S. R. & Elkinton, J. S.,2004).**

Finally, it is important to consider the following themes :

- Very few virulence factors function as determining factors of virulence.
- Damage is caused either directly by the microorganisms or by a microbial component, or by the host's immune response.
- Immune responses can neutralize several or some of the virulence factors **(Schaechter, M *et* al., 1999**).

h.3.2. The Virulence Mechanisms of *Vibrio*

Whether major or accessory, and whether they play direct or indirect roles in pathogenesis, numerous bacterial virulence factors have been described. Their classification into functional categories may seem reductive given the dynamic complexity of the combinations and interactions that lead to the development of an infection and the expression of disease.

The role of virulence factors in opportunistic pathogens is particularly challenging to characterize, as it is closely dependent on the immuno-physiological state of the infected host, which determines its susceptibility to the infectious agent. These factors participate in various stages of bacterial infection, from colonization of host surfaces to penetration into the host, invasion and colonization within the host environment, inhibition or disruption of the host's immune system, evasion of this immune system, and finally, disruption or destruction of the host's essential functions.

It is clearly established that certain functional categories of virulence factors overlap, and some determinants can be attributed to more than one group Casadevall, A. & Pirofski, L.A., (2009), . Nevertheless, for the sake of synthesis, we can arbitrarily distinguish three types of virulence factors produced by bacteria :

1. Factors for colonization and invasion of the host.
2. Factors capable of disrupting, altering, or destroying essential functions of the host.
3. Factors for evading the host's immune system.

These virulence factors have been particularly identified in the pathogenic *Vibrio* species previously mentioned, whose genomes have been fully sequenced : *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* **(Chen, C., K, 2003),** but also in *V. anguillarum*, *V. harveyi*, and other pathogenic species affecting fish, crustaceans, and mollusks **(Austin, B, 2010).**

a. Factors for Colonization and Invasion of the Host

- **The Flagellum**

Mobility is a common characteristic of pathogenic organisms, closely associated with their virulence, providing them with a clear adaptive advantage. It enables migration to preferred ecological niches, such as specific tissues of the host, facilitates contact with host cells, allows penetration of cell membranes, aids in evading toxic substances, and supports escape from intracellular compartments for dispersion in the environment during transmission. Most bacterial cells, particularly those in the *Vibrionaceae* family, are motile thanks to specialized organelles known as flagella (Figure 11) **(Yorimitsu, T. & Homma, M, 2001).**

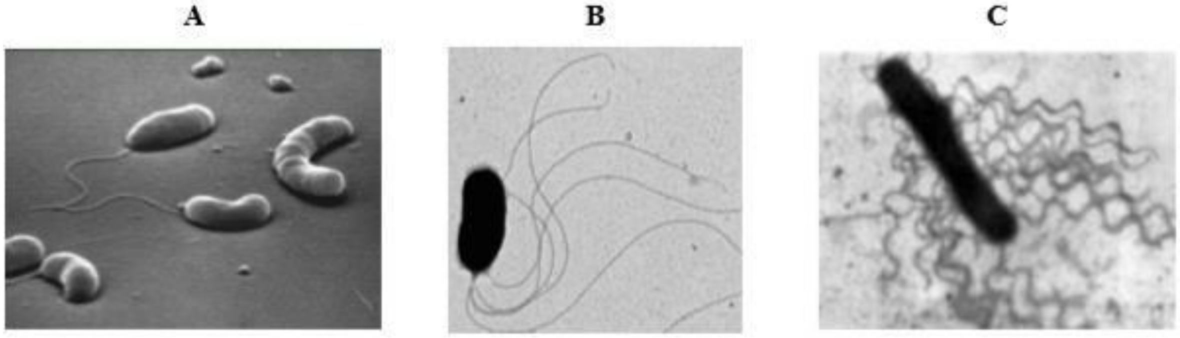


Figure 13 : A. Unique polar flagellum in *V. cholerae,* B. Multiple flagella in *V. fisheri.* C. Multiple lateral flagella in *V. parahaemolyticus* **(Parkinson et *al.*, 2005).**

The flagellum is a structure anchored in the plasma membrane, semi-rigid and rotary, powered by a molecular motor that drives a filament acting like a helix. The ability of bacteria to modulate the direction of flagellar rotation allows for directed propulsion of the prokaryotic cell toward attractive substances or away from repulsive substances . This directed movement (non-random) is called chemotaxis.

Two types of mobility are observed in *Vibrio* : the "swimming" type, facilitated by a single polar flagellum and suited for low-density aquatic environments like seawater, and the "swarming" type, enabled by lateral flagella and adapted to viscous environments such as mucus found at the epithelium of certain hosts. *Vibrio* species typically possess a single polar flagellum, although lateral flagella have been described in *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi* **(Parkinson et *al.*, 2005).**

The movement of the flagellum is driven by an osmo-mechanical coupling, utilizing an electrochemical gradient that allows the passage of Na^+ ions (for most polar flagella) or H^+ ions (more generally for lateral flagella) across the membrane. This process provides osmotic energy that is converted into mechanical energy.

Bacterial mobility is a complex process that requires a significant energy expenditure, which can constitute a considerable portion of the cell's metabolism (about 2% of total energy in *Escherichia coli*). It depends on approximately fifty genes regulated by numerous control mechanisms, and mutations often result in reduced virulence.

For example, in *V. vulnificus*, the creation of mutants with deletions in the flgC and flgE genes, which code for the flagellum, demonstrated its role in mortality processes in mice and in the pathogen's attachment to human epithelial cells. Similarly, the involvement of the flagellum in pathogenicity has also been shown in *V. anguillarum* through targeted mutagenesis techniques: non-motile mutants generated by transposition and tested for their in vivo virulence showed no virulence during bathing assays.

Finally, the flagellum appears to be an accessory virulence factor in *Vibrio*, whose role can sometimes be difficult to demonstrate due to "virulence compensation" phenomena observed in certain flagellum-deficient mutants of *V. cholerae* **(Ran Kim Y. et Haeng Rhee J, 2003).**

- **Pili**

The first step in the colonization process relies on adherence to a target host cell. Many Gram-negative bacteria possess surface appendages that are shorter and finer than flagella, known as pili, which can be classified into two types : common pili and sexual pili. These pili mediate interactions between bacteria, the host, and the environment.

While sexual pili are involved in conjugation and allow for the exchange of genetic material between two bacteria, common pili, which are more numerous, facilitate the attachment of bacteria to host surfaces, thereby conditioning their pathogenicity. Regularly arranged on the surface of the bacterium, common pili are filamentous protein structures composed of the polymerization of a single protein subunit, pilin, assembled with minor polypeptides such as adhesins. The adhesin can interact with receptors, such as glycoproteins or glycolipids, present on the surface of host cells.

The type IV pilus has been extensively described as being involved in adherence, biofilm formation, and mobility **(Bardy S.L, 2003).** Additionally, three different types of pili have been identified in *V. cholerae* and are associated with the pathogenicity of serogroups.

The Toxin-Coregulated Pilus (TCP) is a major virulence factor in the infectious process of *V. cholerae* it is essential for colonization of the human intestinal tract (Figure 12) and also serves as a receptor for the filamentous bacteriophage CTXΦ, which carries the gene for cholera toxin. Fifteen genes located on a pathogenicity island called VPI (39.5 kbp), characteristic of epidemic and pandemic strains of *V. cholerae*, regulate the biosynthesis of this pilus. The mannose-sensitive hemagglutinin (MSHA) pilus also seems to play a role, albeit minor, in virulence.

Other genes coding for adhesion factors have been described, including cep (Core-encoded pili), acf (accessory colonization factor), and ompU (outer membrane protein) **(Sperandio, V et *al.*, 1996).**

Similarly, the involvement of pili in the pathogenicity of *V. vulnificus* has been demonstrated through mutations in the pilD and pilA genes, which notably result in decreased adherence to epithelial cells , **(Paranjpye R.N. et Strom M.S, 2005).**

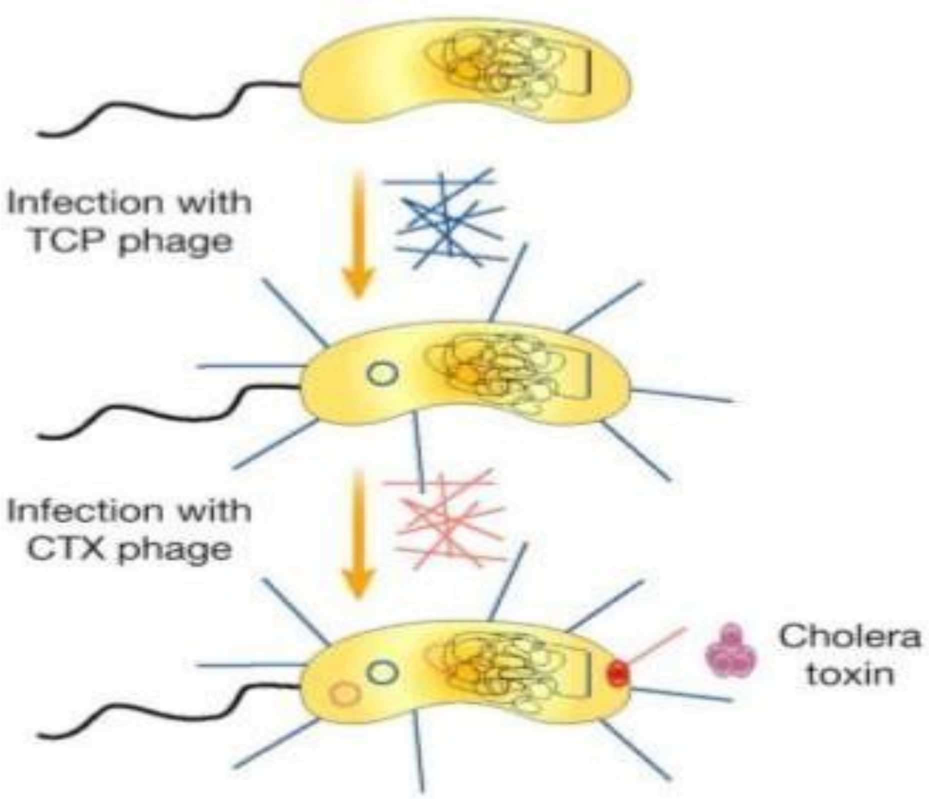


Figure 14 : Description of the role of TCP (Toxin-Coregulated Pilus) in *V. cholerae*, an example of a type IV pilus **(Donnenberg M.S, 2000)**

- **Hydrolytic Enzymes**

In addition to its ability to colonize and adhere to host surfaces, a bacterium's pathogenicity also relies on its capacity to invade its host. To achieve this, many pathogenic bacteria secrete various extracellular proteins known as invasins. These hydrolytic enzymes serve multiple functions, facilitating the bacterium's acquisition of carbon and nitrogen resources, degrading the extracellular matrix, and disrupting tissue organization during invasion. Furthermore, they modulate the host's defense responses and escape mechanisms.

The generic term "invasins" typically refers to enzymes that act at a short distance from the site of cellular multiplication and do not cause cell lysis, distinguishing them from certain extracellular toxins.

Over the past thirty years, a wide variety of hydrolytic enzymes have been described in many *Vibrio* species, including proteases, lipases, nucleases, and hyaluronidases, often associated with the virulence of pathogenic agents. Metalloproteases are proteolytic enzymes whose catalytic activity requires a metal ion. These can be endo- or exopeptidases, mostly zinc-dependent, that cleave peptides or proteins in a more or less specific manner.

The involvement of metalloproteases in the virulence of *Vibrio* bacteria has garnered significant interest, as evidenced by the increasing number of publications on the subject. Various studies have established that metalloproteases exert diverse pathological effects. In addition to their proteolytic power through the degradation of a wide range of host protein substrates—such as lactoferrin, collagen, elastin, plasma proteins, and albumin—metalloproteases lead to increased vascular permeability, trigger hemorrhagic reactions linked to the stimulation of the kallikrein-kinin system, cause agglutination of erythrocytes, and induce tissue necrosis **(Miyoshi, S et *al.*, 2002).**

The various metalloproteases secreted by *Vibrio* species, along with their biological roles in virulence, are referenced in Table 1.4 **(Mersni-Achour, R, 2014).**

- **Siderophores**

There is no doubt that, for bacteria to survive and multiply within the host, they must develop mechanisms to acquire essential nutrients for their growth. Iron is a crucial element involved in numerous biological processes, including gene regulation and certain enzymatic reactions. It is estimated that a bacterial suspension of *E. coli* at approximately 10^9 cells/ml consumes up to 10^{18} iron atoms per liter.

Due to the reduced solubility of iron (about 6×10^6 atoms per liter) and its limited free availability in the environment, bacteria have developed optimized systems for iron acquisition. The most well-known of these is the siderophore. These small molecules with very low molecular weight (<1000 Da) chelate soluble Fe^{3+} in the environment with very high affinity and can also extract it from host carrier proteins, such as transferrin found in mammals **(Binesse J et *al.*, 2008)**

In Gram-negative bacteria, iron-chelating siderophores are internalized through the outer membrane via a complex multiprotein transmembrane system that facilitates the availability of iron for the bacterium. More than 500 siderophores have been described and classified into three groups based on the chemical structure of the functional group that binds to iron **(Crosa J.H., 1997).**

Many *Vibrio* species produce siderophores, such as *V. parahaemolyticus* and *V. cholerae*. In *V. vulnificus*, a specific siderophore known as vulnibactin has been identified. Its role in virulence has been demonstrated by the attenuation of pathogenic phenotypes in mutants that no longer synthesize vulnibactin and cannot extract iron from transferrin **(Litwin C, 1996).**

In *V. anguillarum*, the siderophore, named anguibactin and encoded by an operon located on the plasmid pJM1, constitutes a major virulence factor. This operon also encodes the transport protein complex (FatABCD Complex) that facilitates the internalization of anguibactin into the bacterial cytoplasm, with its involvement in pathogenicity well-documented.

Finally, competition phenomena have been observed among different microorganisms for the uptake of siderophores : some bacteria, like *V. anguillarum*, can intercept siderophores from other bacteria, such as the acinetobactin from *Acinetobacter baumannii*, to dominantly occupy an ecological niche **(López C. et Crosa J., 2007).**

B . Factors Capable of Altering the Host

- **Toxins**

Factors capable of altering the host primarily consist of toxins, which can cause either lesions or cellular dysfunctions. There exists a wide variety of toxin structures and a diverse range of pathological effects, leading to multiple classification possibilities [181]. However, toxins can generally be distinguished as exotoxins, which are secreted or released into the environment, and endotoxins, which are integral components of microbial bodies.

- **Exotoxins**

Exotoxins are toxic bacterial proteins secreted into the extracellular environment or released after bacterial lysis, typically exhibiting catalytic biological activity and high specificity of action. They are generally thermolabile and sensitive to denaturing agents. Three classical types of exotoxins can be distinguished based on their structure and mode of action **(Ménez A et *al.*, 2006):**

-AB Type Toxins : These consist of two modules : one that binds to the receptor on the host cell, determining specificity of action, and the other that carries the catalytic site responsible for toxicity. In *V. cholerae*, the B module of the CT (Cholera Toxin) binds to a specific receptor, the GM1 ganglioside, present on the surface of enterocytes. The A module catalyzes the ADP-ribosylation of the Gsα protein, which activates adenylate cyclase in a permanent and uncontrolled manner within the targeted eukaryotic cell's cytoplasm. The consequent increase in intracellular cAMP leads to a loss of control over ionic flux and results in the passive loss of water from the cells, which is the main symptom of cholera.

After binding to the GM1 receptor, the A subunit is translocated into the cytosol, where it is activated by thiol-dependent reduction. The truncated subunit, A1, catalyzes the ADP-ribosylation of a G protein. This activation of adenylate cyclase results in the release of cAMP, a ubiquitous intracellular messenger. cAMP inhibits the active absorption of NaCl and increases the secretion of water and chloride ions.

-Lytic Toxins

Lytic toxins, among which hemolysins are prevalent in pathogenic vibrios **(Zhang X.H. et Austin B, 2005),** cause lysis of various vertebrate cell types, including erythrocytes and immune cells, earning them the more generic name cytolysins **(Shinoda S, 1996)**. Their action is characterized by the formation of pores in the plasma membranes of target eukaryotic cells, leading to cell lysis and the release of iron-binding proteins (such as hemoglobin, transferrin, or lactoferrin), tissue damage, or destruction of host immune cells.

In *V. cholerae*, most non-O1 strains produce a cytolytic toxin secreted into the external environment : the El Tor cytolysin/hemolysin (HlyA). This toxin exhibits enterotoxic activity and is a major cause of gastroenteritis caused by non-O1 *V. cholerae* strains. In *V. vulnificus*, a hemolysin-type exotoxin (VvhA) has been identified. In addition to its role in releasing iron from hemoglobin and forming pores in cell membranes, this toxin has cytotoxic effects. Intradermal injection of purified VvhA into mice caused damage to adipose, endothelial, and muscle cells, as well as moderate inflammatory infiltrations. Other studies have shown that VvhA stimulates increased vascular permeability, apoptosis of endothelial cells, and hyperproduction of nitric oxide **(Horseman, M.A., Surani, S, 2011).**

Similarly, in *V. parahaemolyticus*, a thermostable direct hemolysin (TDH) and a related hemolysin (TRH) have been described. Both toxins exhibit enterotoxic, cardiotoxic, and cytotoxic activities **(Nair, G. B, 2007)**. In *V. harveyi*, the hemolysin (VHH) of phospholipase type demonstrated cytotoxicity against fish cells and significant pathogenicity toward flounder by injection, resulting in hemorrhages in the peritoneal cavity and necrotic zones at the injection site [188]. Additionally, VHH caused structural changes and induced apoptosis in flounder cells . In *V. anguillarum*, four hemolysins (VAH 2, VAH 3, VAH 4, and VAH 5) have been identified. Purified hemolysins showed hemolytic activities against erythrocytes from fish, sheep, and rabbits. Single-gene mutants of these hemolysins were found to be less virulent than the wild-type strain in trout (Oncorhynchus mykiss), indicating that each hemolysin gene contributes to the virulence of *V. anguillarum*. A hemolysin (Vh-rTDH) produced by *V. hollisae*, similar to the

TDH hemolysin from *V. parahaemolyticus*, has also been identified and shown to have cytolytic activities against several types of animal erythrocytes. Finally, in *V. tubiashii*, a cytolysin similar to that of *V. vulnificus* capable of lysing various cell types and erythrocytes has been described **(Kothary, M.H,2001).**

- **Injected Toxins**

Certain protein and cytotoxic toxins are injected directly into host cells through complex secretion systems, disrupting cellular processes and lysing defense cells. The action of these toxins allows evasion of the host's immune system. The type III secretion system (T3SS), one of the most complex described to date, requires the expression of over 20 genes **(Winstanley C. et Hart C.A., 2001 ; Mota L.J et *al.*, 2005)** and facilitates the secretion and translocation of virulence factors into the cytosol of target eukaryotic cells **(Sekiya K., 2001)**. This secretion system is common among many Gram-negative bacteria, including *Yersinia spp.*, *Pseudomonas aeruginosa*, *Salmonella spp.*, as well as enteropathogenic and enterohemorrhagic *E. coli*. Complete sequencing of a strain of *V. parahaemolyticus* revealed two operons encoding two T3SS: one, T3SS1, is involved in vitro in cytotoxicity, while the other, T3SS2, found in human pathogenic strains, is responsible for enterotoxic activity that induces intestinal fluid accumulation. T3SS-coding operons have been identified in various *Vibrio* strains, such as *V. cholerae* and *V. harveyi* [Henke J.M. et Bassler B.L, 2004 ; Dziejman M et al., 2005], while homologous genes to T3SS have been found in *V. alginolyticus* and *V. tubiashii* . Lastly, a novel type IV secretion system has been described in *V. cholerae*, potentially responsible for the defense of *V. cholerae* against eukaryotic predators like the amoeba *Dictyostelium discoideum* **(Pukatzki S, 2006).**

- **Endotoxins**

Endotoxins comprise the structural toxic compounds of the cell wall of Gram-negative bacteria, known as lipopolysaccharides (LPS). The LPS, a component of the outer membrane, consists of three parts :

- **Lipopolysaccharide A** : Responsible for the toxicity of LPS and its immunomodulatory capabilities.
- **Core Polysaccharide** : Maintains the integrity of the outer membrane.
- **Antigen**: The hydrophilic side chain exposed to the external environment, which exhibits significant variability, contributing to serological specificity **(Caroff M. et Karibian D, 2003).**

The LPS becomes toxic after the lysis of the bacterial cell, triggered by the host's immune response or antibiotic treatment. This endotoxin shock, caused by the massive release of LPS, leads to their entry into the circulatory system, where they bind to a protein known as LBP (LPS binding protein). The LPS/LBP complex is recognized by various receptors on the surface of macrophages (from the Toll-like receptor family), activating the transcription factor NF-κB (nuclear factor κB). This activation results in the massive production of pro-inflammatory cytokines.

The toxicity of LPS is thus attributed to their ability to induce apoptosis in host cells and also to cause over-stimulation of the immune system, leading to septic shock, characterized by symptoms such as tissue edema, fever, hemorrhage, and hypotension (as described in *V. vulnificus*). In *V. cholerae*, other structural components, such as the porins OmpU and OmpT, have also been associated with virulence **(Mathur J., 2007).**

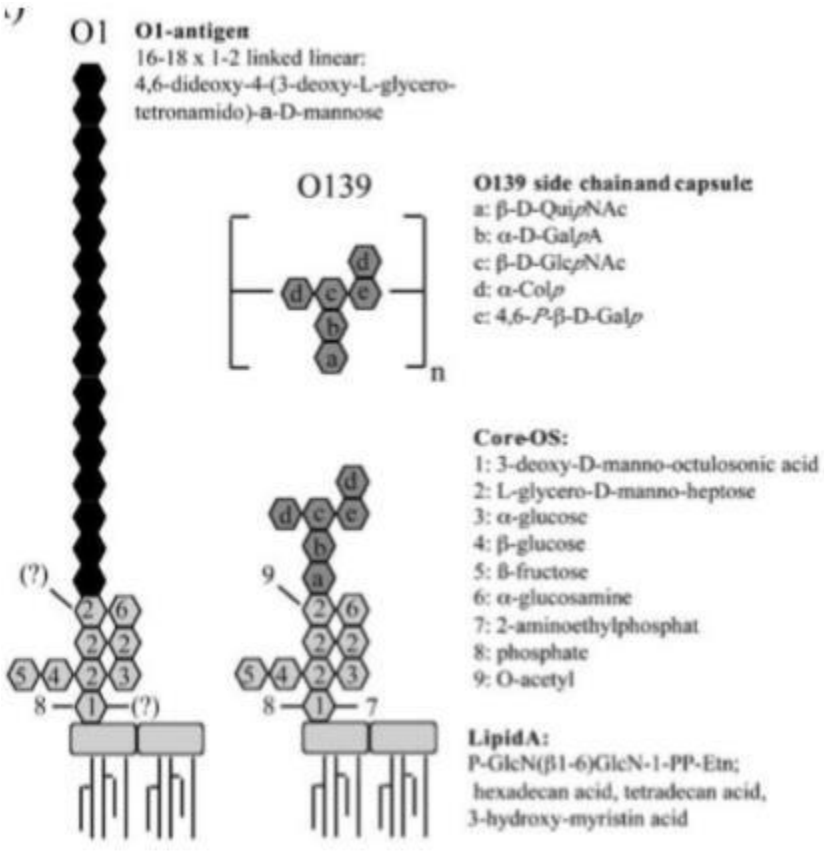


Figure 15 : Structure of LPS and the capsule of serogroups O1 and O139 of *V. cholerae*.

The lipid A and core are identical for both serogroups. The few chemical differences are indicated by "?" **(Reidl J. et Klose K.E, 2002).**

It has recently been demonstrated that LPS, along with capsule proteins and outer membrane proteins (OMPs), interact with the host's innate immune system through conserved motifs referred to as MAMPs (microbe-associated molecular patterns) or PAMPs (pathogen-associated molecular patterns). Indeed, MAMPs or PAMPs are recognized by the host's pattern recognition receptors (PRRs), and these interactions lead to the activation of the host's defense mechanisms **(Medzhitov R, 2007).**

i.Brucella

Brucella is a facultative intracellular Gram-negative coccobacillus, measuring 0.5 to 0.7 µm in diameter and 0.5 to 1.5 µm in length. The cells are non-motile and do not form conventional flagella, capsules, or spores. *Brucella* bacteria are strict aerobes, although some strains require a CO2-enriched atmosphere (5 to 10%) for growth (some biovars of *B. abortus* and *B. ovis*). *Brucella* bacteria can produce catalase, cytochrome oxidase, and nitrite reductase. Most species can hydrolyze urea. The optimal pH for growth ranges from 6.6 to 7.4, and the optimal growth temperature is 37°C, with most strains developing between 20°C and 40°C on suitable media **(Kooh and Lailler, 2006 ; Scholz et *al.*, 2018).**

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

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

Brucella is the agent responsible for brucellosis (Malta fever), an infectious and contagious disease in animals transmissible to humans, with a worldwide distribution. Cases of human brucellosis have been attributed to four of the seven *Brucella* species found in terrestrial mammals. *B. melitensis* and *B. suis* (except biovar 2) are the most virulent species, followed by *B. abortus* and *B. canis*. *B. ovis*, *B. neotomae*, and *B. microti* are not reported as pathogenic to humans. However, some probable cases of human infection linked to a marine mammal *Brucella* strain have been described **(Kooh and Lailler, 2006).**

The main animal reservoirs for *Brucella* include cattle (*B. abortus*), sheep and goats (*B. melitensis*), and domestic pigs (*B. suis*). *Brucella* strains have also been isolated from other domestic species and many species of wild terrestrial mammals and marine mammals **(Kooh and Lailler, 2006)**. *Brucella* spp. can survive for several weeks to months in dust, water, aborted tissues, meat, and dairy products **(Corbel et *al.*, 2006)**.

Brucellosis is a zoonosis. Human contamination can occur through :

- The consumption of contaminated foods (mainly raw milk and dairy products).

- Contact of skin (even seemingly healthy) or mucous membranes (digestive, conjunctival, and nasopharyngeal) with infected animals and their products **(Kooh and**
- **Lailler, 2006).**

j.Actinobacteria

j.1.Definition and Generalities

The former name of actinobacteria was actinomycetes. The term "actinomycetes" comes from the Greek "aktino - mycetes," meaning "ray fungi" or "radiating fungi." Actinomycetes are Gram-positive bacteria characterized by the formation of filamentous hyphae that usually do not fragment and produce asexual spores. Their development leads to circular colonies made up of filaments that radiate outward from the germ that gave rise to them **(Gottlieb, 1973).**

Morphologically, they strongly resemble fungi, partly due to adaptation to similar habitats. Most species live in soil or, less frequently, in fresh water. A small number are pathogenic, and several produce antibiotics.

Some representatives of these microorganisms, especially aerobic ones, have long been excluded from the broader category of bacteria and confused with fungi due to their fungal-like morphology (branched filaments, sporulation organs, etc.) and the mycotic appearance of diseases they cause. This issue has been resolved, and this group of microorganisms is definitively classified among bacteria (prokaryotes) with a guanine-cytosine (G+C) content greater than 55% **(Harir, 2018).**

The main differences between fungi and actinomycetes can be summarized as follows :

- ✓ Their cell walls contain neither cellulose nor chitin, which are found in plants and fungi, respectively.
- ✓ Morphogenesis can be complex (corémy, zonation).
- ✓ The diameter of their mycelium is approximately one-tenth that of most fungal hyphae (generally 0.7 to 0.8 µm).
- ✓ Their sensitivity to attacks by bacteriophages and lysozymes.

The bacterial properties of actinomycetes are as follows : their cytology is prokaryotic ; physiologically, they are generally aerobic bacteria (mostly in soil), but some forms are facultative anaerobes or even anaerobes. They are sensitive to phages and antibacterial antibiotics **(Lechevalier, 1988).** They grow slowly compared to other bacteria, with an average generation time of about 2 to 3 hours. The morphology of different groups of actinomycetes is very variable, ranging from less evolved forms like *Mycobacterium* to highly evolved forms like the genus *Streptomyces*, which forms a true non-fragmented and sporulating mycelium.

Actinomycetes are generally mesophilic ; others are thermophilic, tolerating temperatures around 50°C and can thrive at 60°C or more. They They prefer a neutral or slightly alkaline pH with low to moderate humidity **(Prescott et *al.*, 2010)**.

j.2.Classification

Actinobacteria are classified within the Domain Bacteria or Eubacteria, Phylum Actinobacteria, Class Actinobacteria, and Subclass Actinobacteridae. The phylum Actinobacteria now includes five classes, 21 orders, and numerous families. The genera are characterized by significant morphological diversity, ranging from simple cocci (e.g., *Micrococcus*) to mycelial forms that may or may not be fragmented. The taxonomic position of Actinobacteria, using the species *Actinopolyspora algeriensis* described by Meklat et al. (2012) as an example, is established as follows :

- **Class**: Actinobacteria
- **Order**: Pseudonocardiales
- **Sub-order**: Actinopolysporineae
- **Family**: Actinopolysporaceae
- **Genus**: Actinopolyspora
- **Species**: *Actinopolyspora algeriensis*

The genus *Streptomyces* generally predominates in soils and various other substrates, representing 80 to 95% of the total actinomycetes, followed by *Nocardia* and *Micromonospora*. Other genera constitute only a minimal fraction and are sometimes infrequent or quite rare.

j.3.Importance and Applications of Actinobacteria

Actinobacteria play an ecological role within ecosystems by decomposing organic substances. They represent a significant proportion of the microbial biomass in soil. They have the ability to produce a wide variety of bioactive molecules, including antibiotics and extracellular enzymes. In microbial biotechnology, actinobacteria play a crucial role in agronomy, food, and environmental fields **(Nouioui, 2014),** as well as in pharmacology. The search for new actinomycetes is essential for the discovery of natural product-based medicines with a broad spectrum of biological activity, such as antibacterial and antifungal antibiotics (aminoglycosides, anthracyclines, glycopeptides, beta-lactams, tetracyclines, macrolides, nucleosides, etc.), particularly from the genus *Streptomyces*, as well as vitamins (e.g., B12), toxic, cytotoxic, neurotoxic, antimitotic, antiviral, and anticancer substances **(Williams, 2009).** Recently, new objectives have been added to general screening : the search for new active molecules against AIDS, immunosuppression, Alzheimer's disease, the aging process, and certain tropical diseases; these goals aim at discovering new medications **(Parthasarathi et *al.*, 2011)**. Among the antibiotics synthesized by actinobacteria are streptomycin (*Streptomyces* or *Actinomyces griseus*), chloramphenicol (S. venezuelae), chlortetracycline (S. aureofaciens), terramycin (S. rimosus), oxytetracycline (S. rimosis), erythromycin (S. erythraeus), rifamycin (S. mediterranei), vancomycin (S. orientalis), daunomycin (S. coeruleorubidus), and clavulanic acid (S. clavuligerus) **(Bycroft, 1988 ; Chater, 2006).**

k. Psychrotrophic Bacteria

Psychrotrophic bacteria are bacterial species capable of growing at temperatures of 4 °C or lower, but they can multiply rapidly at temperatures ranging from 10 to 25 °C and even at higher temperatures. Psychrotrophic bacteria (along with various psychrotrophic yeasts and molds) can cause food spoilage. In aerobic storage conditions, aerobic psychrotrophs are the predominant bacteria. Notable examples include *Pseudomonas fluorescens*, *Pseudomonas fragi*, and other species from the *Pseudomonas* genus, as well as *Acinetobacter*, *Moraxella*, and *Flavobacterium*. In anaerobic storage conditions, anaerobic and facultative anaerobic bacteria dominate, including *Brochothrix thermosphacta*, *Lactobacillus viridescens*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Leuconostoc carnosum*, *Leuconostoc gelidum*, *Leuconostoc mesenteroides*, certain *Enterococcus*, *Alcaligenes*, *Enterobacter*, *Serratia liquifaciens*, some *Hafnia*, *Proteus*, and *Shewanella putrefaciens* (formerly *Alteromonas*).

k.1.Genus Pseudomonas

The bacterial genus *Pseudomonas* includes a group of Gram-negative bacilli that are aerobic, motile via polar flagella (monotrichous–peritrichous), and are rarely non-motile and non-sporulating. These bacteria are oxidase positive and are characterized by their ability to utilize a wide variety of hydrocarbon substrates as sources of carbon and energy. Many *Pseudomonas* species do not grow at 37 °C; however, a temperature of 30 °C is suitable for all, both pathogenic and saprophytic. These bacteria exhibit low longevity in culture, even at 4 °C. They are ubiquitous in nature and are characterized by their resistance to antibiotics and antiseptics.

k.1.1. Classification

The family *Pseudomonadaceae* currently encompasses five genera : *Pseudomonas*, *Comamonas*, *Frateuria*, *Xanthomonas*, and *Zoogloea*. A total of 265 species have been documented, but the 1974 edition of the Bergey Manual recognized 29 species, of which 13 are of medical interest. In the third edition of this systematic bacteriology manual, over 40 species have been recorded. Several genetic studies have allowed the classification of the genus *Pseudomonas* into five genetically distinct groups based on DNA-rRNA and DNA-DNA hybridizations :

- Genomic Group I : *fluorescens* group + *stutzeri* group + *alcaligenes* group
- Genomic Group II : *pseudomallei* group + *cepacia* group
- Genomic Group III : *acidovorans* group
- Genomic Group IV : *diminuta-vesicularis* group
- Genomic Group V: *maltophilia* group (*Xanthomonas*)

k.1.2. Pigments Produced by Pseudomonas

The two most common and characteristic pigments produced by *Pseudomonas* are pyocyanin (blue pigment) and pyoverdine (yellow-green fluorescent pigment), both of which are soluble in culture media and can impart color to them. Pigmented species include :

- *P. aeruginosa* : produces both pyocyanin and pyoverdine ; it may possess one, the other, or both, but these can be lost through mutation. There are melanogenic or erythrogenic varieties that produce either black or red pigments.

k.1.3. Pseudomonas Pigments

Pseudomonas fluorescens, *P. putida*, *P. syringae*, and *P. cichorii* produce pyoverdine, although some strains may be apigmented.

- *P. aureofaciens* produces a yellow-orange or purple pigment.

l.Yeasts

l.1.General Characteristics and Taxonomy

Yeasts are heterotrophic eukaryotes that belong to the fungal kingdom, distinguished by their unicellular nature and the absence of true mycelium (at least for the majority of their biological cycle) **(Guiraud, 1998).** The size of the yeast cell or thallus varies widely among species, ranging from 1 to 10 µm in width and 2-3 to 20-50 µm in length. Terms such as spherical, globose, ovoid, elongated, and cylindrical are often used to describe the vegetative forms of yeasts. Each cell is bounded by a rigid cell wall, which constitutes nearly 20% of its dry weight, along with a cytoplasmic membrane and a small nucleus. Yeasts can be haploid or diploid ; their optimal growth temperature ranges from 20 to 28 °C, with a maximum of 35 to 47 °C. They thrive in acidic environments, with a pH range of 4 to 4.5 and an optimal pH between 4.5 and 6.5, and can adapt to more extreme or alkaline pH levels **(Gueroui, 2018).**

Yeasts are chemotrophic, meaning they can derive energy from chemical oxidation-reduction reactions or the fermentation of chemical compounds like sugars. They are widely distributed in nature and are commonly found in water, salts, on plant leaves, and in fruit musts, among other environments.

The classification of yeasts is inherently part of fungal taxonomy and is primarily based on morphological characteristics. Yeasts are categorized into three classes : Ascomycetes, Basidiomycetes, and Deuteromycetes (*Fungi imperfecti*) **(Kurtzman and Fell, 2006 ; Suh et *al.*, 2006).**

- Ascomycetes form the family *Saccharomycetaceae*, which is divided into four subfamilies :
  - **Schizosaccharomycetoideae** : includes the genus *Schizosaccharomyces*, notable for its role in brewing.
  - **Saccharomycetoideae** : encompasses many species relevant to the food industry, including *Saccharomyces* (bread and wine production), *Kluyveromyces* (cheese production), *Zygosaccharomyces*, *Hansenula*, *Debaryomyces*, and *Pichia* (spoilage).
  - **Nadsonioideae** : contains a few interesting species belonging to the genus *Hanseniospora*.
  - **Lipomycetoideae** : of lesser interest, with some species from the genus *Lipomyces*.
- Basidiomycetes contain few yeasts of industrial importance, classified into three families : *Filobasidiaceae*, *Sirobasidiaceae*, and *Tremellaceae*.

Deuteromycetes or *Fungi imperfecti* comprise the family *Cryptococcaceae*, which is divided into four subfamilies :

- **Cryptococcoideae** : includes significant genera such as *Brettanomyces*, *Candida*, *Torulopsis*, and *Loeckera*, whose species serve as fermentation agents, contaminants, or protein sources.
- **Rhodotoruloideae** : includes pigmented species like *Rhodotorula*.
- **Trichospororoideae** : encompasses frequently encountered contaminants like *Trichosporon*.
- **Sporobolomycetoideae** : includes several species from the genus *Sporobolomyces*, which can sometimes contaminate food products.

Based on their applications, yeasts are grouped into seven categories **(Reed, 1981):**

1. **Baker's Yeasts and Baking Products** : strains derived from *Saccharomyces cerevisiae*, continuously improved through somatic hybridization, cell fusion, and various screenings (Larpent, 1990).
2. **Brewing Yeasts** : primarily *Saccharomyces cerevisiae* or *Candida utilis*.

3. **Winemaking Yeasts** : the most commonly used yeast in winemaking is *Saccharomyces cerevisiae*, although other strains like *Saccharomyces ellipsoideus* and *Saccharomyces fragilis* (for champagne) and other genera like *Kloeckera apiculata*, *Candida vini*, *Pichia*, and *Brettanomyces* can also be utilized.

I.2.Distillery Yeasts : in grain alcohol distillation, these yeasts replace brewing yeasts. Two strains of *Saccharomyces cerevisiae* are used—one for rapid ethanol production and the other for slower fermentation—along with *Hansenula polymorpha*.

I.3. Food Yeasts : used in cider production (*S. apiculatus*), vinegar production (*Mycoderma aceti*, "acetic acid bacteria" following alcoholic fermentation by yeasts), cocoa (*Candida krusei*, *Candida famata*, *Pichia membranefasciens*, etc.), coffee (*Saccharomyces*), and soy (*Zygosaccharomyces rouxii*).

I.4.Yeast Derivatives (Autolysates, etc.) : fractions obtained from yeast degradation via plasmolysis, hydrolysis, or autolysis, yielding various yeast derivatives such as inactivated yeasts, yeast extracts, mannoproteins, and protein extracts.

I.5.Industrial Ethanol and Fuel : bioethanol, a biofuel produced from grains (wheat, corn) or sugar beets, is intended for gasoline engines. It is obtained through an industrial fermentation process that converts sugars in these plants into alcohol. The raw alcohol (ethanol) is then distilled and dehydrated to produce bioethanol, which is blended with gasoline by petroleum companies **(Bourgeois et *al.*, 1996).**

Yeasts used in food products are non-pathogenic and do not cause food poisoning ; however, their growth can lead to alterations in the commercial quality of these foods. A small number of pathogenic yeasts should be noted, particularly *Candida albicans*, responsible for thrush in young children and vaginitis, and *Cryptococcus neoformans*, disseminated by pigeons and capable of causing fatal meningitis **(Rambaud, 2004).**

I.6.Role in Food Production

The historical role of yeasts in the food industry remains significant, as they are involved in the production of numerous food products (brewing, winemaking, cheesemaking, etc.). They also contribute to the valorization of agricultural and industrial waste, as well as the production of proteins, enzymes, lipids, vitamins, and biofuels.

Currently, yeasts are widely utilized in biomedical research and biotechnology due to their dual status as microorganisms and eukaryotes. Unlike bacteria, yeasts possess the ability to produce glycosylated proteins. Genetically modified yeasts produce the surface antigen of the hepatitis B virus, which is used in the hepatitis vaccine. Other yeasts produce human serum albumin, hormones, growth factors, and other therapeutic proteins **(Walker, 2009 ; Reski-Bekki, 2014).**

l.7.Industrial Uses of Yeasts

- **Alcoholic Beverages** : Wine, beer, cider, sake, etc.
- **Recombinant Proteins** : Hormones (such as insulin), antiviral vaccines (hepatitis B vaccine), growth factors (tumor necrosis factor), blood proteins (bovine serum albumin), interferons (leukocyte interferons), antibodies (IgE receptors), enzymes (gastric lipase and chymosin).
- **Industrial Alcohols**: Used in cosmetics, chemical industry, pharmaceutical industry, bioethanol, glycerol.
- **Enzymes**: Alpha-amylase, glucoamylase, protease, invertase, pectinase, lipase, inulinase.
- **Biomass**.
- **Baker's Yeast, Feed Yeast, Yeast Extract, Food Colorants**.
- **Vitamins**.

m.Molds

m.1.General Characteristics and Taxonomy

Molds are microscopic fungi ; like yeasts, they lead a saprophytic lifestyle, developing on inert or decomposing substrates. However, they are distinguished from yeasts by their vegetative structure, which consists of mycelial filaments. Molds are non-photosynthetic and immobile. Their body or thallus comprises two parts : the mycelium (a network of filaments called hyphae) and spores. They are acidophilic (pH between 3 and 7), mesophilic (20 to 30 °C), and some are psychrotrophic (< 15 °C) (Gueroui, 2018).

The classification of molds is based on purely morphological characteristics and the septation of hyphae. They are divided into four main subdivisions :

- **Zygomycetes** : Asexual reproduction is most often ensured by sporocystospores or sometimes by exogenous conidia, while sexual reproduction occurs via zygospores, such as *Mucor*, *Rhizopus*, and *Absidia*.
- **Ascomycetes** : Asexual reproduction occurs through conidia, and sexual reproduction via spores contained in asci (ascospores), such as *Aspergillus fumigatus* and *A. nidulans*.
- **Basidiomycetes** : Asexual reproduction occurs through conidia, while sexual reproduction is via spores borne on basidia (basidiospores), such as *Clitocybe nebularis*.
- **Deuteromycetes** (Fungi imperfecti) : This is a vast group encompassing fungi whose sexual reproduction is unknown or asexual varieties of Ascomycetes. They are classified based on the characteristics of conidial structures and hyphal grouping. *Aspergillus* and

Penicillium, for instance, have rare sexual reproduction and are frequently classified in this group, including *Aspergillus flavus*, *A. niger*, etc.

Equipped with powerful and complex enzymatic systems, molds can alter our foodstuffs, leading to irreversible spoilage. Their biochemical activities also enable them to act as agents of biodegradation, biosynthesis, or bioconversion, transforming low-value, unappealing substrates into nutrient-rich food with pleasant flavors or tasty condiments **(Bourgeois et *al.*, 1996).**

m.2.Role in Food Production

The mold used in the preparation of soft cheeses with white rinds (such as Camembert and Brie) is *Penicillium camemberti*. Its essential role during the aging process of the cheese lies in its ability to consume lactic acid, thereby deacidifying the curds. Additionally, it releases enzymes that alter the development of flavors and aromas in various cheeses. The green mold, *Penicillium roqueforti*, has long been used in the production of Roquefort cheese and other so-called "blue" cheeses **(Gueroui, 2018).**

Molds are also employed in the manufacture of food additives. Thom and Curie (1916) produced citric acid using *Aspergillus niger*. They are further utilized for the production of enzymes (α-amylase, catalase, cellulase, lactase, pectinase, etc.), pigments and vitamins (β-carotene, riboflavin), essential oils, flavors, and fragrances **(Bourgeois et *al.*, 1996).**

m.3.Harmful Effects

However, filamentous fungi can have detrimental effects in the food industry at several levels. Phytopathogenic fungi pose significant challenges to the production of raw food materials, such as fruits and vegetables. For example, *Penicillium expansum* causes blue mold on apples, and the green and blue molds found on citrus fruits are generally *Penicillium digitatum* and *P. italicum*. Saprophytic molds contaminate and degrade food quality. Some of these molds are toxinogenic, releasing mycotoxins into food, which present substantial health risks :

- ✓ *Aspergillus flavus* produces aflatoxins, with aflatoxin B1 being a potent carcinogen.
- ✓ *Aspergillus clavatus* produces patulin, a congestive agent that can cause pulmonary and cerebral hemorrhages.
- ✓ *Fusarium sporotrichioides* and related species produce various toxins, including trichothecenes, zearalenone, and fumonisins. Some of these toxins are highly thermostable (30 minutes at 200 °C) **(Guiraud, 1998).**

Microbial Spoilage of Food and Control Measures

3. Microbial Spoilage of Food and Control Measures

3.1. Factors Influencing Food Spoilage Microflora

Bacteria only grow if their environment is suitable. If conditions are not optimal, they may grow at a slow rate, not at all, or even die, depending on the species and conditions. Various physicochemical characteristics of the food and its environment condition the development of microorganisms **(Cuq, 2007).**

3.1.1. Characteristics Specific to Food

3.1.1.1. Biological Structures

The presence of coverings, shells, skins, etc., provides certain foods with excellent protection against microbial proliferation (e.g., seed coats, fruit skins, nut shells, egg shells, animal skins). The alteration of these natural protections often results in contamination and proliferation. Packaging primarily aims to protect stabilized or non-stabilized food from contamination. It is worth noting that edible packaging exists.

3.1.1.2. Naturally Occurring Antimicrobial Agents

Fresh milk contains lactenins and anti-coliform factors with limited activity duration. Eggs contain lysozyme, which is active against Gram-positive germs. Cranberries contain benzoic acid, which is effective against yeasts and molds ; compounds such as thymol (thyme), eugenol (cloves), and cinnamaldehyde (cinnamon) have antimicrobial activities.

3.1.1.3. Chemical Composition of Food

Microorganisms must find nutrients in food to proliferate.

- **From Carbohydrates** :
    - **Polymers (starch, cellulose)**: Hydrolysis modifies texture.
    - **Dimers and Monomers (sucrose, lactose, glucose, fructose, etc.)**: Fermentation leads to the formation of acids and carbonyl compounds, impacting taste and aroma.
- **From Proteins** :
    - **Polymers (proteins)** : Hydrolysis modifies texture.
    - **Amino Acids** : Decarboxylation, deamination, desulfuration, etc., can lead to modifications in taste and odor, as well as the formation of toxic catabolites.

- **From Lipids** : Oxidation and lipolysis affect taste **(Cuq, 2007).**

3.1.1.4. pH

pH significantly influences the ionic balance of an environment, affecting cell permeability, substrate availability, and extracellular enzymatic activities, to a lesser degree intracellular activities (De Roissart and Luquet, 1994). For a given microorganism, growth rate varies with pH, often peaking at an optimum level. Enzymatic activities sensitive to pH frequently limit microbial growth **(Cuq, 2007).**

Microorganisms with an optimum pH below 5.5, capable of growing at low pH, are referred to as acidophiles. This group includes yeasts and molds and certain bacteria classified based on the type of acid they produce (acetic, lactic, propionic bacteria, etc.) (Table 5). The pH of the food will promote proliferation, especially if it is close to the optimum growth pH. Most pathogenic bacteria cannot grow at pH below 4.5, making acidic foods relatively safe. Indeed, at pH 4.5, the toxin production of *Clostridium botulinum* is halted. Gram-negative bacilli are acid-sensitive and cannot thrive at low pH **(Guiraud, 1998).**

Table 8 : Growth pH of Selected Microorganisms **(Bourgeois et *al.*, 1996)**

MICROORGANISMS	MINIMUM	OPTIMUM	MAXIMUM
Molds	**1.5 - 3.5**	**4.5 - 6.8**	**8.0 - 11.0**
Yeasts	**1.5 - 3.5**	**4.0 - 6.5**	**8.0 - 8.5**
Bacteria	**4.5**	**6.5 - 7.5**	**11**
Acetic bacteria	**2.0**	**5.4 - 6.3**	**9.2**
Lactic bacteria	**3.2**	**5.5 - 6.5**	**10.5**
Lb. plantarum	**3.5**	**5.5 - 6.5**	**8**
Le. cremoris	**5.0**	**5.5 - 6.0**	**6.5**
Le. lactis	**4.1 - 4.8**	**6.4**	**9.2**
Lb. acidophilus	**4.0 - 4.6**	**5.5 - 6.0**	**6.5**

Pseudomonas	**5.6**	**6.6 - 7.0**	**8.0**
P. aeruginosa	**4.4 - 4.5**	**6.6 - 7.0**	**8.0 - 9.0**
Enterobacteria	**5.6**	**6.5 - 7.5**	**9.0**
S. typhi	**04/04/05**	**6.5 - 7.2**	**8-9.6**
E. coli	**4.3**	**6.0 - 8.0**	**9.0**
Staphylococcus	**4.2**	**6.8 - 7.5**	**9.3**
Clostridium	**4.6 - 5.0**		**9.0**
C. botulinum	**4.8**		**8.2**
G. perfringens	**5.5**	**6.0 - 7.6**	**8.5**
C. sporogenes	**5.0 - 5.8**	**6.0 - 7.6**	**8.5 - 9.0**
Bacillus	**5.0 - 6.0**	**6.8 - 7.5**	**9.4 - 10.0**
L. monocytogenes	**04/03/05**	**6.5 - 7.5**	

Table 9 : pH of Some Food Products **(Cuq, 2007)**

Food	**pH**	**Food**	**pH**	**Food**	**pH**
Beef	5.1-6.2	Peas	5.0-6.1	Orange	3.4-4.4
Ham	5.9-6.1	Beans	5.0-6.0	Peach	3.4-4.6
Veal	5.7-6.1	Broad beans	5.0-6.0	Apricot	3.5-4.6
Chicken	6.0-6.4	Brussels sprouts	6.0-6.5	Plum	3.4-4.6
Sausage (Frankfurt)	5.0-6.0	Celery	5.0-5.5	Raisins	3.6-4.1

Fish (general)	6.0-6.5	Carrot	5.8-6.1	Date	3.5-4.8
Cod	6.3-6.5	Lettuce	5.8-6.2	Strawberry	3.4-4.0
Mackerel	6.0-6.2	Olives	4.5-5.5	Raspberry	3.2-3.9
Salmon	6.0-6.3	Parsley	5.0-5.5	Blackberry	3.2-3.8
Crab	6.5-6.9	Cucumber	5.1-5.7	Blueberry	3.1-3.5
Shrimp	6.5-6.9	Potato	5.4-5.9	Grapefruit	3.0-3.6
Ground meat	5.6-6.2	Turnip	5.5-6.0	Soup (meat)	6.0-6.5
Milk	6.4-6.7	Apple	3.9-4.6	Soup (vegetable)	6.0-6.5
Parmesan	5.0-5.6	Tomato	4.3-4.9		
Roquefort	4.7				

3.1.1.5. Water Activity

Microorganisms require available water for multiplication ; the availability of water is characterized by its activity. This parameter corresponds to the ratio of the partial pressure of water in the food to that of pure water (adjusted for activity coefficients) :

Aea u=Peau aliment Peau purea_{eau} = \frac{P_{eau\aliment} {P_{eau\, pure}}aeau=Peau pure.

Peaualiment.

- Aeaua {eau} aeau varies between 0 and 1.
- The relative humidity (H.R.) is equal to aeau×100a {eau} \times 100aeau×100.

Microorganisms capable of growing in products with low aeaua {eau} aeau are termed xerophiles, while those in highly sugary or salty environments are known as osmophiles and halophiles, respectively.

Methods to lower water activity include :

- **Physical** : Freezing, dehydration.
- **Additives** : Salting, sugaring.

These methods lead to frozen, dried foods, cured meats, jams, and candies. For aw<0.65a_{w} < 0.65aw<0.65, no microorganisms can grow (though they may survive). For aw<0.85a_{w} < 0.85aw<0.85, no pathogenic microorganisms can grow, except for certain mycotoxin-producing molds.

The high percentage of microbial mortality observed during dehydration, salting, sugar addition, or freezing is largely due to the reduction in water activity. There are correlations between awa_{w}aw and temperature or composition. At a given temperature, the growth rate of a specific microorganism decreases as awa_{w}aw is lowered. The presence of abundant nutrients increases the awa_{w}aw limits compatible with microorganism survival **(Cuq, 2007).**

Table 10 : Water Activity and Microorganism Growth in Food Products According to FDA **(Nsren, 2010)**

awa_{w}aw Value	**Maximum Limits for Microorganisms**	**Food Products Included in These Values**
1.00 - 0.95	*Pseudomonas*, *Escherichia*, *Proteus*, *Shigella*, *Klebsiella*, *Bacillus*, *Clostridium perfringens*, some yeasts	Highly perishable products (fresh), canned fruits and vegetables, meat, fish, dairy, cooked sausage, baked bread ; foods containing up to 40% sugar or 7% salt
0.95 - 0.91	*Salmonella*, *Vibrio parahaemolyticus*, *C. botulinum*, *Serratia*, *Lactobacillus*, *Pediococcus*, some molds, yeasts (*Rhodotorula*, *Pichia*)	Certain cheeses (cheddar, Swiss, Munster, provolone), smoked meat (ham), some concentrated fruit juices, foods containing up to 55% sugar (saturated) or 12% salt

0.91 - 0.87	Many yeasts (*Candida*, *Torulopsis*, *Hansenula*), *Micrococcus*	Dry sausage (salami), puddings, dry cheeses, margarine, foods containing up to 65% sugar (saturated) or 15% salt
0.87 - 0.80	Most types of molds (*Penicillium*, mycotoxin-producing), *Staphylococcus aureus*, most *Saccharomyces* spp., *Debaryomyces*	Most concentrated fruit juices, sweetened condensed milk, chocolate, maple and fruit syrups, flours, rice, and legumes with 15-17% water; fruitcakes; smoked sausages, fondants
0.80 - 0.75	Most halophilic bacteria, mycotoxin-producing *Aspergillus*	Jams, fruit jellies, marzipan, candied fruits, some marshmallows
0.75 - 0.65	Xerophilic molds (*Aspergillus chevaliers*, *A. candidus*, *Wallemia sebi*), *Saccharomyces bisporus*	Oat flakes with 10% water, nougats, fondants, marshmallows, porridge, molasses, raw sugar, some dried fruits, nuts
0.65 - 0.60	Osmophilic yeasts (*Saccharomyces rouxi*), some molds (*Apergillus echinulatus*, *Monascus bisporus*)	Dried fruits with 15-20% water ; some toffees and caramels; honey
0.5	No high microbial growth	Pasta with 12% water ; spices with 10% water
0.4	No high microbial growth	Powdered eggs with about 5% water

0.3	No high microbial growth	Cakes, dry biscuits, crusty bread, etc., with about 3-5% water
0.2	No high microbial growth	Milk powder with about 2-3% water ; dried fruits with about 5% water, cereal flakes with about 5% water, fruit cakes, rustic cakes, dry biscuits

3.1.1.6. Redox Potential

Depending on their respiration mode, microorganisms can be classified as strict aerobes, strict anaerobes, facultative anaerobes, or microaerophiles.

These Properties Explain the Diversity of Spoilage We May Encounter **:**

- Molds and Aerobic Yeasts : These develop on the surface, forming thicker or thinner films.
- Fermenting Yeasts : They multiply deeper within the food, producing gas.
- Clostridium : These organisms only develop in the absence of oxygen (e.g., in mass or canned foods).
- Pseudomonas : These bacteria only develop in the presence of oxygen (surface growth).
- Microaerophilic Lactobacillus : These grow only with reduced oxygen levels.

In foods, the presence or absence of oxygen can be considered a fundamental parameter regarding microorganisms **(Cuq, 2007).**

3.2. External Parameters Affecting Food

These parameters are closely linked to the characteristics of the food's environment and influence both the stability of the product and the behavior of the microorganisms it contains.

3.2.1. Temperature

Generally, a specific type of bacteria grows more rapidly at a certain temperature, known as the optimal growth temperature. The growth rate decreases when the temperature deviates from this optimum. For all bacteria, there is a maximum and minimum temperature beyond which growth ceases **(Bourgeois et *al.*, 1996)**.

In microorganisms, temperature increases the rate of all reactions, including both anabolism and catabolism ; thus, growth rate tends to increase with temperature, following Arrhenius's law. However, as temperature rises, the rate of denaturation of bacterial proteins (particularly enzymes) also increases. When all metabolically active protein molecules are denatured, the germ's growth rate drops to zero, and often, if enzymes involved in genome expression are inactivated, it dies.

For temperatures below the optimal growth temperature, the rate of reactions involved in metabolism and thus the growth rate declines. However, "cold" does not typically lead to significant denaturation of microbial components, allowing metabolic activities to resume as soon as temperatures approach the optimal growth range. Slow freezing, however, can lead to high mortality rates among microorganisms.

Thus, variations in growth rate based on temperature correspond to the combination of these two phenomena **(Cuq, 2007).**

Different cases can be distinguished. Psychrophilic (or psychrotrophic) microorganisms are capable of growing below 15-20 °C, with some able to thrive at negative temperatures. There are facultative psychrophiles and obligate psychrophiles (strict psychrophiles) ; the latter generally cannot grow at 20 °C. Among facultative psychrophiles, many bacteria from the Gram-negative saprophytic flora (e.g., Achromobacter, Flavobacterium, Pseudomonas, etc.) and pathogenic germs capable of growing between 0 and 10 °C include : Listeria monocytogenes, Clostridium botulinum, Bacillus cereus, Yersinia enterocolitica, Vibrio parahaemolyticus, Aeromonas hydrophila, Plesiomonas shigelloides, and even enteropathogenic E. coli, as well as molds (Cladosporium, Sporotrichum, etc.). Cryophiles can grow at negative temperatures, with an optimal growth temperature of -5 °C. Mesophiles include the majority of microorganisms that develop between 15 and 45 °C (most pathogenic germs belong to this category). Thermophiles can grow above 45 °C, and extreme thermophiles can thrive at 75-80 °C and even higher. Among obligate thermophilic microorganisms are lactic bacteria (Lactobacillus thermobacterium, Streptococcus thermophilus) and spore-forming *bacteria (Clostridium thermosaccharolyticum, Bacillus stearothermophilus). It is important not to confuse thermophily with thermoresistance, which is the ability to withstand a given thermal treatment* ***(Bourgeois et al., 1996 ; Guiraud, 1998 ; Cuq, 2007 ; Djidel, 2017).***

-Cold as a Means of Controlling Microorganism Growth

Cold is a widely used method today to control the growth rate of microorganisms. In a refrigerator, the shelf life of food is approximately 3 to 5 days, a period corresponding to the unfavorable proliferation of psychrophilic germs. Freezing at -18°C completely stabilizes the food, preventing any growth of microorganisms. For meats, no harmful germs develop below 5°C, and when the temperature rises by 5°C, the "shelf life of the product" is halved **(Cuq, 2007).**

3.2.2. Relative Humidity

The relative humidity of the storage environment affects both the water activity of the food (dynamic equilibrium) and the growth of microorganisms on its surface. For example, when a food has a water activity of 0.6, it is essential to avoid conditions of relative humidity in the surrounding atmosphere that could lead to an increase in surface water activity to a level compatible with microbial growth **(Cuq, 2007).**

3.2.3. Presence and Concentration of Gases

The concept of controlled atmosphere is not new. Increasing the carbon dioxide content (up to 10%) and decreasing the oxygen content allows for better preservation of fruits and vegetables (fourth range), delaying the development of certain microorganisms, particularly molds. A nitrogen atmosphere or vacuum packaging helps prevent contamination by aerobic microorganisms **(Cuq, 2007).**

3.2.4. Antimicrobials Produced During Food Manufacturing

These are substances that are either bacteriostatic or bactericidal (e.g., ethanol, organic acids such as lactic, acetic, citric, tartaric, malic acids, etc.). The addition of antimicrobial compounds to food products (as additives) or the use of various antimicrobial agents in the food production environment (disinfectants, cleaning agents, etc.) is regulated and will be addressed in the following course **(Cuq, 2007).**

3.3. Control Measures

The use of antimicrobial agents allows for the control of microorganism development, particularly pathogenic microorganisms and those responsible for food degradation. Methods to combat microorganisms are numerous and can be schematically classified into :

3.3.1. Physical Means

3.3.1.1. Temperature

3.3.1.1.1. Heat

The use of heat is a very common method for destroying microorganisms. Cooking, boiling, and blanching are ancient processes, along with industrial processes such as pasteurization and sterilization, tyndallization, etc. **(Bourgeois et *al.*, 1996).**

a. Pasteurization

Pasteurization involves killing all non-spore-forming pathogenic germs. It results in semi-preserved foods (to be kept cold, except when acidic) and is gentler on food than sterilization (e.g., milk, eggs, fruit juices). It is typically practiced between 60 and 75°C and will be discussed in the course on technological treatments. You will learn that the pasteurization value is the time in minutes at 70°C required to destroy 13 log of *Streptococcus faecalis* **(Corpeta, 2014).**

Table 11 : Pasteurization Standards **(Leyral et Vierling, 2007)**

Food product	**Temperature and time required**
Milk	30 mn to 62mn ^{0}C or 15s to 72 ^{0}C
Cream, Dessert cream	30 mn to 71^{0}C or 16s to 20s to 82^{0}C
Bottled apple juice	30mn to 77 ^{0}C
Carbonated drink made from fruit juice	30mn to 66^{0}C
Beer	1 to 2 mn to 82-88^{0}C

b. Sterilization

Sterilization by heat involves exposing food to a temperature generally above 100 °C for a sufficient duration to inhibit enzymes and all forms of microorganisms, including spore-forming bacteria (destruction of microorganisms in a food product to prevent degradation of its sanitary qualities over time).

Sterilization of the food and its container can be achieved in two ways.

Note : The original text mentions two methods for sterilizing food and its container, which are typically appertization (simultaneous sterilization of the container and content) and aseptic packaging (separate sterilization of the container and content followed by aseptic packaging). However, these specific methods are not detailed in the provided translation request.

b.1.Separate Sterilization of the Container and Content

- ✓ Also Known as Bulk Pasteurization : In this method, the food product (content) is sterilized through heat treatment before being sealed in its container. The container itself is sterilized separately, either by heat or other methods such as ultraviolet light, prior to holding the product.
- ✓ Aseptic Packaging : After sterilization, the product is hermetically sealed in the sterilized container within a controlled environment that prevents contamination by environmental microorganisms. This process is referred to as aseptic packaging.
- ✓ Applications : This technique is primarily used for preserving liquid products (e.g., milk, juice) in packaging materials that cannot withstand traditional appertization processes, such as plastic pouches or cartons.
- ✓ UHT Sterilization : When the product is sterilized at very high temperatures (135 °C to 150 °C) for a short duration (15 seconds to 1 second), it is termed Ultra High Temperature (UHT) sterilization. This method preserves the organoleptic and nutritional qualities of the product but is limited to liquid products like milk **(Piar and Lanoisellé, 2000).**

c. Tyndallization

Tyndallization is a thermal treatment equivalent to repeated pasteurizations, separated by intervals of 12 to 24 hours at temperatures of 30 to 40°C. During pasteurization, only the vegetative forms are inactivated, while during the intervals, most of the heat-resistant spores germinate and become sensitive to subsequent pasteurization. This process is used for fragile culture media.

The operation involves heating the medium to 60°C or 70°C for 30 minutes to 1 hour, repeated three times consecutively, with a 24-hour interval between each heating cycle **(Prescotte et *al.*, 2010)**

d. Thermization

Thermization is a mild form of pasteurization. Its primary objective is to destroy pathogenic bacteria that may be present in the product without significantly altering its technological characteristics. Thermization is a thermal treatment applied to raw milk.

Key Features of Thermization :

- Temperature Range : Typically between 57°C and 68°C.
- Duration : The milk is heated for at least 15 to 20 seconds.
- Purpose : To reduce the microbial load, particularly pathogenic bacteria, while preserving the milk's natural properties as much as possible.
- Difference from Pasteurization : Thermization is less intense than pasteurization and does not fully inactivate enzymes like phosphatase, which remains active after the process

  This is a milder form of pasteurization. Its main objective is to destroy pathogenic bacteria that may be present in the product, without significantly altering its technological characteristics. Thermization is a thermal treatment applied to raw milk. The milk must be heated to a temperature of at least 63ºC for 16 seconds **(Cuq, 2007).**

e. Dry Heat.

The material to be sterilized is placed in an electric or gas oven (Pasteur, Poupinel ovens) at 180°C for 1 hour or at 160°C for 2 to 3 hours. There is oxidation of cellular components and denaturation of proteins. This method does not corrode glassware or surgical metal instruments and allows for the sterilization of powders and oils **(Meyer et *al.*, 1984).**

3.3.1.2. Cold (Freezing, Refrigeration, Lyophilization)

- -Refrigeration : Lowering the temperature to a value below room temperature but above the freezing point of the product (water remains liquid). It :

  - Reduces the growth rate (increases the doubling time).
  - Decreases the startup rate (increases the lag phase).
  - Significantly increases the preservation of food (for example, meat lasts 1 day at 22°C but 10 days at 0°C).
  - Selects psychrophilic bacteria (e.g., although "slow," Pseudomonas and Listeria thrive in the fridge).
  - Forces bacteria to adapt (but this process is slow) (increased polyunsaturated fatty acids in membranes, pigments, Pseudomonas lipases in milk).

- Freezing : Lowering the temperature to a value below the freezing point (zero degrees in pure water, but for meat: <-1.4°C). It :
  - Stops bacterial growth (free water disappears, membrane lipids become solid).
  - Kills some bacteria (9 out of 10 Gram-negative cells) : partial and selective lethal effect (cocci and Gram-positive bacteria are more resistant) **(Corpeta, 2014).**
- Lyophilization : Sublimation of water at low temperatures ; it is a method of vacuum drying at low temperatures for previously frozen liquid products (solid phase) by transitioning directly to the vapor phase, without passing through the liquid phase. This change of state is called sublimation **(Ait Abdelouahab, 2001).**

-How does freezing kill certain bacteria ?

- Spores are very resistant, Gram-positive cocci are also quite resistant, while Gram-negative bacilli are generally less so.
- Cells in the growth phase are more sensitive than those in the lag phase.

Destruction occurs at the moment when the water freezes. After that, the number of bacteria remains stable.

- Slow freezing kills more bacteria than very rapid freezing (a "flash" freezing is done in liquid nitrogen to preserve strains).
- Certain substances protect bacteria = cryoprotectants (starch, sugar, milk, glycerol).
- What kills (1) : Disruption of permeability due to solidification of membrane lipids.

- What kills (2): Alteration of the saline concentration of the medium because ice crystals are made of pure water. The unfrozen water therefore contains all the salts, raising its osmotic pressure.
- What kills (3): Mechanical action of ice crystals that crush or puncture the cells **(Corpeta, 2014).**

Freezing at -18°C and quick freezing (-40°C and even -80°C) allows for total stabilization against microorganisms and leads to varying degrees of mortality depending on the nature of the germs and the cooling rate.

3.3.1.3. Radiations.

Solar radiation, or more specifically ultraviolet radiation, is a valuable natural sterilizing agent. The main types of radiation are electromagnetic, electronic, and sonic **(Gueroui, 2018).**

3.3.1.3.1 Electromagnetic Radiation (Ionizing).

These are characterized by their wavelength. Their action on microorganisms has dual significance : food preservation (sterilization) and understanding their mode of action on bacteria can be translated to what is observed in human cells. Irradiation is a very broad generic term that covers the range of wavelengths from infrared to cosmic rays. Ionizing irradiation results from the use of UV radiation but especially X and γ rays, and it is unrelated to radioactivity **(Cuq, 2007).** The wavelengths of electromagnetic radiation are as follows :

-Infrared (> 800 nm), visible (400 to 800 nm), Ultraviolet (≤ 400 nm): these are also the least effective since their wavelength is larger. UV rays are the most commonly used despite their low efficiency. The most active region of their emission spectrum is located between 260 and 270 nm. Their penetration is halted by even the slightest particle of suspended matter.

Rayons X et γ qui libèrent une énergie plus importante et qui pénètrent aussi plus profondément dans la matière. Ces rayonnements agissent essentiellement au niveau de l'ADN. Industriellement, on distingue **(Gueroui, 2018) :**

- ✓ Radappertisation : dose d'irradiation comprise entre 20 et 50 kGy (kilo Gray). Correspond à la stérilisation.
- ✓ Radicidation : dose appliquée suffisante pour éliminer les bactéries pathogènes et la flore d'altération à l'exception des bactéries sporulées. Les doses employées sont inférieures à 10 kGy.

- ✓ Radurisation : doses en général comprises entre 1 et 5 kGy. Ce traitement peut s’appliquer aux denrées emballées.

-Les doses de réduction en KGy permettant de réduire de 90% le nombre de germes vivants sont indiquées, pour certains microorganismes, dans le tableau ci-dessous.

Table 12 : Actions of Radiation on Microorganisms **(Cuq, 2007).**

Microorganism	Dose (KGy)	Microorganism	Dose (KGy)
Pseudomonas aeruginosa	**0,1**	***Staphylococcus aureus***	**0,8 - 1,9**
Lactobacillus	**0,1 - 0,2**	***Clostridium botulinum type E***	**1,2 - 3**
Escherichia coli	**0,15 - 0,3**	***Spores de Cl. botulinum***	**3,5 - 5**
Shigella sp	**0,25 - 0,4**	***Micrococcus radiodurans***	**5 - 8**
Salmonella sp	**0,5 - 1**	***Levures***	**0,8 - 1,2**
Streptococcus faecalis	**0,75 - 1**	***Moisissures***	**0,4 - 1,3**
Moraxella	**0,8 - 1,3**	***Poliovirus***	**14**

3.3.1.3.2. Electronic Radiation.

A continuous emission of electrons accelerated at high speed has a sterilization power identical to that of rays. Electrons can be directed precisely to the desired target, but their penetration ability is significantly weaker than that of γ rays. Their main drawback is the alteration of organic substances subjected to their action **(Gueroui, 2018)**.

3.3.1.3.3. Sonic Radiation.

Ultrasound has the ability to kill microorganisms suspended in a liquid by releasing their intracellular contents through a "mechanical" vibration effect. The application domain includes a wide range :

- Preservation of grains, dried fruits, and vegetables (0.4 to 1 kGy) ;
- Extension of the shelf life of fresh fruits and vegetables (low doses) ;
- Preservation and improved hygiene of meats and fish ;
- Treatment with ionizing radiation can be coupled with other preservation methods (e.g., in the case of processed meat products, it helps reduce the amount of added nitrates) **(Gueroui, 2018).**

3.3.1.4. Bactofugation and Filtration.

3.3.1.4.1. Sterilizing Filtration.

This process is known as cold sterilization. Sterilizing filtrations can be performed using porous organic supports (derived from cellulose, polyamide, Teflon, etc.) or mineral supports (filters made of asbestos, alumina, porcelain, sintered glass, etc.), where the size of the "pores" is perfectly controlled and is smaller than that of most microorganisms to be retained (diameter ≤ 0.5 µm). The oldest filter is the Chamberland filter or candle filter, which uses the properties of unglazed porcelain. Currently, sintered glass filters, diatomaceous earth filters (Berkefeld filters), or cellulose acetate membrane filters are used.

However, these methods are limited to liquids with low organic matter content in suspension (such as beer, wine, and certain fruit juices). They have the advantage of not altering the organoleptic qualities, except in some cases where retention of aromatic compounds on the support occurs **(Gueroui, 2018).**

3.3.1.4.2. Bactofugation.

Centrifugation above 5000 g allows for a reduction in microbial load. In the dairy industry, this process is referred to as bactofugation, which is a centrifugal purification of milk at high speed and at pasteurization temperature, enabling the elimination of spore-forming bacteria. For non-sterilizable milk :

- At 60°C, 95% of spores are eliminated through a reaction with agglutinins associated with fat globules, resulting in their presence in the lighter phase.
- At 80°C, agglutinins are quickly denatured and lose their activity within about ten minutes ; 98 to 99% of spores are then eliminated in the sediment **(Meyer et *al.*, 1984; Cuq, 2007).**

3.3.2. Chemical Means.

Not all chemical compounds with antimicrobial effects can be used as antiseptics or disinfectants. Some, such as fluorides or cyanides, are potent cellular poisons whose toxicity prohibits their use. Others, such as antibiotics and sulfonamides, are treated separately due to their therapeutic role **(Cuq, 2007).**

3.3.2.1. Antiseptics, Disinfectants, Food Preservatives, and Antibiotics.

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

3.3.2.1.1. Oxidizing Agents.

a. Hydrogen Peroxide.

Hydrogen peroxide (or H_2O_2) is an effective antiseptic. At 3% (10 volumes) in aqueous solution, it serves as a good disinfectant. Its use is limited due to its rapid decomposition. It is an odorless, colorless compound. It leads to the oxidation of cysteine and methionine residues in proteins, causing them to lose their biological function. Other substances are also oxidized under these conditions : peroxides appear in unsaturated lipids ; certain vitamins (especially A, D, C, and B1) are oxidized during this treatment **(Cuq, 2007).**

b. Chlorine and Its Derivatives.

Chlorine and especially its chemical derivatives are some of the most common antiseptics or disinfectants. They are used for the treatment of drinking water, swimming pools, disinfection of premises, contaminated objects, etc.

For disinfecting surfaces and equipment in industries, sodium hypochlorite is generally used at doses of 100 to 300 ppm of active chlorine. Antiseptic preparations of chlorine derivatives, such as Labarraque's or Dakin's solutions, are partially prepared with a dilution of hypochlorite.

Chloramines (such as chloramine T) provide more lasting actions than those produced by hypochlorites, but their effectiveness is lower. They are primarily used for disinfecting swimming pool water (Cuq, 2007).

c. Iodine and Its Derivatives.

Iodine is one of the oldest disinfectants. It is both bactericidal and fungicidal. Although it is poorly soluble in water, it is easily soluble in alcohol or aqueous solutions of potassium or sodium iodides. Iodo-iodide solutions (iodine and iodide) or iodine tinctures, such as Lugol's solution, are used to disinfect superficial wounds.

Certain detergents can solubilize iodine and serve as a support for it : iodophores. Iodophores are effective at 150 ppm of iodine. The alcoholic solution (50%) of iodine at 1500 ppm exhibits a very clear sporicidal activity **(Cuq, 2007).**

3.3.2.1.2. Heavy Metals and Their Salts.

Some metals have significant microbicidal effects. The most commonly used heavy metal salts are those of silver, mercury, copper, zinc, and gold. Their effectiveness is greater than that of the corresponding metals. They inactivate the cell by precipitating protein molecules, particularly those with enzymatic activity, or by combining with SH groups.

Silver and some of its derivatives are used for sterilizing swimming pool water and preparing antiseptic dressings **(Cuq, 2007).**

3.3.2.1.3. Alcohols.

Alcohols exhibit bactericidal and fungicidal effects but are not sporicidal. Ethanol shows maximum antiseptic effectiveness at dilutions around 50%. Methanol is less active and more harmful, acting as an inhibitor of brain monoamine oxidase, leading to irreversible damage.

Higher alcohols (propyl, butyl, amyl) have increasing bactericidal power with higher molar mass, but their solubility in water decreases accordingly, which limits their use. The effectiveness of alcohols is enhanced when combined with formaldehyde (final concentration of 10%) or sodium hypochlorite (2000 ppm).

Alcohols (ethanol and propanol) are effective at concentrations around 70%. They are not effective against fungal spores and are quickly inactivated by proteins **(Cuq, 2007).**

3.3.2.1.4. Phenols.

Phenol is bactericidal and fungicidal but has low activity against spore forms. Its action increases in the presence of sodium or potassium salts and decreases in the presence of caustic soda and organic matter.

3.3.2.1.5. Soaps and Detergents.

3.3.2.1.5.1. Soaps.

The antiseptic power of soaps varies depending on the microbial species. Their action is primarily mechanical. They reduce surface tension and increase the wetting ability of water. Germs are eliminated through rinsing.

3.3.2.1.5.2. Detergents

- **Anionic Detergents** : Such as sodium lauryl sulfate (SDS), which generally have low antimicrobial activity.
- **Cationic Detergents** : These are primarily quaternary ammoniums, which exhibit the most pronounced effect and are bacteriostatic at low concentrations **(Cuq, 2007).**

3.3.2.1.6. Dyes.

Dyes are substances with highly variable antiseptic power. They are used only for local applications and allow for the creation of selective culture media in analytical microbiology.

Table 13 : Action of Certain Dyes **(Cuq, 2007).**

Family	**Dye**	**Use**
Thiazines	Methylene Blue	Weak antiseptic
Triphenylmethane	Malachite Green	Wound disinfection
	Brilliant Green	Urinary antiseptic
	Methyl Violet	
	Gentian Violet	
Acridine	Trypaflavine	Local disinfectants
	Gonacrine	

3.3.2.1.7. Food Preservatives.

A preservative is an additive incorporated into a food product to slow the development of its microbial flora. Generally, these are compounds used in low doses to avoid any toxicological risk. Under these conditions, most are only bacteriostatic.

3.3.2.1.7.1. Mineral-Based Preservatives

a. Sodium Chloride (NaCl).

Its main effect on bacteria is to inhibit growth by reducing water activity (Aw), particularly on Gram-negative bacilli. Only halophilic microorganisms can thrive in salted food. Salting is one of the preservation methods for charcuterie products, which are immersed in brine **(Gueroui, 2018 ; Corpet, 2014).**

b. Nitrates and Nitrites.

Nitrates (NO_3) and nitrites (NO_2) of Na or K (E250 and following) are used in salting (max 150 ppm) to inhibit *Clostridium botulinum* (spore germination and growth) and to provide a nice red color and good flavor when combined with myoglobin. Cases of botulism have become rare since this additive has been used. The inhibitory action is due to nitrites. Nitrates are effective because they are reduced to nitrites by bacteria possessing nitrate reductase. The use of nitrite is generally coupled with that of salt in cured meats. It is also used on certain cheeses (Holland) to inhibit the germination of gas-producing clostridia that can cause spoilage. The antibacterial mode of action is not well understood **(Corpet, 2014).**

c. Sulfur Dioxide (SO_2) and Sulfites (SO_3).

Their use is widespread in winemaking. Compounds like sodium sulfite (E220) inhibit many bacteria and molds, while yeasts are more resistant **(Corpet, 2014)**. They are used for the preservation of various preparations based on fruit juices, peaches, peeled tomatoes, fruit concentrates, nuts, dried fruits, etc. **(Gueroui, 2018)**.

3.3.2.1.7.2. Organic-Based Preservatives

a. Organic Acids

-Saturated Organic Acids

By acidifying the food, which results from their addition, they are detrimental to many microorganisms. For example, acetic acid and acetates (E260) are used to preserve onions, pickles, and fish marinades in vinegar.Due to the inhibitory action of their ionized form, this is the case for calcium propionate (E280), which acts as an antifungal agent in plastic-wrapped pastries, with no effect on baker's yeast **(Gueroui, 2018 ; Corpet, 2014)**.

-Unsaturated Organic Acids.

The presence of a double bond increases the antimicrobial activity of organic acids. The main representative of this group is sorbic acid (E200) and its potassium, sodium, or calcium salts. Its action extends, to a lesser degree, to yeasts and spore-forming bacteria, including *C. botulinum*. It is used to preserve fats (butter, margarine, mayonnaise), fruit juices, baked goods, and pastries **(Gueroui, 2018).**

b. Benzoic Acid and Its Derivatives.

Unlike the previous acids, benzoic acid (E210) in its ionized form (thus in an acidic medium) is primarily active against bacteria and yeasts, with its inhibitory action on molds being less pronounced.

c. Other Organic Acids.

The bacteriostatic properties of citric acid (E330), ascorbic acid (E300), and tartaric acid (E334) are utilized in many products to lower pH, alongside lactic acid (E270) for fermented products **(Gueroui, 2018; Corpet, 2014)**.

3.3.2.1.7.3. Condiments and Spices.

Thyme, mint, pepper, cloves, garlic, onion, lemon, and olive oil contain antimicrobial molecules. Their effect is not very strong, but it complements other factors. They are often powerful antioxidants, preventing rancidity **(Corpet, 2014).**

3.3.2.1.8. Antibiotics.

Some antibiotics are used as food preservatives : nisin, subtilin, tylosin, primycin, certain tetracyclines, and polymyxin. Those that are effective against spores are particularly interesting for non-sterilizable products **(Cuq, 2007).**

a-Nisin : Produced by *Lactococcus lactis*, it is added to cheeses in the USA to combat *C. perfringens* and *Listeria* **(Corpet, 2014).**

b-Subtilin : Produced by *Bacillus subtilis*, it is active against Gram-positive bacteria and some Gram-negative bacteria. Its sporostatic activity makes it useful in the canned food industry.

c-Tylosin : Produced by *Streptomyces fradiae*, it is effective against Gram-positive bacteria, some Gram-negative bacteria, and alcohol-acid-resistant bacilli. Its sporicidal power allows its use in canning **(Cuq, 2007).**

d-Tetracycline : Produced by a bacterium of the genus *Streptomyces*, it is active against various bacterial infections, including Gram-positive, Gram-negative, and anaerobic bacteria, as well as other microorganisms such as *Chlamydia*, *Mycoplasma*, and *Rickettsia*. In Israel, tetracycline was added to strawberries **(Corpet, 2014).**

REFERENCES

A

- Adriouch, S, Julia, C, Kesse-Guyot, E, Ducrot, P, Péneau, S, Méjean, C, Assmann, K,E, Deschasaux, M, Hercberg, S, Touvier, M, Fezeu, L, K. 2017. Association between a dietary quality index based on the food standard agency nutrient profiling system and cardiovascular disease risk among French adults. *International Journal of Cardiology*. 234(1) : 22–27.
- Agence Régionale de Santé d'Auvergne (ARSA). 2015.La qualité de l'eau destinée à la consommation humaine en auvergne.
- Ait Abdelouahab, N, 2001. Microbiologie alimentaire. Alger. Office des Publications Universitaires. Algérie.
- Alsina, M, Blanch, A,R.1994. "A set of keys for biochemical identification of environmental Vibrio species», J. Appl. Bacteriol. V.76, 79–85. doi:10.1111/j.1365-2672.1994.tb04419.x.
- Amiot-Carlin, M, J, Georgé, S, 2017. Qualités nutritionnelles des produits végétaux : le cas des fruits et légumes. *Agronomie, Environnement & Sociétés*. 7(1) : 43–49.
- Aubry P, Gaüzère B, A, 2020. Les salmonellosis. Cours médecine tropicale, Centre René Labusquière, Institut de Médecine Tropicale, Université de Bordeaux, 33076 Bordeaux, France, 9 p.
- Arcusa, R, Carrillo, J,Á, Xandri-Martínez, R, Cerdá, B, Villaño, D, Marhuenda, J, ZafrillaM.P, 2021. Effects of a Fruit and Vegetable-Based Nutraceutical on Biomarkers of Inflammation and Oxidative Status in the Plasma of a Healthy Population : A Placebo- Controlled, Double-Blind, and Randomized Clinical Trial. *Molecules (Basel, Switzerland)*. 26(12) : 3590-3604.

B

- Bag, P,K, Nandi, S, Bhadra, RK, Ramamurthy, T, Bhattacharya, S.K, Nishibuchi, M, Hamabata, T, Yamasaki, S, Takeda, Y, Nair, G.B.1999. "Clonal diversity among recently rmerged strains of Vibrio parahaemolyticus O3:K6 associated with pandemic spread», J. Clin. Microbiol. V. 37. 2354–2357.
- Baker-Austin, C, Stockley, L, Rangdale, R and Martinez-Urtaza, J. 2010 : Environmental occurrence and clinical impact of Vibrio vulnificus and Vibrio parahaemolyticus : a European perspective. (en anglais) Environmental Microbiology Reports, 2: 7–18. doi:10.1111/j.1758- 2229.2009.00096.x

Balikó, G, Vernyik, V, Karcagi, I, Györfy, Z, Draskovits, G, Fehér, T., Pósfai, G. 2018.Rational efforts to streamline the *Escherichia coli* genome. *Synthetic Biology : Parts, Devices and Applications*, 49-80.

Bazinet L., Castaigne F. (2011). Concepts de génie alimentaire. Procédé associés et applications à la conservation des aliments. Université LAVAL. Ed. Tec & Doc, Lavoisier, Paris. ISBN. pp 571.

Beaz-Hidalgo, R, Balboa, S, Romalde, J,L, Figueras, M,J.2010. ”Diversity and pathogenecity of Vibrio species in cultured bivalve molluscs», Environ. Microbiol. Rep. V. 2. 34–43. doi:10.1111/j.1758-2229.2010. 00135.x

Ben-Haim, Y, Rosenberg, E.2002. “A novel Vibrio sp. pathogen of the coral Pocillopora damicornis», Mar. Biol. V. 141. 47–55. Doi :10.1007/s00227-002-0797-6.

Bergeron, N, 2009. Caractérisation phénotypique et génotypique d’isolats de *Salmonella Typhimurium* provenant de porcs sains ou septicémiques. Thèse Philosophiae Doctor (Ph.D.) en sciences vétérinaires option microbiologie, Faculté de médecine vétérinaire, Université de Montréal. 263 p.

Bottazzi, v, 1988. An introduction to rod-shaped lactic bacteria, Biochimie. 70:303-315.

Bolotin, A, Wincker, P, Mauger, S, Jaillon, O, Malarme, K, Weissenbach, J, Ehrlich, S,D, Sorokin, A. 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis ssp. Lactis* IL1403. Genome Res. 11, 731-753.

Boudjemaa, N. 2016. Microbiologie alimentaire, Polycopie du cours Licence-Master, Département de Biologie et Physiologie Cellulaire, Université de Blida 1. 63 p.

Bourel, G, Henni, S, Krantar, K, Oraby, M, Divies, C, Garrmyn, D.2001. Métabolisme sucre-citrate chez *Lenconostoc mesenteroides*. Le lait 81 : 75-82.

Bourgeois, C, M, Mescle, J, L, Zucca, J. 1996. Microbiologie Alimentaire. Tome 1. 2éme édition. Lavoisier, Paris. 672 p.

Brat, P, Reynes, M, Brillouet, J.-M. 2003. Valeur-santé des aliments : les caroténoïdes : nature, sources et propriétés. *Journal of Agricultural and Food Chemistry.* 51 : 3442-3447.

- BfR .2022 : Bakterielle Lebensmittelinfektionen durch Vibrionen : Gesundheitliche Bewertung zum Vorkommen von Vibrio spp. (Nicht-Cholera-Vibrionen) in Lebensmitteln, prise de position n° 011/2022 du BfR du 13 avril 2022 (en allemand)

- Bycroft, B, W. 1988. Dictionnary of antibiotics and related substances. London : Chapman and Hall, 944 p.

C

- Casadevall, A, Pirofski, L. 1999." Host-pathogen interactions : redefining the basic concepts of virulence and pathogenicity". Infect. Immun. V. 67. 3703–3713.

- Catteau, M. 1996. Microbiologie alimentaire Tome 1, aspect microbiologique de la sécurité et de la qualité des aliments, Edité par : Bourgeois C.M., Mescle J.F., Zucca J., TEC & DOC, Paris, 672 p.

- Chater, K, F. 2006. *Streptomyces* inside out : a new perspective on the bacteria that provide us with antibiotics. Phil. Trans. R. Soc. B, 361, 761-768 p.

- Chen, C, K, Wu, Y, Chang, C, Chang, H, Tsai, T, Liao, Y, Liu, H, Chen, A, B, Shen, J, Li, T, Su, C, Shao, C, Lee, L, Hor, and Tsai. S. 2003. "Comparative Genome Analysi of Vibrio vulnificus, a Marine Pathogen". Genome Res. V. 13. 2577-2587.

- Chocat, B, Levi, Y, Brelot, E. 2015. L'eau du robinet est-elle différente de l'eau en bouteille ? .Méli-Mélo. Démêlons les fils de l'eau.

- C.I.Eau, Centre d'Information sur l'Eau. 2004. Guide des ressources sur le thème l'eau, Document pédagogique sur l'eau potable. P. 2-8.

- Collin JJ.2004. Les eaux souterraines : connaissance et gestion, Ed BRGM Paris, P.17. 18,27-31.

- Commission du Codex Alimentarius. (2007). *L'Eau*. Première Edition, Rom.

- Corbel, M, J. 2006. Food and Agriculture Organization of the United Nations, W. H. Organization and W. O. f. A. Health. Brucellosis in humans and animals.

- Court L.1986. Le coût Economique et sociale de la pollution de l'eau, Tome 1 : les Micropolluants et la Législation. Ed. Lavoisier - Tec. Et Doc., Paris. P.83-104.

- Corpeta, D. 2014. Ecologie Microbienne, ENVT Cours HIDAOA poly, France. 27 p.

Corpetb, D. 2014. Bases scientifiques et techniques de la conservation des DAOA : autres procédés de conservation, Autres moyens d'inhiber & détruire les bactéries des aliments. ENVT Cours HIDAOA poly - A3 - Bases scient. & tech. Conserv. DAOA, France. 14 p.

Cunha, M, P, V, Saidenberg, A, B, Moreno, A,M, Ferreira, A, J, P, Vieira, M, A, M, Gomes, T, A, T., Knöbl, T. 2017. Pandemic extra-intestinal pathogenic *Escherichia coli* (ExPEC) clonal group O6-B2-ST73 as a cause of avian colibacillosis in Brazil. *PLoS One*. *12*(6).

D

Daniels, N, A, MacKinnon, L, Bishop, R, Altekruse, S, Ray, B, Hammond, R,M, Thompson, S, Wilson, S, Bean, N,H, Griffin, P,M, Slutsker, L.2000. "Vibrio parahaemolyticus Infections in the United States, 1973–1998», *J. Infect. Dis*. V. 181.1661–1666. Doi :10.1086/315459.

De Carli, S, Ikuta, N, Lehmann, F, K, M, da Silveira, V, P, Melo, Predebon, G, Fonseca, A, S, K, Lunge, V, R 2015. Virulence gene content in *Escherichia coli* isolates from poultry flocks with clinical signs of colibacillosis in Brazil. *Poultry science*. *94*(11), 2635-2640.

Dedet, J, P. 2007. La Microbiologie, de ses origines aux maladies émergentes. Dunod, Paris. 289 p.

Dejardins R.1990. Le traitement de l'eau, 2eme Ed. Ecole polytechnique de Montréal,

Denis, F, Ploy, M, C, Martin, C, Bingen, E, Quentin, R. 2011. Bactériologie médicale : Techniques usuelles. 2^{eme} édition. *Issy-les-Moulineaux : Elsevier Masson*.

De Roissart, H, Luquet, F, M. 1994. Bactéries lactiques. Aspects fondamentaux et appliqués, Vol 1. Lorica (ed). Uriage/ France.

Desalme, A, Quilliot, D, Ziegler, O. 2004. Les catégories d'aliments. Cah. Nutr. Diét.. 39, 3, 217-228.

Dess, Q. 1999. Sociologie de l'alimentation, Edition 2000, Paris (France). 121 p.

Djidel, Saliha. 2017. Production des métabolites, Polycopie du cours Biochimie appliquée, Université Ferhat Abbas de Sétif. 72 p.

Dho-moulin, M, Fairbrother, J, M. 1999. Avian pathogenic *Escherichia coli* (APEC).
Veterinary Research. 30,299-316.

Dorel, C, Lejeune, P, Panoff, J, M. 2020. Microbiologie, Encyclopædia Universalis [en ligne], consulté le 19 juin 2020. URLhttp://www.universalis.fr/encyclopedie/microbiologie.

Dreher, M, L. 2018. Whole Fruits and Fruit Fiber Emerging Health Effects. *Nutrients*. 10(12): 1779-1833.

Duchêne-Massias, A. 2015. Valorisation fonctionnelle et antioxydante des épidermes de pommes Golden Delicious. Thèse, Université de Bordeaux (Bordeaux, France). 265 p.

Dupin, H, Cuq, J,L, Malewiak, M,I, Leynaud, R,C, Berthier, A,M. (1992). Alimentation et nutrition humaines. Paris : ESF éditeur. pp 1533.

Duriez, P, Clermont, O, Bonacorsi, S, Bingen, E, Chaventre, A, Elion, J, Denamur, E. 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. *Microbiology*. *147*(6), 1671-1676

E

Ebabhi, A, Adebayo, R. 2022. Nutritional Values of Vegetables. *Ertan Yildirim and Melek Ekinci*, Erzurum, Turquie, 292 p.

Eichholzer, M, Lüthy, J, Gutzwiller, F, Stähelin, H, B. 2001. The role of folate, antioxidant vitamins and other constituents in fruit and vegetables in the prevention of cardiovascular disease : the epidemiological evidence. *International Journal for Vitamin and Nutrition Research.*71(1) : 5-17.

Enjalbert, F. 1993. Alimentation et composition du lait de vache. Point vet. 25 (156): 769-778.

F

FAO. 2020. Fruits et légumes pour la santé. Rapport de l'atelier conjoint FAO/OMS, Japon.52 p

FAO. 2021. Fruits et légumes : éléments essentiels de ton alimentation. Année internationale des fruits et des légumes, Rome, Italy. 86 p.

FAO/WHO.2002. "Food Safety Consultation, Risk assessment of Campylobacter spp in broiler chickens and Vibrio spp. in seafood», Report of a Joint FAO/WHO Expert Consultation, Bangkok-Thailand. 59.

Farto, R, Armada, S,P, Montes, M, Guisande, J,A, Pérez, M.J, Nieto, T,P.2003. "Vibrio lentus associated with diseased wild octopus (Octopus vulgaris)», J. Invertebr. Pathol. V. 83. 149–156.

- Favier, J,C, Ireland, R,J, Toque, C, Feinberg, M. 1995. Répertoire général des aliments. Table de composition des aliments. CIQUAL INRA, 2e éd, Éditions Lavoisier- Tec & Doc, Paris. 897 p.
- Federighi, M. 2005. Bactériologie alimentaire compendium d'hygiène des aliments. 2ed. Economica. Paris. pp 224-233.
- Festy, B, Hartemann, P, Lerdrans, M, Partick, L. 2003.Qualité de l’eau. In : Environnement et santé publique- Fondement et pratiques. Chapitre 13, p333-368.
- Fournaud, J. 1982. Type de germes rencontrés aux différents stades de la filière. In : Hygiène et technologie de la viande fraîche. Centre national de la recherche scientifique Editeur, Paris. pp 353.
- Frans, I, Michiels, C,W, Bossier, P, Willems, K,A, Lievens, B and Rediers, H.2011. “*Vibrio anguillarum* as a fish pathogen : virulence factors, diagnosis and prevention“, *J. Fish Di*, V. 34**,** n° 9, 643–661.
- Fournier et Quilici.2002. “Infections à Vibrions non cholériques”, Encycl Méd Chir, Editions Scientifiques et Médicales Elsevier SAS, Maladies infectieuses. 8-026-F- 15, Paris, 7.

G

- Garry, P et Le guern, L. 1999. Les bactéries lactiques. Bull. Liaison. CTSCCV. 9, (6): 423-429.
- Gay, M, Renault, T, Pons, A.M, Le Roux, F.2004. “Two Vibrio splendidus related strains collaborate to kill Crassostrea gigas : taxonomy and host alterations», Dis. Aquat. Organ. V. 62. 65–74. Doi :10.3354/dao062065.
- Gerard, G, Philippe, H. 2014.Eaux et santé. Hegel .Vol 4, N°3.3p.DOI :10.4267/2042/54108
- Gollucke, A, P, B, Peres, R, C, Odair, A,N, Ribeiro, D, A. 2013. Polyphenols : a nutraceutical approach against diseases. *Recent Patents on Food, Nutrition & Agriculture*. 5(3) : 214- 219.
- Gottlieb, D. 1973. General consideration and implication of actinomycétales in actinomycétale caractiristics and pratical importance edited by G syks and F.A skinner. academic press, London newyork.
- Grasselli, E, François, P, Gutacker, M, Gettler, B, Benagli, C, Convert, M, Piffaretti, J, C. 2008. Evidence of horizontal gene transfer between human and animal commensal *Escherichia coli* strains identified by microarray. *FEMS Immunology & Medical Microbiology*. *53*(3), 351-358.

Gabriel, I, Mallet, S, Sibille, P. 2005. La microflore digestive des volailles : facteurs de variation et conséquences pour l'animal. *INRA Productions Animales*. *18*(5), 309-322.

Grigoraş, C, G. 2012. Valorisation des fruits et des sous-produits de l'industrie de transformation des fruits par extraction des composés bioactifs. Thèse de Doctorat en Chimie, Faculté des Sciences et Technologie, Université d'Orléans (Orléans, France), 235 p.

Guabiraba, R, Schouler, C. 2015. Avian colibacillosis : still many black holes. *FEMS microbiology letters*. *362*(15).

Gueroui, Y. 2018. Aspect Microbiologique de la Sécurité et de la Qualité, Polycopie du cours Qualité des produits et sécurité alimentaire, Université 8 mai 1945-Guelma, 105 p.

Guiraud, J, P. 1998. Microbiologie alimentaire, DUNOD. Paris. 652 p.

H

Harir, M, Bellahcen, M, Baratto, M, C, Pollini, S, Rossolini, G, M, Trabalzini, L, Fatarella, E, Pogni, R. 2018. Isolationand characterization of a novel tyrosinase produced by sahara soil actinobacteria and immobilization on nylon nanofiber membranes. Journal of Biotechnol. 10; 265:54-64.

Hejnova, J, Dobrindt, U, Nemcova, R, Rusniok, C, Bomba, A., Frangeu L., Buchrieser, C. 2005. Characterization of the flexible genome complement of the commensal *Escherichia coli* strain A0 34/86 (O83: K24: H31). *Microbiology*. *151*(2), 385-398.

Hillel D.1988. L'eau et le sol : principe et processus physiques, 2ène Ed ACADEMIA Louvain-la-Neuve, P.143.

Holzapfel, W,H, Haberer, P, Geisen, R, Bjorkroth, J, Chillinger, U. 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. Am J Clin Nutr (73): 365S-373S.

Horseman, M.A, Surani, S.2011. "A comprehensive review of Vibrio vulnificus : an important cause of severe sepsis and skin and soft-tissue infection". Int. J. Infect. Dis. V. 15, n° 3. 157–166. doi:10.1016/j.ijid.2010.11.003.

Huerta, M, G, Roemmich, J,N, Kington, M, L, Bovbjerg, V,E, Weltman, A, L, Holmes, V,F, Patrie, J,T, Rogol A,D, Nadler, J,L. 2005. Magnesium Deficiency Is Associated With Insulin Resistance in Obese Children. *Diabetes Care*, 28(5) : 1175–1181.

I

ICMSF.1996. International Commission on Microbiological Specifications for Foods., “Characteristics of Microbial Pathogens.London”. ICMS. Microorganisms in Foods Blackie Academic and Professional, V. 5.

J

Jayasree, L, Janakiram, P, Madhavi, R.2006. “Characterization of Vibrio spp. Associated with diseased shrimp from culture ponds of Andhra Pradesh (India)»,J. World Aquac. Soc. V. 37. 523–532. doi:10.1111/j.1749- 7345.2006.00066.x

Jean-Louis, Cuq. 2007. Microbiologie alimentaire. Université Montpellier II, Sciences et Techniques du Languedoc, Polytech’Montpellier, France. 134 p.

Jeannequin B, Plénet, D, Carlin, F, Chauvin, J.-E, Dosba, F. 2015. Pertes alimentaires dans les filières fruits, légumes et pomme de terre. *Innovations Agronomiques*. 48 : 59-77.

Jeantet, R, Croguennec, T, Schuck, P, Brule, G. 2007. Sciences des Aliments 2- Technologie des Produits Alimentaires. *Technique et documentation*, Lavoisier, France. 456 p.

Johnson, T, J, Wannemuehler, , Johnson, S, J, Stell, A, L, Doetkott, C, Johnson, J, R, Nolan, L, K. 2008. Comparison of extraintestinal pathogenic *Escherichia coli* strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. *Applied and environmental microbiology*. *74*(22), 7043-7050.

Journal officiel de la république Algérienne N° 45. 2004.

K

Kaas, R, S, Friis, C, Ussery, D,W, Aarestrup, F, M. 2012. Estimating variation within the genes and inferring the phylogeny of 186 sequenced diverse *Escherichia coli* genomes. *BMC genomics*. *13*(1), 1-13.

Kouassi, J,B, Massara, C, C, Sess, D, E., Tiahou, G, G, Djohan, F, Y. 2013. Détermination des teneurs en Magnésium, Potassium, Manganèse et Sodium de deux variétés de gombo.
Journal of Applied Biosciences. 67 : 5219-5227.

- Kunert Filho, H, C, Brito, K, C, T, Cavalli, L, S, Brito, B, G. 2015. Avian pathogenic *Escherichia coli* (APEC)-an update on the control. *The battle against microbial pathogens : basic science, technological advances and educational programs*. *2*, 598- 618

- Kihal, M. 1996. Etude de la production du dioxyde de carbone par *Leuconostoc mesenteroides*, éléments d‟application en technologie fromagère type fromage bleu. Thèse de Doctorat. Université d’Oran.

- Kimmons, J,E, Brown, K,H, Lartey, A, Collison, E, Mensah, P,P, Dewey, K, G. 1999. The effects of fermentation and/or vacuum flask storage on the presence of coliforms in complementary foods prepared in Ghana. Int. J. Food Sci. Nutr. 50, 195-201.

- Kooh et Lailler, R. 2006. Fiche de description de danger transmissible par les aliments : *Brucella spp*. Afssa : Agence Française de sécurité des produits alimentaires. pp 1-4.

- Kurtzman, C,P, Fell, J.W. 2006. Yeast systematics and phylogeny - Implications of molecular identification methods for studies in ecology. In : C. Rosa., G. Péter (Eds), Biodiversity and Ecophysiology of Yeasts. Springer-Verlag, Berlin. pp. 11-30.

L

- Labadi, A.S., Hammache, H. (2016).*Etude comparative des eaux minérales et des eaux de sources produites en algérie*. Larhyss Journal, N°28, p319-342.

- Lacasse, D. 2002. Introduction à la microbiologie alimentaire. Éditeur Saint-Martin, 792 p.

- Lachassagne, P. 2019. Eau minérale naturelle. In : encyclopédie l’environnement, p9.

- Lambert, C.2002. “Etude des infections à Vibrionaceae chez les mollusques bivalves à partir d’un modèle larves de Pecten maximus”. UEB - Université européenne de Bretagne. Heidelberg, J,F, Heidelberg, K,B, Colwell, R,R. “Bacteria of the gamma-subclass Proteobacteria associated with zooplankton in Chesapeake Bay», Appl. Environ. Microbiol. V. 68. 5498–5507.

- Lanham-New, S, A. 2008. Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment. *The Proceedings of the Nutrition Society*. 67(2) : 163-176.

- Larpent J.P. (1990). Biotechnologie des levures masson, Paris. P 132-315.

- Lechevalier, M, P. 1988. Actinomycètes in agriculture and forestry. In : Actinomycetes in

Biotechnology. Goodfellow, M.G., Williams, S.T. and Modarski, M. Ed., Academic Press London, New-York. 327 – 358 pp.

Lelong, H. 2017. Relations entre Mesures Non Médicamenteuses et Pression Artérielle.
Analyse des données de l'étude NutriNet-Santé. Thèse de Doctorat en Santé publique,
Université Sorbonne (Paris, France). 220 p.

Le Roux, F. et Austin, B.2006. Vibrio splendidus. Ed. Thompson F.L., Austin B. et Swings J., "In The Biology of Vibrios". ASM Press, Washington, DC. 285-296.

Le Roux, F, Gay, M, Lambert C, Nicolas, J, L, Gouy M. et Berthe F. "Phylogenetic study and identification of Vibrio splendidus-related strains based on gyrB gene sequences". Diseases of Aquatic Organisms. V. 58, 143-50.

LeStrange, K, Markland, S, M, Hoover, D, G, Sharma, M, Kniel, K, E. 2017. An evaluation of the virulence and adherence properties of avian pathogenic *Escherichia coli*. *One Health*. *4*, 22-26.

Leveau, J, Y, Bouixmriell, E et De-roissart, H. 1991. La flore lactique In Technique d'analyse et de contrôle dans les industries agroalimentaire. Bourgeois C.M., Leveau JY. Tec & Doc, Lavoisier. 52-186.
Leyral, G, Vierling, E. 2007. Microbiologie et toxicologie des aliments : Hygiène et sécurité alimentaires. Wolters Kluwer, France. 287p.

Li, T.W, Ding, M.J, Zhang, J, Xiang, J.H, Liu, R.Y.1998. "Studies on the 610 pustule disease of abalone (Haliotis discus hannai Ino) on the Dalian coast", J. Shellfish Res, V. 17. 707-711.

Luquet, F, M. 1990. Laits et produits laitiers : vache, brebis, chèvre. Volumes2, transformation et technologie (2ed)., technique et documentation, Lavoisier. Paris. pp 664.

Luo, L, Xu, L. 2003. Vitamin K and osteoporosis. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao.Acta Academiae Medicinae Sinicae*. 25(3) : 346-349.

Lefsrud, M, Kopsell, D, Sams, C, Wills, J, Both, A, J. 2008. Dry Matter Content and Stability of Carotenoids in Kale and Spinach During Drying. *HortScience*, 43(6) : 1731-1736.

M

Mabillot A.1986. Le forage de l'eau : Guide Pratique. Ed. Johnson, Filtration systémis, P. 220, 225,335, 340.

Madigan, M, Martinko J. 2007. Biologie des microorganismes. Person Education France.11 edition, Paris. pp 1047.

Mainil, J. 2003. Facteurs de virulence et propriétés spécifiques des souches invasives d'*Escherichia coli* : I) les adhésines et facteurs de colonisation. *Ann. Méd. Vét., 147, 105-126*

Martin, A. 2001. Apports nutritionnels conseillés pour la population française, 3e éd., Tec & Doc, Lavoisier, Paris. pp 660.

Martinez-Urtaza, J, Simental, L, Velasco, D, DePaola, A, Ishibashi, M., Nakaguchi, Y, Nishibuchi, M, Carrera-Flores, D, Rey-Alvarez, C, Pousa, A.2005. "Pandemic Vibrio parahaemolyticus O3:K6", Europe. Emerg. Infect. Dis. V. 11. 1319–1320. doi:10.3201/eid1108.050322.

Marie, G. 2012. L'eau minérale naturelle une alternative pour compléter les apports alimentaires en calcium des adolescents. Nestlé waters France.

Maurin, C, LeDantec.1979. "The culture of *Crassostrea gigas* in France", In: Mann R (ed) Exotic species in mariculture.The MIT Press, Cambridge.106–122.

Matsumoto, C, Okuda, J, Ishibashi, M, Iwanaga, M, Garg, P, Rammamurthy, T, Wong, H.-C, Depaola, A, Kim, Y.B, Albert, M.J, Nishibuchi, M.2000. "Pandemic spread of an O3:K6 clone of Vibrio parahaemolyticus and emergence of related strains evidenced by arbitrarily primed pcr and toxrs sequence analyses". J. Clin. Microbiol. V. 38. 578–585.

McCarter, L.1999."The multiple identities of Vibrio parahaemolyticus», J. Mol. Microbiol. Biotechnol. V. 1. 51–57.

Meklat, A, Bouras, N, Zitouni, A, Mathieu, F, Lebrihi, A, Schumann, P, Sproer, C, Klenk, H,P, Sabaou, N. 2012. *Actinopolyspora algeriensis sp*. nov., a novel halophilic actinomycete isolated from a Saharan soil. Extremophiles. 16:771–776.

Mellata, M. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne pathogens and disease*. *10*(11), 916-932.

Mensah, P, Tomkins, A, M, Drasar, B,S, Harrison, T, J.1990. Fermentation of cerealsfor reduction of bacterial contamination of weaning foods in Ghana. Lancet. 336, 140-143.

Meyer, A, J, Deiana et H, Leclerc. 1984. Cours de microbiologie alimentaire. Doin éditeur. Paris. pp 307.

Mizehoun, A, G, C. 2015. Apport alimentaire de sodium, potassium et iode, relation avec l'état nutritionnel au Bénin. Thèse de Doctorat en Santé Publique/Nutrition-Diététique, Université de Limoges (Limoges, France), 157 p.

N

Nair, G, B, Ramamurthy, T, Bhattacharya, S, K, Dutta, B, Takeda, Y, Sack, D, A.2007. "Global dissemination of Vibrio parahaemolyticus serotype O3:K6 and its serovariants», *Clin Microbiol Rev*. V. 20. 39–48.

Nouioui, I. 2014. Phylogénie et évolution de genre *Frankia*. Thèse de Doctorat, Biologie végétale. Université Claude Bernard, Lyon I. 102 p.

Nolan, L, K, Barnes, H J., Vaillancourt J P., Abdul-Aziz T., Logue C M. 2013. Colibacillosis *In* Diseases Of Poultry, 13th edition, wiley-blackwell, 751-805.

Nsren, A. 2010. Etude comparative des procédés de séchage couplés à la texturation par Détente Instantanée Contrôlée DIC, en termes de cinétique et de qualité nutritionnelle. Applications à la valorisation des déchets agro-industriels. Thèse de Docteur de L'Université de La Rochelle. Discipline : Génie des Procédés Industriels. pp 52.

O

Observation Régional de l'Environnement (ORE). 2016. L'eau en Poitou-Charentes.

OMS. 2011. Lait et produits laitiers, codex alimentaire, 2ed FAO et OMS, Rome. pp 226.

Organisation mondiale de la Santé (OMS). 2015.. La qualité des eaux conditionnées en France.

Owens, L et Busico-Salcedo, N.2006. Vibrio harveyi : Pretty problems in paradise. Ed. Thompson F.L., AustinB. et Swings J., "In The biology of Vibrios". Washington, DC.266-280.

P

Paixão, A, C, Ferreira, A, C, Fontes, M., Themudo, P, Albuquerque, T, Soares, M, C, Sá M C. 2016. Detection of virulence-associated genes in pathogenic and commensal avian *Escherichia coli* isolates. *Poultry science*. *95*(7), 1646-1652.

Pedersen, K, Grisez, L, Houdt, R. v, Tiainen, T, Ollevier, F, Larsen, J, L.1999. “Extended serotyping scheme for Vibrio anguillarum with the definition and characterization of seven provisional”.O-serogroups, Curr Microbiol. V. 38. 183-189.

Percival, S, L, Williams, D, W. 2014. *Escherichia coli. In* Microbiology of waterborne diseases, *Academic Press*. 89-117.

Parthasarathi, S, Parthasarathi, S, Kim, C,J, Lee, J.C, Sathya, S, Manikandan, M, Manikandan T, BalakriShnan, K. 2011. Taxonomic characterization and UV/VIS analysis of antagonistic marine actinomycete isolated from South Pacific Coast of Philippines. Int. J. Med. Res. 1, 99-105 p.

Patrignani, F, Lanciotti, R, Mathara, J,M , Guerzoni, M, E, Holzapfel, W.H. 2006. Potential of functional strains, isolated from traditional Maasai milk, as starters for the fermented food. Dairy Products 32-45-01.

Pennec, Y,L., Garré, M. 2003. Salmonelloses de l’adulte. EMC – Maladies Infectieuses. 8-018-A-15, 9 p.

Piar, G., Lanoisellé, J, L. 2000. Appertisation des denrées alimentaires. In Colloque annuel-SFT (pp. 3-33).

Porter, L, Butler IV, M, Reeves, R H. 2001. “Normal bacterial flora of the spiny lobster Panulirus argus and its possible role in shell disease», Mar. Freshw. Res. V. 52. 1401–1405

Prescott, L,M, J,P, Harley, D, Klein, J,M.Wiley, L,M. Sherwood et C,J, Woolverton. 2010. Microbiologie. 3emédition, Groupe de Boeck s.a. Bruxelles.

K

Kita-Tsukamoto, K, Oyaizu, H., Nanba, K. et Simidu, U.1993. “Phylogenetic relationships of marine bacteria, mainly members of the family Vibrionaceae, determined on the basis of 16S rRNA sequences”. International Journal of Systematic Bacteriology.
V. 43.8-19.

R

Rambaud, J, C. 2004. Flore microbienne intestinale : Physiologie et pathologie digestives. Edit John Libbey Eurotext, Paris. 247 p.

Reed, G. 1981. Use of microbial cultures : Yeast products. Food Technol. 35: 89–94.

Reichelt J.L., Baumann P., Baumann L.1976. “Study of genetic Relationships among marine species of the genera Beneckea and Photobacterium by means of in vitro DNA/DNA hybridization”, Arch. Microbiol, V. 110, 101-120.

Reski-Bekki, M, A. 2014. Production de metabolites par les levures : caractérisation et identification des arômes et des alcools. Thèse de doctorat, Université d’Oran. Algérie.

Roberts, T, A. 1999. Predictive microbiology applied to chilled food preservation. In : La microbiologie prévisionnelle appliquée à la conservation des aliments réfrigérés, Office des publications officielles des Communautés européennes Editeur, Luxembourg, pp 333.

Robineau, B, Moalic, P, Y. 2010. Une maladie d’actualité en production aviaire : la colibacillose. *Bulletin de l'Académie Vétérinaire de France*.

Rodier J. al. 2005. L'analyse de l'eau Eaux naturelles, eaux résiduaires, eaux de mer &Ed Dunod, Paris, P .189,190, 194, 215, 825,838,

Roignant, F. 2007. L’eau en mediterranne : usage enjeux.

Romalde, J,L, Dieguez, A,L, Lasa, A, Balboa, S.2014. “New Vibrio species associated to molluscan microbiota”. a review. Front. Microbiol. V. 4. doi:10.3389/fmicb.2013.00413

Rosenberg, E, Ben-Haim, Y.2002. “Microbial diseases of corals and global warming. Environ”. Microbiol. V. 4. 318–326.

Ross, R, P, Morgan, S, Hill, C. 2002. Preservation and fermentation : past, present and future. Int J Food Microbiol, 79 : 3-16.

Rubio-Portillo, E, Yarza, P, Peñalver, C, Ramos-Esplá, A , A, Antón, J.2014. “New insights into Oculina patagonica coral diseases and their associated Vibrio spp”. Communities . ISME J. V. 8.1794–1807. doi:10.1038/ismej.2014.33

Ruimy, R, V, Breittmayer, P, Elbaze, B, Lafay, O, Boussemart, M., Gauthier and R Christen.1994. “Phylogenetic analysis and assessment of the genera Vibrio, Photobacterium, Aeromonas and Plesiomonas deduced from small-subunit rRNA sequences», Int. J. Syst. Bact. V. 44. 416-426.

S

Sarowska, J, Futoma-Koloch, B, Jama-Kmiecik, A, Frej-Madrzak, M, Ksiazczyk, M, Bugla-Ploskonska, G, Choroszy-Krol, I. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources : recent reports. *Gut pathogens*. *11*(1), 10.

Sakazaki, R, Iwanami, S, Fukumi, H.1963. "Studies on the enteropathogenic facultatively halophilic bacteria, Vibrio parahaemolyticus, (Morphological, cultured and biochemical properties and its taxonomical position", *Japan Journal of Medical Science and Biology*, V. 16, 161-188.

Sawabe, Tomoo, Ogura, Y, Matsumura, Y, Feng, G, Amin, A,R, Mino, S, Nakagawa, S, Sawabe, Toko, Kumar, R, Fukui, Y., Satomi, M, Matsushima, R, Thompson, F,L, Gomez-Gil, B, Christen, R, Maruyama, F, Kurokawa, K, Hayashi, T.2013. "Updating the Vibrio clades defined by multilocus sequence phylogen : proposal of eight new clades, and the description of Vibrio tritonius sp". nov. Front. Microbiol. V. 4. doi:10.3389/fmicb.2013.00414.

Schaechter, M, Medoff, G, Eisenstein, B. I.1999. "Microbiologie et pathologie infectieuse". De Boeck Supérieur.

Schleifer, K,H, Ludwig, W. 1995). Phylogeny of the genus *Lactobacillus* and related genera. System Appl Microbiol 18, 461-467.

Scholz H.C., Banai M., Cloeckaert A., Kämpfer P., Whatmore A. M. (2018). Brucella. Bergey's Manual of Systematics of Archaea and Bacteria : 1-38 p.

Shapiro-Ilan, D. I, Fuxa, J. R, Lacey, L. A, Onstad, D. W, Kaya, H, K.2005. "Definitions of pathogenicity and virulence in invertebrate pathology», J Invertebr Pathol. V. 88.1–7.

Singleton, P. 1999. Bactériologie. 4 éme Edition, Dunod, Paris. 415 p.

Slavin, J, L L loyd, B. 2012. Health benefits of fruits and vegetables. *Advances in Nutrition*.
3(4) : 506-516

Slimestad R., Fossen T. & Brede C. (2020). Flavonoids and other phenolics in herbs commonly used in Norwegian commercial kitchens. *Food Chemistry*, 309 : 1-28.

Suh, S, O, Kurtzman, C, P., Lachane, M. 2006. Phylogenetics of Saccharomycetales, the Ascomycete yeasts. Mycologia. 98: 1006-1017.

Sutra, L, Federighi, M, Jouve, J. 1998. Manuel de bactériologie alimentaire, polytechnica. 570 p.

Stackebrandt, E, Frederiksen, W, Garrity, G.M., Grimont, P,A, Kampfer, P, Maiden, M,C, Nesme, X, Rossello-Mora, R, Swings, J, Truper, H,G, Vauterin, L, Ward A.C. et Whitman W.B.2002. "Report of thead hoc committee for the re-evaluation of the species definition in bacteriology". International Journal of Systematic and Evolutionary Microbiology. V. 52.1043-1047.

Stordeur, P, Mainil, J. 2002. La colibacillose aviaire. *Ann. Méd. Vét.*146, 11-18.

T

Taleb, S. 2014. Confrontation des normes Algériennes des eaux potables aux directives *de l'organisation mondiale de la santé (OMS).*

Tenaillon, O, Skurnik, D, Picard, B, Denamur, E. 2010. The population genetics of commensal *Escherichia coli. Nature Reviews Microbiology. 8*(3). 207-217.

Thu, H, T, N. 2008. Étude de la flore lactique du nem chua, produit carné fermenté cru traditionnel du sud Vietnam et maîtrise du processus de fermentation par ajout de souches lactiques sélectionnées spécifiques du produit, Thèse de Doctorat, Spécialité : Sciences des Aliments et Nutrition, l'Université Bordeaux 1, France. 201 p.

Thomas, S, R, Elkinton, J, S.2004. "Pathogenicity and virulence". J Invertebr Pathol.
V. 85. 146–151.

Thompson, F, L, Lida, T et Swings, J.2004. "Biodiversity of Vibrios". Microbiology and Molecular Biology Reviews. V. 68.403-31.

Thompson, J, R, Randa, M, A, Marcelino, L, A, Tomita-Mitchell, A, Lim, E et Polz, M, F 2004 ."Diversity and dynamics of a north atlantic coastal Vibrio community". Applied and Environmental Microbiology. V. 70.4103-10.

Tirilly, Y, Bourgeois, C, M. 1999. Technologie des légumes. Agriculture et agroalimentaire. Librairie Eyrolles, Paris, France, 558 p.

Titchenal, C, Dobbs, J. 2005. Nutritional Value of Vegetables. *Handbook of Food Science, Technology, and Engineering.* 4(1) : 1-14.
Toyofuku, H.2014. "Vibrio parahaemolyticus Risk Management in Japan", in : Sauvé, G. (Ed.), Molluscan Shellfish Safety, Netherlands. 129–136. doi:10.1007/978- 94-007-6588-7_11

Toranzo, A,E, Magariños, B, Romalde, J,L.2005.. "A review of the main bacterial fish diseases in mariculture systems". Aquaculture. V. 246. 37–61. doi:10.1016/j.aquaculture.2005.01.002.

Travers, M,A, Boettcher Miller, K, Roque, A, Friedman, C, S.2015. "Bacterial diseases in marine bivalves". J. Invertebr. Pathol. V. 131.11-31. doi:10.1016/j.jip.2015.07.010.

Travers, M.A., Mersni Achour, R., Haffner, P., Tourbiez, D., Cassone, A.L., Morga, B., Doghri, I., Garcia, C., Renault, T., Fruitier-Arnaudin, I., Saulnier, D.2014. "First description of French V. tubiashii strains pathogenic to mollusk : I. Characterization of isolates and detection during mortality events", J. Invertebr. Pathol. V. 123. 38–48. doi:10.1016/j.jip.2014.04.009.

U

Uwamungu, J, Jiang, Y. 2010. Analyse physico-chimique et bactériologique des eaux de la rivière

V

Vanhove, A.S, Duperthuy, M, Charrière, G,M, Le Roux, F, Goudenège, D, Gourbal, B, Kieffer-Jaquinod, S, Couté, Y, Wai, S.N, Destoumieux-Garzón, D.2015. "Outer membrane vesicles are vehicles for the delivery of Vibrio tasmaniensis virulence factors to oyster immune cells», Environ. Microbiol. V. 17. 1152– 1165. doi:10.1111/1462-2920.12535

Vandamme, P, Pot B, Gillis, M, de Vos P, Kersters, K et Swings J.1996. "Polyphasic taxonomy, a consensus approach to bacterial systematics», Microbiology and Molecular Biology Reviews. V. 60.407-438.

Varoquaux, P. 2002. Fruits frais prêts à l'emploi dits de 4ème gamme. In Technologies de Transformation des Fruits. Albagnac édition, Lavoisier, Paris(France) : 119-156.

Vierling, E. 2003. Aliments et boissons : filières et produits.Doin Centre régional de documentation pédagogique d'Aquitaine, Rueil-Malmaison, Bordeaux, France. 270 p.

Viland, M., Montiel, A., Duchemin, J., Zarrabi, P. (2001). *Eau et Santé*. Editions du Gret. Paris (France) : Groupe de recherche el d'échanges technologiques, p109.

Vuddhakul, V, Chowdhury, A, Laohaprertthisan, V, Pungrasamee, P, Patararungrong, N, Thianmontri, P, Ishibashi, M, Matsumoto, C, Nishibuchi, M.2000. "Isolation of a Pandemic O3:K6 Clone of a Vibrio parahaemolyticus Strain from environmental and clinical sources in Thailand”. Appl. Environ. Microbiol. V. 66. 2685–2689. doi:10.1128/AEM.66.6.2685-2689.2000.

W

Wang, S, Meckling, K, A, Macrone, F, M, Kakuda, Y, Tsao R. 2011. Can phytochemical antioxidant rich foods act as anti-cancer agents ? *Food Research International Journal*. 44 : 2545-2554.

Wang, Z, Lu, X, Liang, Y., Wang, C.2010. “Haplosporidium nelsoni and H. costale in the Pacific oyster Crassostrea gigas from China’s coasts”. Dis. Aquat. Organ. V. 89.223–228. Doi : 10.3354 /dao02196.

Walker, G, M. 2009. Yeasts. University of Abertay Dundee, Dundee, Scotland. Elsevier Inc. 1174-1187.

Wall, D, Kaiser, D.1999. “Type IV pili and cell motility», Mol. Microbiol. V. 32. 01-10. doi:10.1046/j.1365-2958.1999.01339.x

Watnick, P, I, Kolter, R.1999. “Steps in the development of a Vibrio cholerae El Tor biofilm», Mol. Microbiol. V.34. 586–595.

Weber, P. 2001. Vitamin K and bone health. *Nutrition*. 17(10) : 880-887.

Williams, P, G. 2009. Panning for chemical gold : marine bacteria as a source of new therapeutics. Trends Biotechnol., 27, 45-52 p.

X

Xenou, D, Tzelves, L, Terpos, E, Stamatelopoulos, K, Sergentanis, T,N , Psaltopoulou, T. 2022. Consumption of Fruits, Vegetables and Bladder Cancer Risk : A systematic review and meta-analysis of prospective cohort studies. *Nutrition and Cancer*. 74(6) : 2003-2016

Z

Zhang, H, Cai, Y. 2014. Lactic Acid Bacteria Fundamentals and Practice. Springer Dordrecht Heidelberg New York London. P: 535.


Hautausschlag
Allergische Reaktionen
Schlafstörungen
Erbrechen
Kopfschmerz
Ohnmacht
Schwindel