Chapter 3

Main Groups of Microorganisms of Relevance for Food Safety and Stability: General Aspects and Overall Description

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3.1 INTRODUCTION

Food preservation, since ancient times, played a central role in survival of mankind enhancing the safety and stability of different foodstuffs. Traditional techniques used to prevent food deterioration in the past as salting, drying, fermentation, and heating prevail in modern food industry. The knowledge about the main causes of deterioration has increased substantially since then and losses due to spoilage and food contamination were reduced (Gram et al., 2002).

The knowledge about the composition, origin, pH, water activity (a_w) of food and storage conditions (e.g., temperature, atmosphere, and pressure) along with the information about the characteristics of most prevalent and resistant microorganism predicts the composition of microflora during processing and storage. From this scenario, the adequate use of technology (such as pasteurization and sterilization), hygienic strategies (e.g., good hygiene practices and good manufacture practices), and traceability (prevent and reduce the distribution of unsafe and poor quality food) can prevent and delay the colonization of spoilage and pathogenic microorganisms in foodstuff. However, food remains susceptible to spoilage and contamination of pathogenic microorganism throughout food chain, retail shops, restaurants, and house of consumers (Aung & Chang, 2014; Gram et al., 2002).

The damage from spoilage and contaminated food affects the food industry (economic loss, damage to reputation, and punishment according to local

law) and consumers (Akkerman, Farahani, & Grunow, 2010). The spoilage of food by microbial activity involves many and complex mechanisms wherein the acceptance of sensory attributes decay and consumers reject the food. The presence of visible growth as slime and colonies changes in texture due to degradation of proteins/carbohydrates/lipids, and the perception of off-odors and off-flavors may indicate spoilage by microorganisms (e.g., bacteria and molds) (Gram et al., 2002).

On the other hand, the development, the cross-contamination, and toxin production of pathogenic microorganisms impair the safety of food and impose a risk to health of consumers. The ingestion and colonization of pathogen microorganisms in human digestive tract usually cause gastroenteritis, which is a condition characterized by diarrhea, intestinal cramps, nauseas, vomiting, and fever. The estimation of foodborne diseases in the world is complex but international, national, and local efforts have been made to estimate the burden of foodborne diseases worldwide (Flint et al., 2005). The main agents involved in gastroenteritis are bacteria, although parasites, virus, yeasts, and molds are underrecognized causes of gastroenteritis and they have been becoming more frequent in last decade (Acheson, Bresee, Widdowson, Monroe, & Glass, 2002; Dorny, Praet, Deckers, & Gabriel, 2009; Fleet, 2007).

This chapter is focusing on the characteristics of the main microorganisms (bacteria, yeasts, molds, virus, and parasites) involved in food spoilage or contamination as known and their recently discovered species, defects, and alterations in foodstuff, most common food associated with each foodborne disease, resistance to thermal processing, occurrence in different countries, outbreaks, and associated symptoms.

3.2   SPOILAGE NONSPORE-FORMING BACTERIA

Food spoilage causes not only economic loss, but also loss of edible foods. In countries where foods are produced and procured from many countries much more than the need, spoilage up to a certain level is not considered serious. However, in many countries where food production is not efficient, food spoilage can adversely affect the availability of food. With the increase in world population, serious consideration needs to be given on not only increasing food production but also reducing food spoilage, because in some countries it could reach 25% or more. The acceptance qualities of a food can be lower because of infestation with insects and rodents, undesirable physical and chemical actions, and microorganisms' growth. Microbial spoilage occurs either because of microbial growth in a food or because of the action of some microbial enzymes present in the food (Ray, 2005).

The food microbiological profile is quite different from an unspoiled and nonsterile food, to the same food spoiled. The first case generally contains many types of microorganisms, such as bacteria, yeasts, and molds (also

viruses) from different genus, maybe more than one species from the same genus, and even more than one strain from the same species. However, when the same food is spoiled, it is found to contain predominantly one or two types, and they may not even be present initially in the highest numbers in the unspoiled or fresh product. Among the different species initially present and capable of growing in a particular food, only those with the shortest generation time under the storage conditions attain the numbers rapidly and cause spoilage.

Theoretically, any microorganism (including microorganisms used in food fermentation and pathogens) that can multiply in a food to reach a high level (spoilage detection level) is capable of causing it to spoil. Yet, in reality, bacterial species from only several genera have been implicated more with spoilage of most foods. This is dictated by the bacterial characteristics, food characteristics, and the storage conditions (Ray, 2005).

This point describes a limited number of nonspore-forming bacteria as representative of the major groups of food spoilage. The predominant bacteria associated with spoilage are *Brochothrix thermosphacta*, *Carnobacterium spp.*, *Lactobacillus spp.*, *Lactococcus spp.*, *Leuconostoc spp.*, *Pediococcus spp.*, *Stretococcus spp.*, *Kurthia zopfii*, and *Weisella spp*. The main defects that these bacteria cause in foodstuffs are off-odors and off-flavors, discolorations, gas production, slime production, and decreases in pH.

3.2.1 *Brochothrix* spp.

Brochothrix spp. is a common spoilage organism of meat and meat products stored at chilled temperatures. *B. thermosphacta and Brochothrix campestris are the two species assigned to the genus Brochothrix. B. thermosphacta* is the predominant spoilage organism in pork, lamb, and fish, particularly on fatty surfaces, chilled raw, and processed products stored aerobically or under modified atmospheres (Borch, Kant-Muermans, & Blixt, 1996; Gardner, 1981). It is a Gram-positive rod, nonsporing, nonmotile, and facultative anaerobe. There is no evidence to support that it is pathogenic (Kilcher, Loessner, & Klumpp, 2010).

As a facultative anaerobe, *B. thermosphacta* is well suited for growing under modified atmosphere environments. The successful spoilage of chilled products is due mainly to its psychotropic nature. It has a growth range from $0°C$ to $30°C$, with an optimum of $20-25°C$. Refrigeration temperatures will selectively favor its growth. Its pH growth range (pH $5-9$) is well adequate within most meat products. These factors, along with its ability to tolerate low a_W values and remain relatively resistant to curing agents, increase its ability to outgrow with respect to other food spoilage microflora.

Spoilage is greatest in depleted aerobic conditions, often aided by increased carbon dioxide levels. Such conditions are common in vacuum-packed products (Pin, García de Fernando & Ordóñez, 2002), where it

produces undesired volatile compounds such as acetoin, diacetyl (aerobic growth), or lactic acid and ethanol (anaerobic growth) (Pin et al., 2002; Stanley, Shaw, & Egan, 1981). Moreover, it can lead to the formation of slime textures on cured and processed meat products (frankfurters, bologna, sausage, and luncheon meats) (Jackson, Marshall, Acuff, & Dickson, 2001).

3.2.2   *Carnobacterium* spp.

Carnobacterium is a Gram-positive rod-shaped lactic acid bacteria (LAB). Although they are lactic acid−producing bacteria, they grow in a pH range of 7−9. Most of the species produce lactic acid through the fermentation of carbohydrates such as glucose. *Carnobacterium* is frequently a predominating element of the microflora of chilled vacuum or modified atmosphere-packed meat and seafood, as well as dairy products (Groth Laursen et al., 2005). Its genus is currently composed of 11 species that have been isolated mostly from water or sediment and/or cold environments. Among these species, *Carnobacterium divergens* and *Carnobacterium maltaromaticum* (formerly *Carnobacterium piscicola*) are also dominant in meat (beef, pork, and poultry), seafood (fish and shrimps), and dairy (raw milk and cheese) food products (Leisner, Laursen, Prévost, Drider, & Dalgaard, 2007). These two species can act as food spoilage bacteria when an improperly storage is realized, due to their tolerance to freezing temperatures and thawing, or as protective cultures, depending on the strain and food product (Barakat, Griffiths, & Harris, 2000; Paludan-Müller, Dalgaard, Huss, & Gram, 1998).

The growth and/or presence of high numbers of these species have been associated with sensory spoiled products (Leisner et al., 2007), including cooked sliced ham inoculated with *C. maltaromaticum* (Budde, Hornbaek, Jacobsen, Barkholt, & Koch, 2003), sterile beef inoculated with *C. divergens* or *C. maltaromaticum*, then stored under vacuum and subsequently transferred to aerobic conditions (Leisner, Greer, Dilts, & Stiles, 1995), frozen/thawed and modified atmosphere-packed fish (Emborg, Laursen, Rathjen, & Dalgaard, 2002), various lightly preserved seafood (Basby, Jeppesen, & Huss, 1998), and high pressure processed squid mantle (Paarup, Sanchez, Peláez, & Moral, 2002).

3.2.3   *Lactobacillus* spp.

Lactobacillales or LAB are an order of Gram-positive, acid-tolerant, generally nonsporulating, nonrespiring, either rod or coccus-shaped bacteria that share common metabolic and physiological characteristics. These bacteria are usually found in decomposing plants and milk products, produce lactic acid as the major metabolic product of carbohydrate fermentation. Furthermore, lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item (Garvie, 1980).

3.2.3.1 Lactobacillus casei

Lactobacillus casei is a species of genus *Lactobacillus* found in dairy products as nonstarter LAB, which has a wide pH and temperature range. It is present in ripening cheddar cheese and in Sicilian green olives. Their subspecies *casei* and *pseudoplantarum* have been associated with soft body defects and gas production in Mozzarella cheese, respectively (Hull, Roberts, & Mayes, 1983; Hull, Toyne, Haynes, & Lehmann, 1992). Regarding *alactosus* and *rhamnosus* species, they have been associated with the development of a phenolic flavor in Cheddar cheese (Hull et al., 1992).

3.2.3.2 Lactobacillus curvatus (Lactobacillus curvatum)

Lactobacillus curvatus is a Gram-positive, rod shape, nonsporulating and facultative heterofermentative, which is not able to grow well in high salt concentration. It can be present in a wide range of foods including raw sausage, milk, grapes, and plant material coming into the winery. As mentioned previously, these bacteria can lead to the formation of slime textures on cured and processed meat products (Marshall & Bal'a, 2001).

3.2.3.3 Lactobacillus delbrueckii subsp. bulgaricus

Lactobacillus delbrueckii subsp. bulgaricus (until 2014 known as *Lactobacillus bulgaricus*) is one of several bacteria found in other naturally fermented products. It is a Gram-positive rod, nonmotile, and does not form spores. It is regarded as aciduric or acidophilic, since it requires a low pH (around 5.4−4.6) to grow effectively with an optimal range of 43−46°C. *L. delbrueckii subsp. bulgaricus* is commonly used alongside *Streptococcus thermophilus* as a starter for making yogurt (Courtin & Rul, 2003). However, it produces some defects such as pink discoloration on cheese (Ride, 1983). Moreover, it has also been considered as a contaminant of beer due to its homofermentative production of lactic acid, an off-flavor in many styles of beer. In other styles of beer, however, LAB can contribute to the overall appearance, aroma, taste, and/or mouthfeel, and generally produce an otherwise pleasing sourness (Priest & Campbell, 2002).

3.2.3.4 Lactobacillus plantarum

Lactobacillus plantarum is a widespread member of the genus *Lactobacillus*, commonly found in meat and processed foods, in many fermented food products as well as anaerobic plant matter. *L. plantarum* has one of the largest genomes known among the LAB and is a very flexible and versatile species. It is Gram-positive aerotolerant bacteria that grows at 15°C and with concentration of 4% of NaCl, but not at 45°C, and produces both isomers of lactic acid (D and L). *L. plantarum* is commonly found in many fermented food products including sauerkraut, pickles, brined olives, Korean kimchi,

Nigerian Ogi, sourdough, and other fermented plant material, some cheeses, fermented sausages, and stock fish. The high levels of this organism in food also make it an ideal candidate for food spoilage. Its growth rates have been found to be higher on fat than on lean beef and pork tissues (Vanderzant, Savell, Hanna, & Potluri, 1986).

Regarding processed products, slimy, souring, and greening defects can be generated. Slimy spoilage is usually confined to outside product surface and may start developing as discrete colonies that expand to form a uniform gray layer of slime (Jackson et al., 2001). The role of LAB in wine spoilage is well recognized, to assess the risk associated with the residing species, it is important to identify and enumerate the bacteria during the different stages of vinification (du Toit & Pretorius, 2000). Spoilage by this organism is common in musts and wines, and is caused by its heterofermentative property of converting malic acid into compounds other than lactic acid. Of these, acetic acid is of concern and biogenic amines and ethyl carbamate may be produced (du Toit & Pretorius, 2000).

3.2.3.5   Lactobacillus sakei

Lactobacillus sakei is a bacterium species of the genus *Lactobacillus*. It is a facultative heterofermentative able to produce either alcohol or lactic acid from sugars. *L. sakei* is used in Europe for the production of traditional dry sausage as starter and can be used for the conservation of fresh meat (Bredholt, Nesbakken, & Holck, 2001). It was therefore of interest for its application in cooked ham and sausages during processing in the factory. Regarding spoilage effect, they have negligible effect on the sensory properties of the products; however, it can lead to the formation of slime textures on cured and processed meat products (Marshall & Bal'a, 2001).

3.2.4   *Pediococcus* spp.

Pediococcus is a genus of Gram-positive LAB usually occurring in pairs or tetrads, and purely homofermentative (Haakensen, Dobson, Hill, & Ziola, 2009). Its bacteria are usually found in fermented products and are considered contaminants of beer and wine. Thereby, the species *Pediococcus damnosus* frequently grow in wine and beer, where they overproduce glucan and spoil products by increasing their viscosity (Delaherche, Claisse, & Lonvaud-Funel, 2004).

3.2.5   *Streptococcus* spp.

Streptococcus is one of nonspore-forming bacteria responsible for fermentative spoilage of dairy products (Frank, 2007). *S. thermophilus* is one of the most widely used bacteria in the dairy industry. It is a Gram-positive, anaerobic fermentative facultative. It is nonmotile and does not form endospores

with an optimal growth temperature range of 35−42°C. Although its genus, *Streptococcus*, includes some pathogenic species, food industries consider *S. thermophiles* a safer bacterium than many other *Streptococcus* species. *S. thermophiles* is found in fermented milk products, and is generally used in the production of yogurt and cheese. It helps make reduced-fat cheese with similar characteristics to regular, full-fat cheese. These bacteria are selected because they produce exopolysaccharide that give reduced-fat cheese a similar texture and flavor as regular cheese. In addition, *S. thermophiles* produced low moisture cheese and decreased the cheese bitterness (Awad, Hassan, & Muthukumarappan, 2005).

3.2.6   *Lactococcus* spp.

Lactococcus is a genus of LAB that are known as homofermentors. It means that they produce a single product, lactic acid in this case, as the major or only product of glucose fermentation. Their homofermentative character can be altered by adjusting environmental conditions such as pH, glucose concentration, and nutrient limitation. They are Gram-positive, catalase-negative, nonmotile cocci that are found single, in pairs, or in chains. The genus contains strains known to grow at or below 7°C (James, 1992). These organisms are commonly used in the dairy industry in the manufacture of fermented dairy products such as cheeses. They can be used in single-strain starter cultures, or in mixed-strain cultures with other LAB such as *Lactobacillus* and *Streptococcus*. Special interest is focused on the study of *Lactococcus lactis* and their subspecies *lactis* and *cremoris*, as they are the strains used as starter cultures in industrial dairy fermentations (Vos & Simons, 1994).

   L. lactis is a Gram-positive bacterium used in dairy industry that has a homofermentative metabolism and it has been reported to produce exclusively L-(+)-lactic acid (Roissart & Luquet, 1994). However, Åkerberg, Hofvendahl, Zacchi, and Hahn-Hägerdal (1998) reported that D-(−)-lactic acid can be produced when cultured at low pH. On the other hand, *L. lactis subsp. lactis*, formerly *Streptococcus lactis* (Chopin, Chopin, Rouault, & Galleron, 1989) is used in the early stages for the production of many cheeses, including Brie, Camembert, Cheddar, Colby, Gruyère, Parmesan, and Roquefort (Coffey & Ross, 2002). Spoilage by these bacteria can produce malty flavors and "ropy" textures in fluid milk and dairy foods (Morgan, 1976).

3.2.7   *Leuconostoc* spp.

Leuconostoc is a genus of Gram-positive bacteria, heterofermentative, and are able to produce dextran from sucrose. They are generally slime forming (Björkroth & Holzapfel, 2006). Regarding its species, it is important to highlight that *Leuconostoc carnosum* is a lactic acid bacterium. Its name derives

from the fact that it was first isolated from chill-stored meats. It is also a spoilage bacterium in vacuum-packaged, cooked meat products as sliced cooked ham. Its significance that it thrives in anaerobic environments with a temperature around 2°C, thus, it has been known to spoil vacuum-packed meat (Björkroth, Vandamme, & Korkeala, 1998). Spoilage by *L. carnosum* produces typical sensory changes, such as souring, gas formation, and/or slime formation, at the stationary phase; and a vacuum-packaged product is usually expected to maintain good sensory quality for at least 3−4 weeks. However, due to an increased level of contamination or particularly active spoilage strains, spoilage may occur during the shelf-life period, subjecting to defects aforementioned (Korkeala, Suortti, & Mäkelä, 1988).

3.2.8 *Kurthia* spp.

Kurthia is the little known genus of the coryneform bacteria group. Young cultures form chains or rods, rather than the usual V-shaped appearance. Its bacteria are strictly aerobic and so far there is no evidence of pathogenicity (Gardner, 1969). The species *Kurthia zopfiienus* is mainly known as food spoilage organisms predominately associated with the off-flavors and taints of both cured and fresh meat and meat related products that are stored at elevated temperatures (Holzapfel & Schillinger, 1992).

3.2.9 *Weissella* spp.

Weissella is a genus of Gram-positive bacteria catalase-negative, nonendospore forming cells with coccoid or rod-shaped morphology (Björkroth, Dicks, & Endo, 2014) and belongs to the group of bacteria generally known as LAB. Its species are important from a technological point of view, and should be taken into account in any envisaged biotechnological applications (Fusco et al., 2015). *Weissella* species have been isolated from a wide range of habitats, for example, on milk, vegetables, as well as from a variety of fermented foods such as European sourdoughs and Asian and African traditional fermented foods. Spoilage by *Weissella minor* produces typical slime textures and due to be heterofermentative, producing CO_2 from carbohydrate metabolism with either $D(-)$-, or a mixture of $D(-)$- and $L(+)$-lactic acid and acetic acid as major end products from sugar metabolism (Nychas, Drosinos, & Board, 1998).

3.3 SPOILAGE SPORE-FORMING BACTERIA

This point explores the main spore-forming bacteria involved in the spoilage of various processed foods. Due to changes in the design of industrial food processing and increasing international trade, highly thermoresistant spore-forming bacteria are an emerging problem in food production. This type of

bacteria is considered a major threat in heat-treated foods. Spore-formers causing food spoilage are particularly important in low-acid foods (pH ≥4.6) packaged in hermetically sealed containers, which are processed by heat (Setlow & Johnson, 2007). Certain spore-formers also cause various types of spoilage of high-acid foods (pH <4.6), while psychrotrophic spore-formers have also been recognized to cause spoilage of refrigerated foods (Moir, 2001; Setlow & Johnson, 2007). Both, chemical and physical treatments used in the food-processing industry are not enough to eliminate the spores (Carlin, 2011). Endospore formers may thrive in different parts of the food-processing plant (Kalogridou-Vassiliadou, 1992). A number of agents cause spore activation, including low pH, some chemicals, and sublethal heat. Although spores are metabolically dormant and can remain in this state for many years, if they receive the proper stimulus and conditions, they return to active metabolism through the spore germination (Setlow & Johnson, 2007).

The spore-forming bacteria can be divided into two major groups: the aerobic *Bacillus* species and the strictly anaerobic *Clostridium* species (Heyndrickx, 2011). Thermophili acidophilic spore-forming bacteria such as *Alicyclobacillus* can also cause spoilage of acidic beverages. They grow without the production of gas and no changes in the appearance of the beverage containers are observed. Unfortunately, this type of spoilage is discovered only when the consumer opens and begins to consume the product. The economic losses might be extremely high. Generally, bakery products are specifically spoiled by *Bacillus* species while different *Clostridium* species classically contaminate refrigerated vacuum-packed meats (André, Vallaeys, & Planchon, 2016). There are three main differences between *Clostridium* and *Bacillus* genus. The first difference, as commented earlier, is that *Clostridium* is strictly anaerobic, while *Bacillus* is an aerobic bacterium. The second difference is the spores. *Clostridium* forms bottle-shaped endospores, while *Bacillus* forms oblong endospores. The last difference is that *Clostridium* does not form the enzyme catalase, while *Bacillus* secretes catalase to destroy toxic byproducts of oxygen metabolism (Maczulak, 2011). Microbial spoilage of food is usually indicated by changes in texture or the development of off-flavors. The primary sources of food contamination are probably soil and air. Being a soil resident *Bacillus* and *Clostridium* species are part of the microbiota of plant raw materials (used as ingredients for many foods), attached as vegetative cells or spores (Heyndrickx, 2011). The main groups of bacteria that cause deterioration of food are described as follows.

3.3.1   Bacilli

The family *Bacillaceae* represents a very large, diverse set of bacteria that have one common yet distinct feature: the ability to make dormant endospores aerobically when challenged with unfavorable growth conditions

(Zeigler & Perkins, 2009). These are Gram-positive and most of them are mesophiles, psychrotrophs, and thermophiles (Jay, 2000). The genus still contains about 80 species. *Bacillus* species inhabit soils, air, and dust (Jay, 2000). Many species and strains can produce extracellular enzymes that hydrolyze carbohydrates, proteins, and lipids. The main nonpathogenic *Bacillus*, *Geobacillus*, and *Alicyclobacillus* species that produce food spoilage are as follows:

3.3.1.1 Bacillus sporothermodurans

Bacillus sporothermodurans produces highly heat-resistant spores that may survive at ultra-high temperature (UHT) treatment or industrial sterilization (Pettersson, Lembke, Hammer, Stackebrandt, & Priest, 1996; Scheldeman, Herman, Foster, & Heyndrickx, 2006). According to Meier, Rademacher, Walenta, and Kessler (1996), the spores of *B. sporothermodurans* are more resistant than the spores of many thermophiles. Affected milk products include whole, skimmed, evaporated or reconstituted UHT milk, UHT cream, and chocolate milk in different kinds of containers and also milk powders (Hammer, Lembke, Suhren, & Heeschen, 1995; Klijn et al., 1997).

3.3.1.2 Bacillus amyloliquefaciens

B. amyloliquefaciens is a Gram-positive soil bacteria closely related to the species *Bacillus subtilis*. The two species share many homologous genes and appear so similar, that it is not possible to visually separate the two species (Priest, Goodfellow, Shute, & Berkeley, 1987). *B. amyloliquefaciens* and its closely related species are particularly known to be involved in ropy bread spoilage that is characterized by an unpleasant fruity odor followed by enzymatic degradation yielding soft, sticky, and stringy bread crumb making the bread inedible (Valerio et al., 2015). Ropiness is usually occurring in the summer season when the climate is warm (25°C and 30°C) and humid as in the Mediterranean countries. This species is able to grow from 15°C to 55°C and from pH 5 to 9 (Valerio et al., 2015).

3.3.1.3 Geobacillus stearothermophilus

G. stearothermophilus is a common thermophilic spoilage organism that normally produces acid but no gas in spoiled packs that have been held at elevated temperatures around 50−55°C. If readily fermentable sugars are in limited supply, a study has found that this organism can elevate pH. The minimum pH for growth is around 5.3 (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). *G. stearothermophilus* is also significant because of its role in food spoilage, mostly milk and dairy. During the pasteurization process of these products, dairy is often heated to temperatures that denature the molecules of pathogenic bacteria. However, in *G. stearothermophilus*,

unusually heat-tolerant enzymes and proteins allow it to survive this process (Furukawa et al., 2009; Hwang & Huang, 2010).

3.3.1.4 *Bacillus coagulans*

B. coagulans is also a thermophile, but differs from *B. stearothermophilus* in being able to grow at pH values below 4.0. *B. coagulans*, facultative anaerobic, thermotolerant, and acidophilic bacteria, is an important food spoilage microorganism (Haberbeck, Alberto da Silva Riehl, de Cássia Martins Salomão, & Falcão de Aragão, 2012). In the canned vegetable industry where foods are acidified to pH values between 4 and 4.5, this bacterium is frequently found, since spores of *B. coagulans* are able to grow and germinate at pH values as low as 4 (De Clerck et al., 2004; Lucas et al., 2006). It produces off-flavors and souring of product during spoilage. *B. coagulans* has caused considerable economic loss for the food industry because of the "flat sour spoilage," which is a drastic acidification of the food product due to the production of lactic acid without gas formation (Lucas et al., 2006).

3.3.1.5 *Alicyclobacillus acidoterrestris*

A. acidoterrestris is an obligate acidophilic, which grows optimally at pH 3.5−4.0 and has a minimum and maximum pH for growth of 2.5 and approximately 5.5. At these pH values, *Alicyclobacillus* is the only acidophilic spore-producing genus described up to now as a cause of spoilage. *A. acidoterrestris* spores are generally more heat-resistant than those of other acidophilic spore-formers, and cause spoilage of processed fruit and vegetables juices (Evancho & Parish, 2001; Silva & Gibbs, 2004). In addition, *A. acidoterrestris* is able to grow over a temperature range of 25°C to 60°C (Yamazaki, Teduka, & Shinano, 1996). The most likely cause of contamination of the fruit is from soil contamination during harvesting. The heat resistance of the spores is such that pasteurization will not guarantee freedom from the organism (Brown, 2000). Some strains in the genus *Alicyclobacillus* have greater spoilage ability because they can produce large amounts of guaiacol that adversely affects the odor of the product (Danyluk et al., 2011).

3.3.2 Clostridia

Clostridia is one of the largest bacterial class, including more than 150 validly described species. Among these, there are several with enormous biotechnological potential and also a few well-known pathogens (Dürre, 2009). *Clostridia* are obligate anaerobes and not osmotolerant. They are typically involved in the spoilage of foods such as canned or vacuum-packaged foods. *Clostridia* in the public opinion is associated with production of a bad smell. In most cases, this is caused by butyric acid, one of the major fermentation

products (Dürre, 2009). The main mechanism of spoilage is protein hydrolysis. Anaerobic proteolysis by *Clostridium* spp. can result in a noxious putrefaction of the food. *Clostridium* species inhabit soils and the intestinal tract of animals, including humans (Maczulak, 2011). As occurs with some *Bacillus*, some species of *Clostridium* also produce enzymes that hydrolyze carbohydrates and proteins in food processing. The main nonpathogenic *Clostridium* and *Desulfotomaculum* species that produce food spoilage are as follows:

3.3.2.1 Clostridium butyricum, Clostridium beijerinckii, and Clostridium pasteurianum

The butyric anaerobes, as these three *Clostridium* species causing spoilage in low-acid canned foods, are usually associated with spoilage of products with pH values between 3.9 and 4.5 producing blown cans and a butyric odor (Hersom & Hulland, 1980). These bacteria, especially *Clostridium butyricum* and *Clostridium tyrobutyricum*, can also cause spoilage and gas production (blowing) in hard cheeses (Brown, 2000).

3.3.2.2 Clostridium sporogenes

Clostridium sporogenes is closely related to the proteolytic strains of *Clostridium botulinum*. Spoilage from this organism produces typically blown or burst packs with a strong putrefactive odor. According to Brown (2000), if spoilage from *C. sporogenes* is experienced, all suspect packs should be recalled and investigations into the cause of spoilage should be undertaken. A process fault that allowed *C. sporogenes* to survive and proliferate may also have been serious enough to allow spores of *C. botulinum* to survive, germinate, and produce toxin. Therefore, its physiological and genetic similarity to *C. botulinum* Group I is often used as a surrogate for this organism in demonstrating the effectiveness of food processes (Brown, Tran-Dinh, & Chapman, 2012; Diao, André, & Membré, 2014; Taylor et al., 2013).

3.3.2.3 Clostridium thermosaccharolyticum

The most heat-resistant spores are those of *Clostridium thermosaccharolyticum* (Brown, 2000). The spores survive thermal processing to germinate and grow when the product is stored at elevated temperatures around 30−60°C. Canned food products spoiled by this organism are of the swell or gaseous type, with the foods having a depressed pH and cheesy odor (Ashton, 1981). These organisms occur widely in soil and therefore, they are found on raw material such as mushrooms and onion products (Ashton, 1981).

3.3.2.4 Clostridium putrefaciens

Clostridium putrefaciens was of considerable concern to the ham curing industry. Studies by Roberts and Derrick (1975) demonstrated that this

organism was able to grow in 4% NaCl and 100 ppm of $NaNO_2$ at pH 7.0 even at 5°C. The odor produced by this species in meat is very characteristic. One accustomed to these odors has no difficulty in differentiating pure cultures of this anaerobe from other putrefactive organisms by this means. A marked softening of the meat without evident reduction in bulk is characteristic of *C. putrefaciens* (Sturges & Drake, 1927). Modern processing trends are to use lower levels of salt and nitrite, increased pH levels of 6.8−7.0, and chill storage that would tend to favor the growth of *C. putrefaciens.*

3.3.2.5   Desulfotomaculum nigrificans

Desulfotomaculum nigrificans are moderately thermophilic members of the polyphyletic spore-forming genus *Desulfotomaculum* in the family *Peptococcaceae*. Nowadays, the spoilage caused by *D. nigrificans* is quite rare, however, in the past, an entire season's production of canned sweet corn could be lost from this organism (Brown, 2000). *D. nigrificans* causes "sulfur stinker" spoilage often resulting in blackened product when the steel in cans reacts with the H_2S produced.

3.4   PATHOGENIC NONSPORE-FORMING BACTERIA

Although sporogenesis is considered an important environmental survival and food contamination mechanism in bacteria, several pathogenic bacteria that do not form spores remain involved in the main food contamination outbreaks in the last decade. The most relevant bacterial species in this group are *Brucella spp.*, *Campylobacter spp.*, *Salmonella spp.*, *Yersinia spp.*, *Listeria spp.*, and *Escherichia coli spp.* which are involved in most of the 4362 foodborne outbreaks registered in Europe Union in 2015 (European Food Safety Authority, 2016; McCabe-Sellers & Beattie, 2004). The constant evolution, adaptation, and exploitation of new vehicles generated from current technology, new trends of retail shops/food commercialization, and changes in the behavior of food consumption impose challenges in the control of these pathogenic bacteria. In this scenario, the constant monitoring of transmission routes, outbreaks, clinical manifestations, and emerging new strains of well-known food pathogens are necessary to increase knowledge and develop new strategies of prevention and management of food-related diseases (Newell et al., 2010).

3.4.1   *Brucella* spp.

Brucellae are a group of Gram-negative, nonspore-forming, nonencapsulated coccobacilli composed by six recognized species (*B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae*) that can infect animals and humans. Animals are considered as the main reservoirs of these bacteria where *B. abortus* infects bovines, *B. melitensis* causes brucellosis in caprine

and ovine and *B. suis* is the agent associated with swine species which can lead to abortion and economic loss in meat chain. Other *Brucella* species were reported to infect dogs (*B. ovis* and *B. canis*) and rats (*B. neotomae*) although some strains were isolated from bison, wild boars, caribou, seals, and dolphins, and were also reported as carriers of these bacteria. Particularly for marine mammals, two new *Brucella* species were proposed: *B. pinnipediae* and *B. cetaceae* (Cloeckaert et al., 2001; Pathak et al., 2014).

Pasteurization is a well-known thermal processing that effectively reduces *Brucella* spp. counts. The time required to kill 90% of *B. abortus* counts (*D*-value) in milk at 59−63°C was between 4.7 and 7.3 min (Katzin, Sandholzer, & Strong, 1943). Increasing in temperature reduces the time of thermal processing since at 61°C requires 2−6 min, whereas 67°C demands 6−17 s to reduce *B. abortus* counts in milk (Kronenwett, Lear, & Metzger, 1954). Similarly, *B. suis* could also be destroyed by pasteurization as reported for milk heated at 62°C for at least 7 min (Park, Graham, Prucha, & Brannon, 1932). The pH value can also influence the development of *Brucella spp.* since the optimal range of pH is between 6.6 and 7.4, which is close to that observed for milk (pH = 6.6−6.7). However, in pH values lower than 4.1−4.5, the development of these bacteria is inhibited (Falenski et al., 2011). However, the consumption of unpasteurized food increases the risk of brucellosis. The infection in humans mainly occurs by contact or consumption of raw or undercooked meat, unpasteurized milk, and dairy products and raw fish. The occupational exposure is the main cause of transmission in humans in farmers, slaughterhouse workers, veterinarians, and researchers (Franco, Mulder, Gilman, & Smits, 2007). The clinical manifestations associated with brucellosis are wide: intermittent fever, headache, nausea, vomiting, night sweats, progressive deterioration in visual function, periorbital pain, and periodic generalized tonic-clonic seizures, and other impacts in hepatobiliary, genitourinary, musculoskeletal, cardiovascular, and integumentary systems. However, the information available about this disease is limited (Atluri, Xavier, de Jong, den Hartigh, & Tsolis, 2011; Franco et al., 2007; Sohn et al., 2003).

The data from the EFSA regarding the cases of brucellosis revealed that in Europe Union 437 cases were registered in 2015. In food-related cases, milk was the source of infection (European Food Safety Authority, 2016). Regarding studies that evaluated *Brucella* spp. outbreaks, an outbreak in Nigeria in the late 1970s occurred due to the settling of nomadic populations after the local civil war. After this event, improvements were done in intend to reduce future outbreaks but the combination of pastoralist movements and growing demand for food poses the risk of *Brucella* outbreaks in recent days (Ducrotoy et al., 2014). In Arab countries, the incidence of human brucellosis was reported high in Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan, and Oman, where the main species was *B. melitensis*. In addition, the consumption of unpasteurized dairy products was associated with brucellosis

in Algeria, Jordan, Kuwait, Oman, Saudi Arabia, Lebanon, Palestinian Authority, and Syria (Refai, 2002). Brucellosis is also considered as a travel disease. The occurrence of new cases in regions where this illness is under control is usually traced back to endemic regions along with consumption of contaminated nonpasteurized milk products (Norman, Monge-Maillo, Chamorro-Tojeiro, Pérez-Molina, & López-Vélez, 2016). The occurrence of new cases in areas with low or zero cases of brucellosis can also be associated with gastronomic habits of immigrants as reported for Moroccan immigrants in Spain. All nine cases in this outbreak were caused by consumption of unpasteurized raw milk (Ramos et al., 2008).

3.4.2 *Campylobacter* spp.

Campylobacteraceae are a group of microaerophilic Gram-negative, nonspore-forming, cytochrome oxidase positive, curved, and commensal bacteria composed by 27 species: *Campylobacter avium*, *C. canadensis*, *C. coli*, *C. concisus*, *C. corcagiensis*, *C. cuniculorum*, *C. curvus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. hominis*, *C. hyointestinalis*, *C. insulaenigrae*, *C. iquaniorum*, *C. jejuni*, *C. lanienae*, *C. lari*, *C. mucosalis*, *C. peloridis*, *C. rectus*, *C. showae*, *C. sputorum*, *C. subantarcticus*, *C. troglodytis*, *C. upsaliensis*, *C. ureolyticus*, and *C. volucris*. The most common reservoirs of *Campylobacter* spp. are the intestine of many animals such as cattle, sheep, poultry, pets, and wild animals (Huang, Brooks, Lowman, & Carrillo, 2015; Ngulukun, 2017; Silva et al., 2011). Poultry and turkeys are considered as the primary reservoirs of *Campylobacter* spp. and usually show little or no symptoms of infection that could be associated with *Campylobacte*r infection (Lam, DaMassa, Morishita, Shivaprasad, & Bickford, 1992).

Campylobacter species require temperature around 41°C which reduces the ability of these bacteria to develop outside hosts and during processing and storage of food. Thermal processing such as pasteurization is effective in the inactivation of vegetative cells since the *D*-values for 50−55°C are between 0.25 and 8.77 min. Reduction of pH to values below 4.9 can inhibit *Campylobacter* spp. development. The optimal pH range for *Campylobacter* spp. development is 6.5−7.5 (Silva et al., 2011). Gastrointestinal infection in humans caused by *Campylobacte*r species is mainly associated with *C. jejuni*, *C. coli*, and *C. lari* species (Huang et al., 2015). The clinic manifestation of campylobacteriosis includes watery diarrhea (with blood in some cases), fever, and abdominal cramps. Regarding fever, more than 90% of cases reported fever for almost 1 week with temperature lower than 40°C when the diseases resolve without antibiotic treatment (Allos, 2001; Black, Levine, Clements, Hughes, & Blaser, 1988).

According to data from the European Food Safety Authority (EFSA), campylobacteriosis was the leading zoonotic disease in humans with more than 229.000 cases in 2014 with an average of 65.5 cases per 100.000

habitants in Europe Union. The EFSA also highlights that campylobacteriosis rates have been increasing since 2008 and occurrence in broiler meat remains high although the case fatality rate remained low, around 0.03%. The seasonality is also an important factor to consider in campylobacteriosis since the incidence of new cases increases during summer (European Food Safety Authority, 2016).

Regarding cases of campylobacteriosis and *Campylobacter* cross-contamination incidents, an outbreak of campylobacteriosis in east of England in 2011 occurred due to consumption of undercooked chicken liver pâté causing 49 cases. Microbiological assays revealed that this outbreak was caused by *C. jejuni* and *C. coli.* ingestion (Edwards et al., 2014). In a restaurant in Liverpool (England), one confirmed case and two probable cases were associated with *Campylobacter* infection in 2011. Although the microbiological counts of food from the day of infection were not determined due to delay onset and reporting, the most probable infected food were king prawns and chilli sauce. Cross-contamination between raw chicken liver and cooked food was suggested to explain this outbreak (Farmer, Keenan, & Vivancos, 2012). Similarly in Barcelona (Spain), a *Campylobacter* outbreak was caused by poor food handling hygiene and deficient kitchen facilities. Raw chicken meat contaminated cooked food served to primary school children in canteen that means indicated cross-contamination (direct transfer of bacteria to cooked food). A total of 75 cases were registered in this outbreak (Calciati et al., 2012).

3.4.3   *Salmonella* spp.

Salmonellae are a group of facultative anaerobic, Gram-negative, rod-shaped bacteria in only two species: *Salmonella enterica* and *S. bongori* with more than 2600 serovars. The six subspecies of *S. enterica* are: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica* according to World Health Organization (WHO) Collaborating Centre for Reference and Research on Salmonella (Brenner et al., 2000; Issenhuth-Jeanjean et al., 2014). Although these bacteria are present in intestine of mammals, birds (particularly poultry), and man worldwide (Ellis, 1969; Hoelzer, Moreno Switt, & Wiedmann, 2011), the presence of *Salmonella* spp. has also been reported in water, sediments, aquatic flora (Jyoti et al., 2011), and reptiles (Schröter et al., 2004).

Thermal processing such as pasteurization can reduce the *Salmonella* spp. counts since the *D*-value at 60°C is between 0.27 and 1.0 min for whole egg and scrambled egg mix, respectively (Garibaldi, Straka, & Ijichi, 1969). In chicken skin, the *D*-value at 60°C was reported in a range of 1.9−2.5 min (Yang, Li, & Johnson, 2001). The ideal pH for *S. enteritidis* was noticed between 5.9 and 6.5 (Blackburn, Curtis, Humpheson, Billon, & McClure, 1997),

although the growth of *Salmonella* spp. can occur at pH 4.05 (Chung & Goepfert, 1970).

Ingestion of *S. enterica* species is associated with one of the following syndromes: enteric fever, enterocolitis with diarrhea, bacteremia (bacterial infection in the blood), and chronic asymptomatic carriage. The intensity and extension of symptoms depend on host susceptibility and *S. enterica serovar* (Coburn, Grassl, & Finlay, 2007). Salmonellosis was the second most common zoonotic disease in Europe Union in 2015 with more than 94,000 cases (average of 21.2 cases per 100,000 habitants). Among all *Salmonella serovars*, *S. enteritidis* and *S. typhimurium* were responsible for more than half of all confirmed salmonellosis cases. The main contaminated foods with *Salmonella* spp. were poultry (minced or meat preparations) and turkey meat although a smaller number of cases was associated with pig and bovine meat consumption. Eggs were rarely involved in salmonellosis cases. Another important data revealed was the seasonality of salmonellosis because it is a disease with peaks during the summer months when the average of cases increase since 2008 (European Food Safety Authority, 2016).

Outbreaks of foodborne and cross-contamination with *Salmonella* are associated with a variety of foodstuff due to poor sanitation practices, poor equipment design, deficient control of ingredients, and inadequate thermal processing (Carrasco, Morales-Rueda, & García-Gimeno, 2012). *S. enterica* serovar *enteritidis* was the pathogen responsible for 327 cases at a London prison in 2009 when the prison inmates displayed diarrhea, headache, abdominal pain, fever, and vomiting. The most probable cause was associated with consumption of egg cress rolls (Davies et al., 2013). In Turkey, two outbreaks occurred in 2009: four youth hostels and two private schools in Diyarbakir. In youth hostels, a meal composed by chicken and potatoes was the main cause, whereas a green salad was the cause in the private schools. In both incidents, investigation of probable causes revealed that the contaminated food was bought from the same company. A total of 346 cases were registered in these two outbreaks (Dorman et al., 2011).

In Birmingham (United Kingdom) apparently nonrelated gastroenteritis cases were associated with consumption of iceberg lettuce contaminated with *Salmonella* Braenderup in 2003 during a period of 5 days. Among the 145 cases, diarrhea, abdominal pain, and nausea were the predominant symptoms. However, the mechanism of contamination remains unclear (Gajraj, Pooransingh, Hawker, & Olowokure, 2012). In 2007, a *S. enterica* outbreak in Pennsylvania (United States) was caused by inappropriate cooking of turkey pot pie. A total of 396 cases were registered with diarrhea or bloody diarrhea. This incident was associated with inadequate information in products labeled as inconsistent information and undefined microwave wattage since the frozen pie was a not-ready-to-eat microwavable food (Mody et al., 2014).

3.4.4   *Yersinia* spp.

The genus *Yersinia* is characterized by its Gram-negative, rod-shaped, nonspore-forming bacteria. Fifteen species are included in this genus: *Y. pestis*, *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. aldovae*, *Y. bercovieri*, *Y. entomophaga*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii*, *Y. rohdei*, *Y. ruckeri*, *Y. aleksiciae*, *Y. massiliensis*, and *Y. simili* (Hurst, Becher, Young, Nelson, & Glare, 2011). In history, *Y. pestis* is the most relevant species due to the two pandemics events known as the Justinian plague (Roman Empire between 541 and 767 *anno Domini*) and the Black Death (Europe between 1346 and 19th century), which have evolved and may cause isolated cases (Achtman et al., 2004). *Y. enterocolitica* is commonly found in many farm animals (particularly pig), mammals (e.g., rodents, dogs, and cats), surface water, and sewage. This species is the main common cause of yersinosis in humans (Bottone, 1999).

Pasteurization is suitable to reduce the counts and even kill *Y. enterocolitica* in minced beef. The *D*-value at 50°C was reported between 17.4 and 26.3 min, while at 55°C the time was reduced to 0.65−1.96 min, and at 60°C, the *D*-value was in the range of 0.07−0.97 min (Bolton et al., 2000; Doherty et al., 1998). In milk, *D*-values of *Y. enterocolitica* at 57.2°C (4.6−14.7 min) and 62.8°C (0.18−0.96 min) were dependent on the strain (Lovett, Bradshaw, & Peeler, 1982). The control of pH value is also an important factor in the growth of *Y. enterocolitica* since these bacteria can develop in values between 4.5 and 8.5 (Bhaduri, Buchanan, & Phillips, 1995). Yersinosis, particularly from *Y. enterocolitica*, displays diarrhea and abdominal pain as main symptoms although pseudoappendicitis (similar to appendicitis but without inflammation) may occur in older children and young adults (Rosner, Werber, Höhle, & Stark, 2013). Yersiniosis was third most common zoonotic disease in Europe Union in 2015 with 7202 cases (average of 2.20 cases per 100.000 habitants). *Y. enterocolitica* was the most common species in all reported cases. Although yersinosis cases usually increase during May−August period (summer), the average rate decreased since 2008 (from 2.33 to 2.20 cases per 100.000 habitants) (European Food Safety Authority, 2016).

The incidence of yersinosis associated with food contamination was reported worldwide. A case in yersinosis (*Y. pseudotuberculosis*) was noticed in Taiwan. Only one case was registered in that occasion and symptoms were intermittent fever, abdominal pain, diarrhea, and progressive jaundice (yellowish skin pigmentation) for 5 days. The suggested cause of infection was raw food: sashimi (from fish and cattle liver), raw Tako (octopus), and semiboiled pig's ear (Lai et al., 2014). An outbreak in Pennsylvania, United States in 2011, was caused by consumption of glass-bottled pasteurized milk contaminated with *Y. enterocolitica*. A total of 22 cases were registered along with one death. The most common symptoms were diarrhea and fever

although abdominal cramping, nausea, sore throat, and rash were also reported (Longenberger et al., 2014). Similarly, in Finland an outbreak caused by contaminated raw milk affected 43 people in 2014. Microbiological assays revealed that *Y. pseudotuberculosis* was the pathogen and associated the contamination to single producer. The contamination occurred in the filter and after the notification; the producer recalled the milk (Pärn et al., 2015).

In 2014, a yersinosis outbreak was reported from a military base and civilians from the northern Norway. That incident was associated with consumption of iceberg lettuce and radicchio rosso contaminated with *Y. enterocolitica* that produced 133 cases (MacDonald et al., 2016). In Japan, the contamination of *Y. enterocolitica* promoted different clinic manifestations in member of family. Three people in the same family carried the bacteria: two (grandmother and child) suffered from enterocolitis and the other one was asymptomatic (did not show any symptoms). The source of infection was not determined and the most probable explanation is that the grandmother was infected by the consumption of improperly cooked pork and the child by person-to-person (Moriki et al., 2010).

3.4.5 *Listeria* spp.

In the genus *Listeria* comprises rod-shaped, Gram-positive, catalase positive, nonspore-forming, noncapsulated bacteria. This genus includes 17 species: *L. monocytogenes*, *L. ivanovii*, *L. aquatica*, *L. booriae*, *L. cornellensis*, *L. fleischmannii*, *L. floridensis*, *L. grandensis*, *L. grayi*, *L. innocua*, *L. marthii*, *L. newyorkensis*, *L. riparia*, *L. rocourtiae*, *L. seeligeri*, *L. weihenstephanensis*, and *L. welshimeri*. However, only *L. monocytogenes* and *L. ivanovii* are considered pathogens to humans (Orsi & Wiedmann, 2016). The main suggested niches for *Listeria* species are soil and water although plants, animals (particularly farm animals), and man are also considered as reservoirs of *Listeria* spp. (Linke et al., 2014; Pelisser, Mendes, Sutherland, & Batista, 2001).

The pasteurization of food contaminated with *Listeria* spp. can reduce the counts of these bacteria. In mince beef, the *D*-values at 50°C, 55°C, and 60°C are 32.7−36.1, 3.2−3.5, and 0.15−0.33 min, respectively (Bolton et al., 2000; Doherty et al., 1998). The minimum pH value that allows *L. monocytogenes* development was reported around 4.3−5.0 (Farber, Sanders, Dunfield, & Prescott, 1989). When humans are contaminated with *Listeria*, particularly *L. monocytogenes*, a variety of symptoms can be observed as: abdominal cramps, diarrhea, headache, nausea, vomiting, fever, myalgia, general malaise, arthralgia, confusion, and neck stiffness (Awofisayo-Okuyelu et al., 2016). In the case of pregnant women, other symptoms reported were anisocoria (pupils of different sizes), aphasia (communication disorder), facial nerve paralysis, pleural effusion (excessive amount of fluids around the lung), sepsis (infection in the blood), ascites (accumulation of fluid in the membrane of abdominal organs), and tachycardia (Kiefer et al., 2016).

Among countries of UE more than 2200 cases of listeriosis were registered in 2015 (average of 0.46 cases per 100.000 habitants). Although listeriosis affected a less people in Europe Union in 2015, the number of deaths was the highest since 2008: 270 deaths that mainly affected elder people over 65 years old (European Food Safety Authority, 2016). Outbreaks due to contaminated food are registered worldwide with elevated number of deaths. In 2012, an outbreak of listeriosis involved 22 cases and 4 deaths in United States due to consumption of ricotta salata (a semisoft cheese produced from sheep milk) produced in Italy. The investigation revealed that processing plants of Italian cheese producer was contaminated with *L. monocytogenes* (Acciari et al., 2016).

In 2012, a municipal hospital in Vaasa (Finland) reported an outbreak among elderly patients. Initially, two patients displayed septicemia due to *L. monocytogenes* infection and after the evaluation of other patients in the same ward revealed that 8 patients were also infected (febrile gastroenteritis). The investigation revealed that jelly meat was the most probable cause that also infected 10 individuals in Finland in that summer (Jacks et al., 2016). In North-East Scotland, three cases of listeriosis (two elderly people and one baby) were registered due to consumption of ready-to-eat food contaminated with *L. monocytogenes*. The probable cause of infection was linked to inadequate sanitary practices, poor hand hygiene, and cross-contamination in a producer of meat products and ready-to-eat food (Okpo et al., 2015).

3.4.6 *Escherichia coli* spp.

The genus *Escherichia* is characterized by Gram-negative, nonspore-forming, and facultative anaerobes rod bacteria, which is composed by six species: *Escherichia coli*, *E. blattae*, *E. hermannii*, *E. vulneris*, *E. fergusonii*, and *E. albertii* (Abbott, O'Connor, Robin, Zimmer, & Janda, 2003; Gaastra, Kusters, van Duijkeren, & Lipman, 2014). Among all *Escherichia* species, *E. coli* is the most relevant due to the health impact associated with ingestion and colonization in human digestive system. *E. coli* bacteria are considered as worldwide intestinal pathogen with defined phenotypes: diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and Shiga toxin-producing *E. coli* (STEC)/verocytotoxin-producing *E. coli* (VTEC) (enterohemorrhagic *E. coli* [EHEC] is also included in this phenotype) (Cho et al., 2014; Scheutz, 2014). *E. coli* is present in the intestinal microflora of many farm animals (cattle, sheep, and swine), wild animals (birds and deer), pets (dogs and cats), and humans as commensal bacteria (Bach, McAllister, Veira, Gannon, & Holley, 2002). Particularly for cattle, STEC poses an important risk due to production of milk, meat, and derived products (Hussein & Sakuma, 2005).

Thermal processing such as pasteurization of food can reduce the *E. coli* counts. The *D*-value at 50°C in meat and meat products ranges between 49.50 and 115.0 min, while at 55°C the time required is reduced to 6.37−19.26 min and at 60°C less than 1 min is required (0.37−0.58 min) (Ahmed, Conner, & Huffman, 1995). In apple juice, *D*-value at 52°C was 18 min that was shown to be reduced by reduction of pH (3.6) and malic acid (0.8%) to 14 and 15 min, respectively (Splittstoesser, McLellan, & Churey, 1996). However, when thermal processing fails or posterior contamination occurs, the ingestion of *E. coli* imposes a severe risk to host health. Symptoms of intoxication by *E. coli* depend on many factors such as phenotype and host immune susceptibility that may lead to mild diarrhea, infection of urinary tract, bloodstream, and central nervous system (Hussein & Sakuma, 2005).

STEC/VTEC and EHEC induce the hemolytic uremic syndrome (HUS) which is characterized by progressive renal failure and bloody or nonbloody diarrhea induced by Shiga toxins: Stx1 and Stx2. ETEC clinic manifestations involve profuse watery diarrhea in adult travelers although the dehydration can be severe in children as a result of heat-labile and heat-stable toxin produced by this *E. coli* species. EPEC symptoms are characterized by fever, nausea, vomiting, watery diarrhea with mucus and dehydration (severe and even fatal in children). EIEC induces fever, abdominal cramps, and dysentery (Hunt, 2010).

In UE, STEC infection affected 5901 people in 2015 (average of 1.27 cases per 100,000 habitants) with 8 deaths. The main food associated with STEC contamination was meat from sheep, goat, cattle, and wild ruminants followed by raw milk and dairy products. The occurrence of STEC cases displays a seasonal trend when peaks were observed during summer (European Food Safety Authority, 2016). The occurrence of outbreaks was documented in the literature. In 2008, 341 cases were related to STEC in Oklahoma due to consumption of contaminated food in a buffet-style restaurant. Although the most probable cause of infection was cross-contamination, 25 cases developed HUS and 1 case died. The most common symptoms were diarrhea, abdominal pain, nausea, headache, fatigue, myalgia, and blood in stools (Bradley et al., 2012).

In 2011, a large outbreak spread throughout Germany leading to 3816 cases wherein 845 evolved to HUS symptoms and 54 people died due to STEC contamination. Women were particularly affected during the outbreak when gastroenteritis symptoms evolved to HUS (68% of all women). This outbreak was linked to consumption of sprouts and the STEC isolated from confirmed cases indicated that STEC was Shiga toxin-2 producer (Frank et al., 2011).

In 2002, an outbreak of EHEC was reported in Skania (Sweden). Thirty-eight cases were registered with abdominal pains, and/or diarrhea (watery or bloody) and/or HUS. The investigation revealed that a fermented sausage

was contaminated with EHEC. The lack of thermal processing, use of dormant starter culture, and short curing period were indicated as main factors that EHEC contamination (Sartz et al., 2008). A large EHEC outbreak was reported in Japan in 2011 with 86 cases. The most common clinical manifestations were diarrhea and bloody stool wherein 34 cases evolved to HUS, 21 cases displayed acute encephalopathy and 5 cases died from EHEC infection. The investigation revealed that raw beef liver and *yukhoe*, traditional Korean seasoned raw beef rib (Yahata et al., 2015) was the foodborne cause.

An outbreak in Norway 2012 was linked to ETEC contamination. A total of 214 cases displayed diarrhea, abdominal pain, nausea, fever, and vomiting as common symptoms. The investigation revealed that contaminated scramble eggs was the most probable cause of contamination during a Christmas buffet served in the hotel (MacDonald et al., 2015).

3.5   PATHOGENIC SPORE-FORMING BACTERIA

Pathogen spore-forming bacteria are a big challenge for the food industry in its continuing concern to produce safe food, reducing the number of foodborne illness. A particular emphasis is necessary with this class of microorganisms, due to their inherent ability to survive extreme processing conditions. Indeed, the bacterial spores are among the most resistant forms of living organisms. Their resistance favors their survival to food processing and long-term persistence in foods (Carlin, 2011).

Traditional microbiological detection methods used in the food industry have restrictions in terms of time, efficiency, and sensibility. Food microbiology has undergone considerable development in recent years, largely due to predictive modeling (McMeekin et al., 2008) and risk assessment (Augustin, 2011). In spite of that, its application in diverse areas of the food industry to improve the safe food security is still limited. According to Augustin (2011), the microbiological risk assessment related to probabilities and severities of adverse health effects for consumers have to include four steps: (1) hazard identification, (2) exposure assessment, (3) hazard characterization, and (4) risk characterization. This risk analysis for improving food control systems is necessary to develop the different processing methodologies for the elimination of these microorganisms, such as heat treatments, chemical based on acidification or the combination of them. Besides, in the last years, emerging technologies such as plasma and advanced oxidation have been developed. However, not all of them are efficient in eliminating spores due to the morphological features that provide resistance to hostile conditions.

Because of their ubiquitous nature, bacterial spore-formers are widely located in all types of foods. From the beginning of the 20th century, it has been studied the spore-forming bacteria, especially the pathogenic species, but rapidly, spoilage spore-forming bacteria were also investigated (Remize, 2016). Indeed, in recent years, there has been an increasing interest in spore-forming

bacteria, because they are considered a major risk in heat-treated food production plants. Mesophilic spore-forming bacteria belong essentially to two taxonomic groups: the *Bacillales* order and the *Clostridium* genus, depending on whether they are aerobic or not. *Bacillales* gathers aerobic, facultative aerobic, or facultative anaerobic bacteria. The *Bacillaceae* family covers 19 genera including *Bacillus* and *Anoxybacillus*, which comprise several thermophilic species, whereas inside the genus *Clostridium* a total of 168 species are described (Garrity & Bergey, 2009). The order *Bacillus*, *Clostridium*, *Sporolactobacillus*, *Soporosarcin*, and *Desulfotomaculum* are capable of forming endospores. For food microbiology, the first two have a special interest because of the problems caused by some species of these orders.

3.5.1 *Bacillus* spp.

Of the many species of *Bacillus* and related genera, most do not cause disease. However, there are some species that generate important diseases in humans. *Bacillus cereus* is known to be responsible for two types of foodborne diseases: the diarrheal and the emetic type. In addition, *B. cereus* is related to diseases transmitted by food. It has been isolated from other species such as *B. subilis*, *B. licheniformis*, and *B. pumilus* that have gained great importance due to the type of pathology that they produce (Scheldeman et al., 2006). Diarrheal disease is often associated with protein rich foods (meat, vegetables, puddings, and milk products) and is thought to be caused by vegetative cells (ingested as viable cells or spores) that produce enterotoxins in the small intestine (Abee et al., 2011). The emetic disease is often associated with starch-rich foods (fried and cooked rice, pasta). It was identified for the first time in the United Kingdom in the early 1970s, when microorganisms from *B. cereus* were linked to several outbreaks caused by eating cooked rice. The *B. cereus* emetic toxin, cereulide, produces the emetic disease that is present in foods before ingestion (Abee et al., 2011).

Within the genus *Bacillus*, *B. cereus* and its closest relatives form a highly homogeneous subdivision, which has been termed the "*B. cereus* group." This group comprises the species *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, and *B. weihenstephanensis* (Jensen, Hansen, Eilenberg, & Mahillon, 2003; Tourasse, Helgason, Økstad, Hegna, & Kolstø, 2006). *B. cereus* group have been isolated from a broad range of environments, ranging from soils, plant rhizospheres, insects, animals, and obviously humans (Jensen et al., 2003; Vilain, Luo, Hildreth, & Brözel, 2006). Most studies related to genomic analysis have indicated that *B. cereus* is specialized on protein metabolism; therefore, this fact suggests that it has adapted toward a symbiotic or parasitic life cycle (Ivanova et al., 2003; Stenfors Arnesen, Fagerlund, & Granum, 2008).

3.5.2 *Clostridium* spp.

Clostridium are rod-shaped, Gram-positive bacteria capable of producing spores. The genus *Clostridium* gathers all mesophilic anaerobic spore-formers encountered in food. According to the Health Protection Agency, the *Clostridium* genus consists of more than a hundred known species, including harmful pathogens such as *C. botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *C. butyricum*, and *Clostridium sordellii*. The anaerobic status of these bacteria is a strong driver for niche differentiation compared to *Bacillales* representatives. Besides, they can be differentiated based on their biochemical activities, including saccharolysis and proteolysis. The first four members produce powerful toxins released from vegetating (growing) bacteria, that is, *C. botulinum*, produces the most powerful toxin known to man, which is the key virulence factor responsible for the pathogenesis of disease. Its action is the reverse of that of tetanus toxin, that is, it prevents muscle contraction, leading to flaccid paralysis of important muscles. It inhibits the release of acetylcholine at motor nerve endings in the parasympathetic nervous system. This potent toxin is relatively heat-resistant (although destroyed by temperatures $>60°C$). *C. difficile* is found in the feces of $3-5\%$ of humans, in the gut of several animals and in the environment. The organism produces at least two potent toxins that are responsible for severe and occasionally fatal diarrhea. *C. perfringens* produces a number of potent toxins, the most important of which is the a-toxin (phospholipase C), which causes host cell lysis and finally *C. tetani* produces an extremely potent single toxin (tetanus toxin) which underlies the pathogenesis of tetanus. Tetanus toxin consists of two components, including the neurotoxic "tetanospasmin" and the hemolytic tetanolysin. The toxin prevents muscle relaxation, leading to persistent contraction of facial and body muscles.

3.5.3 Sporulation and Germination Process and Morphology Spore

Formation, resistance, and germination of spores have been widely studied, essentially for *Bacillus*. According to several authors (Piggot & Hilbert, 2004; Sonenshein, 2000) sporulation occurs in response to a high cellular density, nutritional limitations, or cellular communication. The resistance of spores is developed during the sporulation. These spores are metabolically dormant and resistant to heat, radiation, desiccation, pH extremes, and toxic chemicals (Setlow, 2000). The dormant spores also monitors the environment around theirs, and when conditions are again favorable (presence of germinants as nutrients such as amino acids, sugars, purine nucleosides, or other agents as lysozyme or salts) for growth, the spore germinates and goes through outgrowth, ultimately being converted back into a growing cell
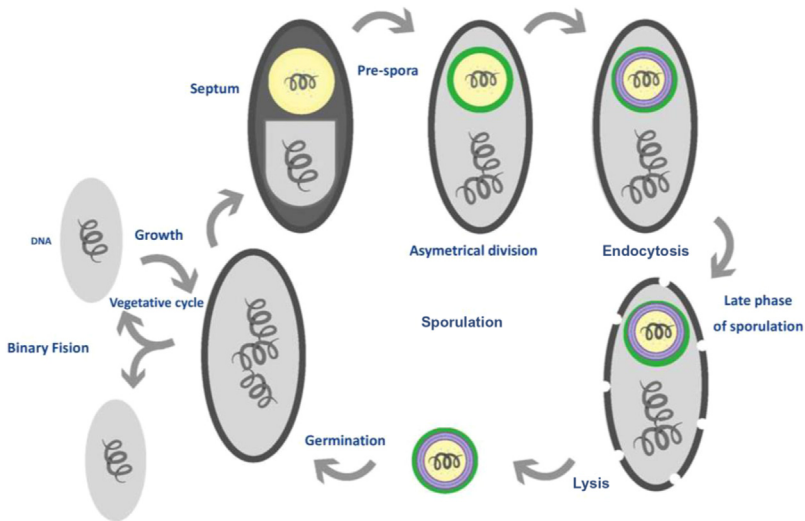
FIGURE 3.1   Sporulation and cycle of germination of the *B. subtilis*. *Adapted from McKenney, P.T., Driks, A., & Eichenberger, P. (2013). The Bacillus subtilis endospore: Assembly and functions of the multilayered coat.* Nature Reviews Microbiology, 11*(1), 33−44 (McKenney, Driks, & Eichenberger, 2013).*

(Paidhungat & Setlow, 2002). The transition from spore to vegetative cell involves three distinct phases: activation, germination, and growth. In Fig. 3.1, schematic diagram of the sporulation and cycle of germination of the *B. subtilis* can be observed.

Spore wraps are multilayered, and usually are distinguished from spore core to external medium. They are composed of an internal membrane, a cortex, an external membrane, a coat, and possibly an exosporium. Each layer presents specific structural, biochemical, and permeability properties (Remize, 2016). Exosporium is a thin delicate covering made of proteins. Inner and outer spore coats are composed of layers of spore-specific proteins. The cortex is composed of loosely linked peptidoglycan and contains dipicolinic acid, which is particular to all bacterial endospores. This acid cross links with calcium ions embedded in the spore coat and contributes to the extreme resistance capabilities of the endospores because it creates a highly impenetrable barrier. Finally, they contain the usual cell wall and, cytoplasmic membrane, nucleoid, and cytoplasm. The core only has 10%−30% of the water content of vegetative cells; therefore, the core cytoplasm is in a gel state. The low water content contributes to the endospores' success in dry environments. Schematic example of spore-forming bacteria structure is depicted in Fig. 3.2.
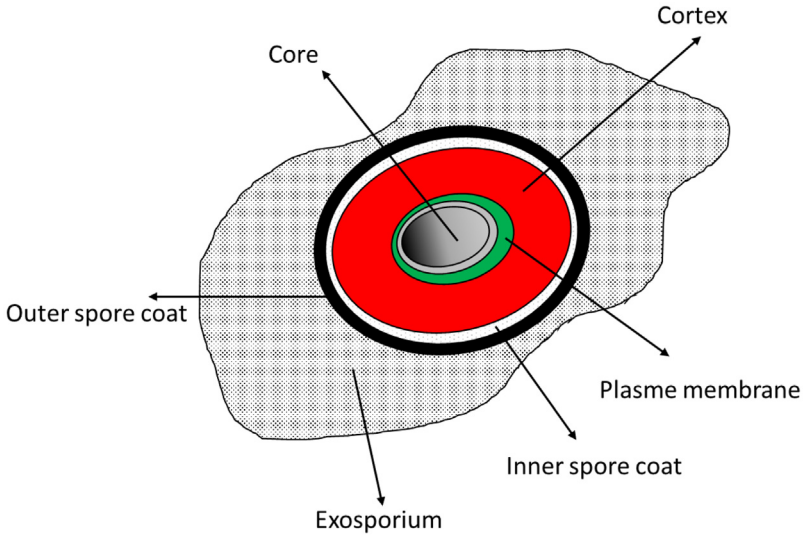
FIGURE 3.2   Structure of spore-forming bacteria.

3.5.4   Contamination of Bacterial Spores to Food and Inactivation Methods

Soil is considered as a major habitat of spore-forming bacteria. *B. cereus* and *C. botulinum* have been detected worldwide in soil samples (Dodds, 1993) with levels in soil that can reach $10^5 - 10^6$ spores/g of soil (Lund, 1986; Te Giffel, Beumer, Slaghuis, & Rombouts, 1995). The spores ingested with fecal and other soil materials colonize the gut of animals and may develop a symbiotic relation with their hosts (König, 2006). Besides, soil contamination can transfer to plant material (silage) and to feed. According to Magnusson, Christiansson, and Svensson (2007), the major cause of contamination into foods is related to dispersion of spores by fecal material of warm-blooded animals. Indeed, the microorganism, *C. perfringens* is a common colonizer of the gastrointestinal tract of mammals (including man) (Brynestad & Granum, 2002) and *C. botulinum* has been identified in cow and pig feces collected in abattoirs (Dahlenborg, Borch, & Rådström, 2003). On the other hand, food-processing facilities can be also the source of spores in foods, in fact milking machinery and apparatus have been indicated as sources of milk contamination (Scheldeman, Pil, Herman, Vos, & Heyndrickx, 2005). Specifically, Svensson, Ekelund, Ogura, and Christiansson (2004) have noticed the presence of a persistent *B. cereus* spore, due to their resistance to cleaning operations because many of them have strong adhering properties and ability to form biofilms (Auger et al., 2009). Overall, it is worthy to say that processed foods are increasingly a

mixture of multiple ingredients and additives, each bringing its own spore-forming bacteria into the final food products. Works in the literature clearly demonstrate that the origins of *B. cereus* spores are very diverse, depending on factors such as the microbial ecology, animal feeding, the farming management, the climate, and the hygienic practices during food processing.

A wide variety of methods have been developed for producing safe foods, classified in (1) physical, (2) chemicals, and (3) others, within which emerging technologies can be mentioned. Physical methods of inactivation of microorganisms are those, based on the use of temperatures and modified pressures with the aim of altering cell structures or essential biomolecules for their metabolism as high-pressures (HPP) and ionizing radiations (ultraviolet and infrared). Regarding the first methodology, Zhang and Mittal (2008) reported successful conditions with HPP for the inactivation of spores of various species. However, these authors affirm that due to the complicate intrinsic and extrinsic factors, HPP conditions should be verified case by case for required sterility. It has been studied that the effect of infrared radiation on the inactivation of spores, due to this radiation is quickly converted to heat, producing an intense local heating on spores (Mamouni, Tang, Wu, Vlahovic, & Yang, 2011). Explanation is based on local heating over the spores produce mechanical abrasion causing the germination by the activation of the lysis enzymes (Dong, Tang, Wu, Vlahovic, & Yang, 2013). These enzymes are present in the spore in an active form, but they do not act until they are activated by the mechanical damage.

Within the chemical processes, the most used are acidification and smoking. Such processes can generate added value to the product by improving sensorial characteristics of the final product. However, these treatments are not effective in spore elimination. Spores are extremely resistant to a variety of chemicals, including acids, bases, oxidant and alkylating agents, aldehydes and organic solvents. However, treatments with strong acids can eliminate spores, probably due to the rupture of the inner membrane (Setlow, 2006). On the contrary, treatment with a strong alkali is less effective because they can be reactivated with an adequate recovery by lysozymes (Setlow, 2006). Within emerging technologies, we can highlight plasma and advanced process of oxidation based on radical generation. Plasma has been reported to inactivate both vegetative cells and bacterial endospores. Three basic mechanisms have been attributed to the inactivation of spores: (1) DNA degradation by UV radiation, (2) volatilization of compounds of the spore surface by UV photons, and (3) erosion of the spore surface by the adsorption of reactive molecules as free radicals (Philip et al., 2002).

Finally, advanced oxidation processes are based on the generation of hydroxyl radicals using different precursors (UV, hydrogen peroxide, ozone, electrons, photo catalysis with titanium dioxide, Fenton reagent, etc.). All of these treatments have been reported as highly efficient in the elimination of microorganisms (Bondala, Castillo, González, & Sanchez-Salas, 2011),

because they produce damage on external spore (mainly in inner membrane). Then when the treated spores germinate, these ruptures in the membrane have a fatal consequence in its viability (Shapiro, Setlow, & Setlow, 2004). In particular, the Fenton reaction has been tested for elimination of spores in water with successful results (Bondala et al., 2008). However, studies to establish whether these processes can be widely used in other foods, without a possible change in sensory characteristics must be developed.

3.6   YEASTS AND MOLDS

The use of microorganisms to obtain different types of food, such as beer, wine, bread, cheeses, and fermented milk is very old. There have been reports of application of fermentation processes for the production of foods from times before Christ. In the 20th century, the industrial microbiology expanded even more, because they perceived new possibilities for obtaining large variety and quantity of products by fermentative processes.

3.6.1   Yeast

Yeasts are fungi that grow as solitary cells that reproduce by budding and can be classified either to *Ascomycetes* (e.g., *Saccharomyces*, *Candida*) or *Basidiomycetes* (e.g., *Filobasidiella*, *Rhodotorula*) (see Table 3.1).

Yeast taxa are distinguished on the basis of the presence or absence of capsules, the size and shape of the yeast cells, the mechanism of daughter cell formation (conidiogenesis), the formation of *pseudohyphae* and true *hyphae*, and the presence of sexual spores, in conjunction with physiologic data. Morphology is employed primarily to distinguish yeasts at the genus level, whereas the ability to assimilate and ferment various carbon sources and to utilize nitrate as a source of nitrogen are used in conjunction with morphology to identify species. Currently, there are approximately 1500 recognized yeast species listed in the latest edition of the yeasts: a taxanomic study (Fell, Kurtzman, & Boekhout, 2011) and estimated total number is around 150,000 forming roughly 5%−10% of estimated fungal species. Of all these yeast species, only about 12 are employed at industrial scale, and around 70−80 species were used at laboratory scale to possess potential value in biotechnology (Deak, 2009; Fell et al., 2011).

Traditionally, the yeast has been used for the production of alcoholic beverages, biomass, and glycerol. In this regard, *Saccharomyces cerevisiae* is famously known for its role in food production. It is the critical component in the fermentation process that converts sugar into alcohol; an ingredient shared in beer, wine, and distilled beverages. It is also used in the baking process as a leavening agent; yeast-releasing gas into their environment results in the spongy-like texture of breads and cakes. Because of its role in fermentation, humans have known about and used *S. cerevisiae* for a long

TABLE 3.1 Biotechnologically Important Yeast Spp.
(Johnson, 2013a, 2013b)

Ascomycetous	Basidiomycetous
Saccharomyces cerevisiae	Rhodotorula spp.
Schizosaccharomyces pombe	Rhodosporidium spp.
Kluyveromyces lactis	Trichosporon spp.
Kluyveromyces marxianus	Xanthophyllomyces dendrorhous
Schwanniomyces occidentalis	Cryptococcus spp.
Lipomyces spp.	Phaffia rhodozyma
Saccharomycopsis spp.	
Debaryomyces hansenii	
Ogataea polymorpha	
Komagataella pastoris	
Scheffersomyces stipitis	
Pichia spp.	
Yarrowia lipolytica	
Candida spp.	
Blastobotrys adeninivorans	

time. Other yeast involved in the common fermented foods and beverages such as breads and bakery products, dairy products (e.g., kefir; yoghurt, fermented milk), fermented meat and sausages, cheeses, beers, wines, etc. are: *Kluyveromyces, Galactomyces, Hyphopichia, Pichia, Saccharomycodes, Rhodotorula, Metschnikowia, Saccharomycopsis, Yarrowia, Cryptococcus, Brettanomyces, Debaryomyces, Hansenula, Schizosaccharomyces, Hanseniaspora, Trichosporon, Torulopsis, Geotrichum, Zygosaccharomyces*, and *Candida*.

In addition to traditional industrial use, there are applications of yeasts in several food fermentations such as alcoholic beverages, sausages, cheese, bakery products, and other fermented foods. Modern applications of yeasts involve the production of single-cell proteins, ethanol, industrial enzymes, foodstuffs and fodder, and small molecular weight metabolites (see Fig. 3.3). Finally, yeasts also have important roles in agriculture as agents of biocontrol, bioremediation, and as indicators of environmental quality.

On the other hand, the spoilage yeasts of drinks and foods have gained an increasing importance in food technology, being responsible for significant

FIGURE 3.3 Modern applications of yeasts. *Adapted from Walker, G.M. (2000).* Yeast physiology and biotechnology. *Chichester: Wiley (Walker, 2000).*

economic losses (Thomas, 1993). In this regard, Pitt an Hocking (1999) noticed that only about 10 species of yeasts (*Dekkera bruxellensis, Zygosaccharomyces bisporus, Schizosaccharomyces pombe, Issatchenkia orientalis, Debaryomyces hansenii, Candida holmii, Pichia membranifaciens, Zygosaccharomyces bailii, Saccharomyces cerevisiae, Zygosaccharomyces rouxii*, and *Kloeckera apiculata*) are responsible for the spoilage of foods that have been processed and packaged. The deterioration of food and drinks caused by yeasts is summarized in Table 3.2.

3.6.2 Molds

The term *mold* is commonly applied to certain multicellular, filamentous fungi whose growth on foods is usually readily recognized by its fuzzy or cottony appearance. Generally, molds are concerned in the spoilage of foods where moldy or mildewed foods are considered unfit to eat. The gross

TABLE 3.2 Principal Spoilage Effects Caused by Yeast Activity in Foods (Loureiro & Querol, 1999)

Food Product	Spoilage Effect							
	Surface Growth	Discoloration	Gas Production	Haze/Cloudiness	Sediments	Films	Off-flavors	Texture Changes
Fresh vegetables	•	•					•	•
Brined vegetables	•	•	•			•	•	•
"Ready-to-eat" vegetables	•		•				•	
Fresh fruits		•	•				•	•
Fruit juices			•	•	•	•	•	
"Ready-to-eat" fruits	•		•				•	
Mayonnaise, salad dressings		•	•			•	•	•
Wine, beer			•	•	•	•	•	
Soft drinks			•	•	•		•	
Confectionery, jams, jellies	•	•	•	•		•	•	•
Syrups, honey, fruit concentrates			•	•		•	•	
Butter, cream		•					•	
Cheeses		•	•				•	
Yoghurts			•			•	•	
Sliced bread	•	•					•	
Unbaked bread dough	•	•					•	
Sausages, meat products	•	•	•				•	

appearance of a mold growing on a food is often enough to indicate its genus. In this regard, some look dry and powdery, some look velvety on the upper surface, and others gelatinous or wet, whereas some molds are compact and others are loose and fluffy. In addition, the pigments in the mycelium (black, purple red, gray, etc.) are also characteristic.

Molds are characterized by the development of *hyphae* (a mass of branching, interwined filaments), which result in the colony characteristics (known as a *mycelium*). In general, most molds require less available moisture than required for most yeast and bacteria and the optimal temperature for most molds range from 25°C to 30°C. In addition, molds grow on the surface of food (they require free oxygen for growth) and in a wide range of pH values (from 2 to 8.5), but the majority is favored by an acid pH. The most important molds from the industrial point of view are as follows:

Mucor that have been used for centuries in food manufacturing for cheese ripening or Asian fermented food production. The genus *Mucor* contains several species. The most common ones are *Mucor amphibiorum, M. circinelloides, M. hiemalis, M. indicus, M. racemosus*, and *M. ramosissimus.*

Aspergillus molds are very widespread, they are involved in the spoilage of foods, and some are useful in preparation of fermented foods. This genus grows well in high sugar and salt concentrations and hence in many foods with low moisture content. The *Aspergillus* genus comprises 344 species (Samson et al., 2014), and the chemodiversity among these species is very high.

Rhizopus are involved in the spoilage of many foods such as vegetables, berries, bread, and fruits. The genus *Rhizopus* includes several species. The most common ones are, namely, *Rhizopus arrhizus, Rhizopus azygosporus, Rhizopus microsporus, Rhizopus schipperae*, and *Rhizopus stolonifer.*

Penicillium is well known and one of the most common fungi. It has a worldwide distribution and a large economic impact on human life. Its main function in nature is the decomposition of organic materials, where species cause devastating rots as pre- and postharvest pathogens on food crops (Frisvad & Samson, 2004; Pitt & Hocking, 2009), as well as producing a diverse range of mycotoxins (Frisvad & Samson, 2004). In addition, some species also have positive impacts, with the food industry exploiting some species for the production of specialty fermented sausages (López-Díaz, Santos, García-López, & Otero, 2001; Ludemann, Greco, Rodríguez, Basílico, & Pardo, 2010) and cheeses, such as Camembert or Roquefort (Giraud et al., 2010).

Alternaria are involved in the spoilage of many foods such as potato and tomatoes. *Alternaria* spp. are among the most well-known producers of diverse secondary metabolites, especially toxins (Montemurro & Visconti, 1992). The most common species are *Alternaria citri, Alternaria tenuis*, and *Alternaria brassicae.*

Bothrytis are important pathogens of many agronomically important crops, such as grapevine, tomato, bulb flowers, and ornamental crops (Jarvis, 1977).

Molds also play an important role in the ripening of some dry-fermented products. The uses of mold cultures contribute to the development of the typical flavor through its proteolytic, lipolytic, β-oxidative, and deaminitive activities (Sunesen & Stahnke, 2003). In addition, molds growth on the surface food products exerts antioxidative effects due to their obligate requirement for oxygen consumption for respiratory purposes and physical barrier effects due to the presence of their mycelial growth, which reduces the penetration of oxygen, and light into the product (Bruna et al., 2003). Additionally, surface molds can also have a protective role against pathogenic or spoilage microorganisms by colonizing product surfaces and out-competing microbial competition (Ludemann, Pose, Pollio, & Segura, 2004).

3.7 VIRUSES AND PARASITES

3.7.1 Viruses

Viruses are obligate intracellular parasites that cause a wide range of diseases in plants, animals, and humans. As obligate parasites, viruses must continue to find new host cells and individuals, and be able to use the biochemical reactions of the cell to replicate. Consequently, virus multiplication will not occur in foods that can act only as a passive vehicle in the transmission of infection (Adams & Moss, 2008). They can be transmitted in different ways but the infection of the cells lining the intestinal tract and dispersed by shedding into the stool or through emesis were the most important foodborne infections. Several different viruses may cause a single disease, as determined by the tissue or organ that is affected, or one virus may cause a unique disease due to the nature of its interaction with the body (Rosenthal, 2009). Numerous viruses can be found in the human gut, but only few are commonly recognized as important foodborne pathogens. According to the type of illness that they produce, viruses can be classified into three groups (Koopmans & Duizer, 2004):

- Viruses that causes gastroenteritis
- Enterically transmitted hepatitis viruses
- Viruses that cause illness after they migrate to other organs, such as the central nervous system or the liver.

The number of foodborne viruses is relatively small, but the Norovirus and Hepatitis A are currently recognized as the most important human foodborne pathogens with regard to the number of outbreaks and people affected in the world (Cliver, 1997). Both viruses are highly infectious and may lead to outbreaks. There are other viruses involved in the transmission of food or waterborne but they occur occasionally: Hepatitis E, Aichivirus, Astrovirus, Coronavirus, Rotavirus, and Saporavirus.

3.7.1.1 Norovirus

Norovirus is a very contagious virus that can infect anyone, from an infected person, contaminated food or water, or by touching contaminated surfaces. The clinical manifestation of the norovirus is relatively mild, about 50% of all outbreaks of food-related illness are caused by norovirus. The virus causes inflammation of stomach or intestines or both. This leads to have stomach pain, nausea, and diarrhea and to throw up. These symptoms can be serious for some people, especially young children and older adults (CDC, 2017a), and many people are infected in a very short time. Most outbreaks of norovirus illness happen when infected people spread the virus to others. However, norovirus can also be spread by consuming contaminated foods or water. Food can be contaminated with norovirus at any point when it is being grown, shipped, handled, or prepared. Foods that are commonly involved in outbreaks of norovirus illness are: leafy greens (such as lettuce), fresh fruits, and shellfish (such as oysters).

3.7.1.2 Hepatitis

3.7.1.2.1 Hepatitis A

Hepatitis A virus (HAV) is classified as a picornavirus. Primates are the only natural host (Balayan, 1992). There is only 1 HAV serotype, and immunity after infection is lifelong (Lemon, Jansen, & Brown, 1992). After ingestion, uptake in the gastrointestinal tract, and subsequent replication in the liver, HAV is excreted in bile, and high concentrations are found in stool specimens. Transmission occurs by the fecal-oral route, by direct contact either with a HAV-infected person or by ingestion of HAV-contaminated food or water (Adams & Moss, 2008). Hepatitis A is an increasing problem because of the decrease in immunity of populations in countries with high standards of hygiene.

HAV contamination of a food product can occur at any point during cultivation, harvesting, processing, distribution, or preparation. HAV can be spread either by eating or drinking food or water contaminated with the virus, including frozen or undercooked food. The food and drinks most likely to be contaminated are fruits, vegetables, shellfish, ice, and water (CDC, 2017b). Recognizing foodborne transmission using routine surveillance data may be difficult because: (1) case patients may have difficulty recalling food histories during the 2−6 weeks before illness, (2) cases may accrue gradually or not be reported, (3) a food item may be focally contaminated, (4) some exposed persons have unrecognized HAV infection, (5) some exposed persons have preexisting immunity (from a previous infection or previous vaccination), (6) persons who acquire infection through contaminated food are not recognized amid an ongoing high incidence in the community, and (7) cases are geographically dispersed over several public health jurisdictions (Fiore, 2004).

3.7.1.2.2 Hepatitis E

Hepatitis E is a liver infection caused by the Hepatitis E virus (HEV). Hepatitis E is common in many parts of the world, especially in oriental and meridional Asia, and it is transmitted from ingestion of fecal matter, even in microscopic amounts, and is usually associated with contaminated water supply in countries with poor sanitation (CDC, 2017b). There are at least four different genotypes: 1 and 2 have only been found in humans, while 3 and 4 circulate in several animals (including pigs, wild boars, and deer) without causing disease, and occasionally infect the human being (WHO, 2016). In developing countries, HEV genotypes 1 and 2 are spread by fecally contaminated drinking water. HEV genotype 3 causes sporadic cases that have occurred following consumption of uncooked/undercooked pork or deer meat. Consumption of shellfish was a risk factor in a recently described outbreak in a cruise ship. HEV genotype 4, detected in China, Taiwan, and Japan, has also been associated with foodborne transmission.

3.7.1.3 Aichivirus

The *Aichivirus* (AiV) is a member of the *Kobuvirus* genus (*Picornaviridae* family) that includes three different species (Aichivirus A, B, and C). The picornaviruses isolated from human were termed *Aichivirus*. Transmission occurs by the fecal-oral route, by direct contact either with a HAV-infected person or by ingestion of HAV-contaminated food or water. Their foodborne diseases have been potentially linked with gastroenteritis (Khamrin, Maneekarn, Okitsu, & Ushijima, 2016).

3.7.1.4 Astrovirus

Astrovirus (AstV) is a type of viruses classified as *Astroviridae* family genus *Mammoastrovirus* that cause gastroenteritis. AstV are widespread globally (Bosch, Pintó, & Guix, 2014). Food plays an important role in its transmission. Foods may become contaminated with AstV at the preharvest stage, as with bivalve mollusks grown in polluted water or fresh produce, lettuce, green onions, raspberries, and strawberries irrigated with contaminated water, observing high level of contamination in oysters and shellfish (Rzezutka & Cook, 2004). AstV is stable in drinking water, fresh surface water, and seawater, indicating another means of transmission. Young children in childcare backgrounds or adults in military barracks are most likely to develop the disease (Lukashov & Goudsmit, 2002).

3.7.1.5 Coronavirus

Coronaviruses are an extensive family of viruses, some of which may be the cause of various human diseases, ranging from the common cold to severe acute respiratory syndrome. Coronaviruses are enveloped viruses with a positive-sense RNA genome. The virus derives its name from the "corona"

seen in electron micrographs around the virion created by the viral glycoproteins. This structure also protects the virion from harsh conditions and allows this enveloped virus to be transmitted by the fecal-oral route in addition to the respiratory route (Rosenthal, 2009). Coronaviruses may represent potential foodborne disease agents (Koopmans & Duizer, 2004).

3.7.1.6   Rotavirus

Rotavirus (RV) is a double-stranded RNA virus of the family *Reoviridae*. RV is a contagious virus that can cause gastroenteritis (inflammation of the stomach and intestines) by the fecal-oral route (CDC, 2017c). Symptoms include severe watery diarrhea, often with vomiting, fever, and abdominal pain. Infants and young children are most likely to get rotavirus disease. RV can be spread by contaminated water and food, especially shellfish (Bishop, 1994).

3.7.1.7   Sapovirus

Sapovirus (SaV) is an important pathogen causing acute gastroenteritis in humans globally, especially in infants and young children. SaV are widespread and its outbreaks have become more frequent recently (Oka, Wang, Katayama, & Saif, 2015; Todd & Greig, 2015).

3.7.2   Parasites

This point describes a limited number of parasitic diseases as representative of the major groups of foodborne parasitic organisms. Numerous parasites can be transmitted by food including many helminths and protozoa. In developing countries, the most common way that parasites are transmitted in foods occur by consumption of undercooked fish, crabs, mollusks, meat, raw aquatic plants such as watercress and raw vegetables that have been contaminated by human or animal feces. Symptoms of foodborne parasitic infections vary greatly depending on the type of parasite. Helminthic infections can cause abdominal pain, diarrhea, muscle pain, cough, skin lesions, malnutrition, weight loss, neurological, and many other symptoms depending on the particular organism and burden of infection. Protozoa such as *Cryptosporidium* spp., *Giardia intestinalis*, and *Cyclospora cayetanensis* most commonly cause diarrhea and other gastrointestinal symptoms. Therefore, the foodborne parasites could be classified as described later.

3.7.2.1   Helminths

A variety of human helminthic infections could be acquired through the consumption of food products from infected animals and plants, through the accidental ingestion of infected invertebrates in foodstuffs or drinking water, or through inadvertent fecal contamination by humans or animals.

3.7.2.1.1 Trichinella

Trichinella is the etiological agent of trichinosis, the roundworm disease that has been of greatest concern from the standpoint of food transmission (Jay, Loessner, & Golden, 2005). People acquire trichinosis by consuming raw or undercooked meat infected with the *Trichinella* parasite, particularly wild game meat or pork. Even tasting very small amounts of undercooked meat during preparation or cooking puts you at risk for infection. Several different species of *Trichinella* can cause human disease; the most common species is *Trichinella spiralis*, which has a global distribution and is the species most commonly found in pigs. However, there is an increasing number of reports of *Trichinella britovi* as a cause of human infection caused by meat from domestic pigs (Gamble, Zarlenga, & Kim, 2007; Pozio, Rosa, & Morales, 2001). Trichinosis is considered a zoonosis because infection occurs after ingestion of raw or poorly cooked meat from infected animals. One to two days after the ingestion of heavily encysted meat, trichinae penetrates the intestinal mucosa, producing nausea, abdominal pain, diarrhea and sometimes, vomiting. The larvae begin to invade striated muscles about 7−9 days after the initial symptoms. About 6 weeks after the initial infection, encystment occurs, accompanied by tissue pain, swelling, and fever (Jay et al., 2005).

3.7.2.1.2 Taenia

Taeniasis is the result of ingesting tissue cyst of either *T. saginata* from cows or *T. solium*/*T. asiatica* from pork. Flatworm and roundworm parasites in humans are their definitive hosts (Jay et al., 2005). People with taeniasis may not know they have a tapeworm infection because symptoms are usually mild or nonexistent. Although most cases of taeniasis are asymptomatic, up to one-third of patients complain of nausea or abdominal pain that is often relieved by eating. Generally, epigastric pain may be accompanied by weight loss, increased appetite, headache, constipation, dizziness and diarrhea. Allergic reactions, such as urticarial and pruritus may also be due to the worm and its metabolites (Gamble et al., 2007).

3.7.2.1.3 Anisakis

Anisakiasis is a parasitic disease caused by anisakid nematodes that can invade the stomach or intestine of humans. This roundworm infection is caused by two closely related genera and species: *Anisakis simplex* and *Pseudoterranova decipiens*. Both of these organisms have several intermediate hosts and generally more than one definitive host (Jay et al., 2005). The transmission of this disease occurs when infective larvae are ingested from fish or squid that humans eat raw or undercooked (Sakanari et al., 1988). The noninvasive form of anisakiasis is generally asymptomatic, resulting in tingling throat syndrome when worms are released from seafood following digestion and migrate up the esophagus into the pharynx, where they

subsequently may be expectorated (Sakanari & McKerrow, 1989). In the invasive form, worms typically penetrate the mucosa of the stomach or small intestine, resulting in epigastric pain, nausea, vomiting, and diarrhea, usually 12 h after consumption of the infected seafood (Hayunga, 2007). In some chronic cases, the only treatment is the removal of the larvae via endoscopy or surgery.

3.7.2.1.4 *Diphyllobothrium latum*

Diphyllobothrium latum are the largest tapeworms that can infect people and can grow up to 9 m long. The definitive hosts of *D. latum* are humans and other fish-eating mammals (Jay et al., 2005). While most infections are asymptomatic, complications include intestinal obstruction, nausea, abdominal pain, diarrhea, and weakness. *D. latum* may also cause pernicious anemia and vitamin B12 deficiency (Hayunga, 2007). Diagnosis is made by identification of eggs or segments of the tapeworm in a stool sample with a microscope. Safe and effective medications are available to treat *Diphyllobothrium*. Infections, as in the case of anisakiasis, are acquired by eating raw or under-cooked fish, usually from the Northern Hemisphere (Europe, North America, and Asia), but cases have also been reported in Uganda and Chile. Fish infected with *Diphyllobothrium* larvae may be transported to and consumed in any area of the world. Adequately freezing or cooking fish will kill the parasite.

3.7.2.2 *Helminths Acquired From Other Food Sources*

Although the four helminths discussed earlier are the most important, there are many of them that can be transmitted by other sources of food (Hayunga, 2007) such as *Clonorchis sinensis, Paragonimus westermani, capillaria philippinensis, Gnathostoma* roundworms, *Heterophyles heterophyles, Metagonimus yokagawai, Echinostomum, Nanophyetus salmincola, Eustrongylides, Phylometra*, and *Nybelinia surmenicola* from fish or marine source, *Faciola hepatica, Fasciolopsis buski, Dicrocoelium, Angiostrongylus costaricensis, Trichostrongylus*, and *Echinococcus granulosus* acquire from vegetation or *Dracunculus medinensis* and *Gnathostoma spinigerum* acquire from drinking water.

3.7.2.3 *Protozoa*

Protozoan parasites have long been associated with foodborne and water-borne outbreaks of disease in humans. Difficulties arise with the inactivation of these organisms because of their resistance to environmental stresses (Ortega, 2007).

3.7.2.3.1 Cryptosporidium

Cryptosporidium is a microscopic parasite that causes the diarrheal disease cryptosporidiosis. There are many species of *Cryptosporidium* that infect

animals, some of which also infect humans such as *C. felis*, *C canis*, *C. meleagridis*, *C. baileyi*, *C. muris*, and *C. parvum* (Ortega, 2007). These species can infect humans, occurring most frequently in human immunodeficiency virus (HIV)-positive individuals (Cacciò, Pinter, Fantini, Mezzaroma, & Pozio, 2002; Fayer et al., 2001; Gatei et al., 2002; Matos, Alves, xiao, Cama, & Antunes, 2004). The parasite is protected by an outer shell that allows it to survive outside the body for long periods and makes it very tolerant to chlorine disinfection. While this parasite can be spread in several different ways (as food contaminated with *Cryptosporidium* oocytes), water (drinking water and recreational water) is the most common way to spread the parasite. Generally, *C. parvum* infects the border of the intestinal epithelium and causes villous atrophy. Immunocompetent patients develop a profuse diarrhea accompanied by epigastric cramping, nausea, and anorexia (Ortega, 2007).

3.7.2.3.2 *Cyclospora cayetanensis*

Cyclospora cayetanensis is a coccidian that is closely related to the cryptosporidia, and some human infections by the latter it has been misdiagnosed as cyclosporiasis (Jay et al., 2005). People become infected with *Cyclospora* by ingesting sporulated oocysts, which are the infective form of the parasite. This most commonly occurs when food or water contaminated with feces is consumed. An infected person sheds immature and noninfective *Cyclospora* oocysts in the feces. The oocysts are thought to require days to weeks in favorable environmental conditions to become infective. Therefore, direct person-to-person transmission is unlikely, as is transmission via ingestion of newly contaminated food or water. Cyclospsoriasis is characterized by mild-to-severe nausea, anorexia, abdominal cramping, mild fever, and watery diarrhea (Ortega, 2007).

3.7.2.3.3 *Toxoplasma gondii*

T. gondii is a coccidian parasite that infects a variety of warm-blooded hosts. Cats are the definitive hosts, while other warm-blooded animals serve as intermediate hosts. The oocytes are environmentally resistant and can survive several years. Infections are acquired principally by ingestion of food and water containing oocysts, ingestion of animal tissues containing cystic forms or transplacental transmission (Dubey & Beattie, 1988). The unsporulated oocysts require 24 h outside the host to differentiate and become infectious. When the oocysts are ingested, the sporozoites are released and they invade epithelial cells and rapidly multiply asexually, producing tachyzoites. Choriorentinitis is frequently observed in adults who acquire the infection. Other symptoms as fever with rash, headache, muscle aches, and pain and swelling of the lymph nodes were described (Jay et al., 2005). In immunosuppressed patients, toxoplasmosis could be reactivated from latent

infections (Reiter-Owona et al., 2000). Toxoplasmosis can also be acquired by transplacental transmission when a pregnant woman becomes infected. Most infected children do not show any signs of the disease until later in life, when they may present with chariorentinitis and mental retardation (Ades, 1991; Brady-McCreery, Hussein, & Paysse, 2003; De Marco, Ceccarelli, Frulio, Palmero, & Vittone, 2003).

3.7.2.3.4 Giardia

Giardia is a protozoan flagellate that belongs to the phylum Sacomastigophora. The prothozoites have eight flagella that arise on the ventral surface near the paired nuclei and give rise to falling-leaf motility (Jay et al., 2005). Most human infections result from the ingestion of contaminated water or food or direct fecal-oral transmission. *Giardia* is protected by an outer shell that allows it to survive outside the body for long periods. Five different species of *Giardia* have been described (*G. muris*, *G. lambia*, *G. agilis*, *G. ardeae*, and *G. psittaci*) (Erlandsen & Bemrick, 1987; Ortega, 2007). *Giardia* can be observed in the form of trophozoite and the cyst. The cyst is the infectious form and is relatively inert. After cysts are ingested, excystation occurs in duodenum after exposure to the acidic gastric pH and the pancreatic enzymes. The majority of *Giardia* infections are asymptomatic, but they can present a chronic diarrhea. Symptomatic patients present with loose, foul-smelling stools, and increased levels of fat and mucus in fecal samples. Flatulence, abdominal cramps, bloating, nausea, anorexia, and weight loss are common. Fever is occasionally present at the beginning of the infection (Ortega, 2007).

3.8 CONCLUSION

There are thousands of different types of microorganisms everywhere in air, soil, and water, and consequently on foods, and in the digestive tract of animals and human. Microbiology is important to food safety, production, processing, preservation, and storage. Microbes such as bacteria, molds, and yeasts are employed for the production of foods (wine, beer, bakery products, dairy, etc.) and food ingredients. On the other hand, microbial contamination of food can occur at any point in the food production process: growth, harvesting, transport, storage, or final preparation. In this regard, the microbial growth in foods can also cause visible changes such as change in color, deposition of powdery growth, making it lose its organoleptic characteristics, and effervescences on food surface.

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