CHAPTER 1
Introduction to the microbial ecology of foods

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1.1 Introduction

Food products become a microbial ecosystem when they are contaminated and colonized by microorganisms. Fresh foods allow rapid microbial growth due to a high content of nutrients whereas processed foods correspond to a harsher environment for growth, reducing the natural microbial population associated with raw food. In addition to natural microbiota related to its origin and environmental conditions, food may be contaminated from outside sources during production, processing, storage, transport, and distribution. Hence, growth and activities of microorganisms (bacteria, yeasts, and molds) are some of the major causes of food spoilage. However, few microorganisms are pathogens while many are useful in producing desirable changes during food fermentation. A large number of microorganisms can simultaneously grow in food if the abundance of nutrients is sufficient. As a consequence, the diversity and occurrence of microorganisms present depend on the composition of food, the extent of microbial contamination, and the treatments applied. Finally, intrinsic and extrinsic factors such as temperature, water content, and oxygen content have a considerable influence on the growth of microorganisms, depending on the properties of the microorganisms and on the interactions among them.

Microbial ecology of food concerns the study of the type of microorganisms present (diversity and structure), their rate of occurrence, activities (functionality), and interactions with each other (microbial communities) and their environment. Ecological studies also help to understand the transmission and dissemination of pathogens and toxins. Microbial ecology is intimately connected with microbial physiology as ecophysiological parameters determine the activities within individual cells and thus the responses of microbial populations to environmental influences. These combined effects control the type of microorganisms capable of growth in a particular food ecosystem (Leistner, 2000; McMeekin *et al.*, 2010).

Quantitative microbial ecology relies on predictive microbiology to forecast the quantitative evolution of microbial populations over time, using models that include the mechanisms governing population dynamics and the characteristics of food environments. In this respect, the diversity of the microbial community of a food ecosystem must be assessed, along with the identification of species and their comparative quantification. Traditional microbiological techniques (culture-dependent methods) have been used for decades for this purpose. However, these methods give a single viewpoint for describing a portion of the microbial dynamics and estimating microbial diversity. Culture-independent techniques based on direct analysis of genetic materials (DNA or RNA) are increasingly being used for characterization of microbial diversity structure and function. The

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development of these molecular methods and their applications in the field of microbial ecology of food has transformed our understanding of the nature and evolution of microbial populations and their metabolic activities (Ndoye *et al*., 2011).

This introductory chapter aims at providing some background in order to set the stage for further study of predictive microbiology, unit operations, processes, and the microbial ecology of specific categories of food products in the subsequent chapters.

1.2 Role of food characteristics and environment on microbial fate

Foods are classified as non-perishable for those that do not need time/temperature control, semi-perishable for those that remain unspoiled for a prolonged period and perishable for those that need time/temperature control to kill or prevent the growth and activities of microorganisms in order to extend their shelf life.

In 1971, Mossel defined four groups of ecophysiological parameters that influence the survival or growth of the microorganisms contaminating a raw or processed food: (i) intrinsic factors that are essentially chemical but with some important intrinsic factors that are physical and structural (e.g., pH, water activity, redox potential, available nutrients, presence of antimicrobial substances, food matrix); (ii) extrinsic factors that include the externally applied factors (e.g., temperature, relative humidity, etc.); (iii) implicit factors that are mostly dependent on the physiological properties of the microorganisms and microbial interactions; and (iv) processing factors (heat destruction, smoke, salts, organic acids, preservatives, and other additives) and conditions affecting foods (slicing, mixing, removing, washing, shredding,etc.) as well as influencing transfer of microorganisms (cross-contamination events) (Gould, 1992; ICFMS, 1980; Mossel, 1971; McMeekin and Ross, 1996).

In the context of quantitative microbial ecology, the growth of microorganisms could be modeled and then predicted as a function of only a few ecophysiological parameters such as temperature, pH, and water activity (a_w), sometimes with other factors such as the presence of preservatives and oxygen. Growth of a specific microorganism also depends on the initial microbial load, the sources of nitrogen and carbon, the processing method used in the food production, and the external environment of the food during storage, distribution, sale and handling. The physicochemical properties of foods in association with environmental conditions determine the selection of microorganisms capable of growing and multiplying at the expense of other less competitive species. As a result, the whole microbial ecology of the food system should be considered to accurately predict food spoilage (Braun and Sutherland, 2006). Such an integrated microbial model must take into consideration all these factors as input variables along with modeling parameters representing the processes applied during food manufacture and storage (Figure 1.1).

1.2.1 Temperature

The lag period and growth rate of a microorganism are affected by temperature as growth can be inhibited by decrease or increase of temperature below or above the optimum growth range. Indeed, every microorganism has a defined temperature range in which they grow, with a minimum, maximum, and optimum within the extended range of –5 to 90°C (Table 1.1). Organisms causing food spoilage can be grouped by temperature preference as (i) mesophiles (optimum temperature 30–45°C, minimum growth temperature ranging from 5 to 10°C and a maximum of 50°C); (ii) psychrophilic organisms (optimum growth range temperature of 12 to 15°C with a maximum range of 15 to 20°C); (iii) psychrotropes (formerly called psychrotrophs with an optimum temperature 25–30°C with a minimum of –0.4 to 5°C); and (iv) thermophiles (optimum temperature 55–75°C with a maximum as high as 90°C and a minimum of around 40°C).

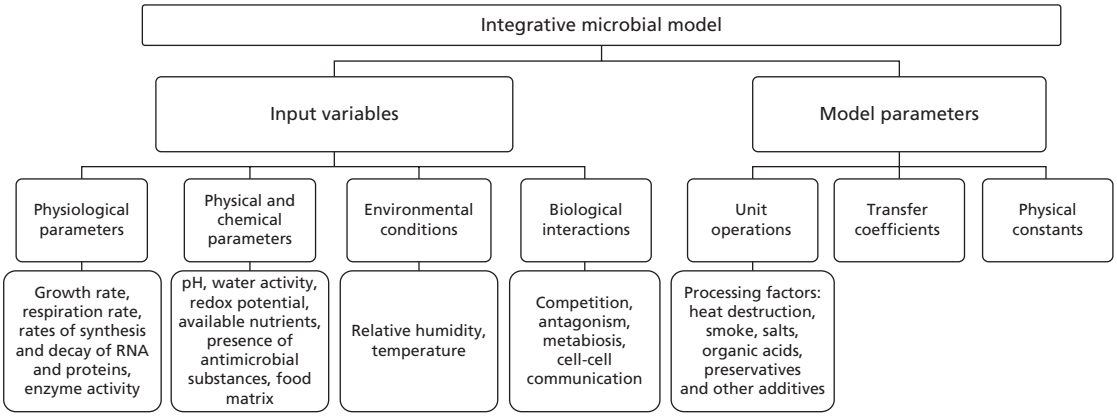

Figure 1.1 Integrative parameters affecting the development of microbial ecosystems in food.

Table 1.1 Psychrophilic, psychrotropic, mesophilic, and thermophilic microorganisms of importance in food.

Group	Temperature (°C)	Examples of bacteria (genus name only)
Psychrophiles	–5 to 20	*Acinetobacter, Bacillus, Clostridium, Flavobacterium, Vibrio*
Psychrotropes	–5 to 35	*Pseudomonas, Enterococcus, Alcaligenes, Shewanella, Brochothrix, Corynebacterium, Lactobacillus, Listeria, Micrococcus, Moraxella, Pectobacterium, Psychrobacter*
Mesophiles	5 to 47	*Bacillus, Carnobacterium, Clostridium, Corynebacterium, Escherichia, Lactobacillus, Lactococcus, Leuconostoc, Listeria, Hafnia, Pseudomonas, Salmonella, Shigella, Staphylococcus, Vibrio, Yersinia*
Thermophiles	40 to 90	*Bacillus, Paenibacillus, Clostridium, Geobacillus, Alicylobacillus, Thermoanaerobacter*

Adapted from ICMFS (1980) and Jay (2005).

Changes in storage temperature as well as the time–temperature relationship have an impact on the evolution of these different groups. Refrigeration and chill temperatures promote growth of psychrophilic microorganisms, of which there are few that affect food spoilage, and psychrotrophic spoilage organisms such as pseudomonas, yeasts, and molds as well as pathogens such as *Listeria monocytogenes*. At high temperatures, spore-forming bacteria and lactic acid bacteria are able to multiply. Thus, temperature changes have an influence on the metabolic activities of some microorganisms and consequently on the biochemistry of the spoilage process. Time has an impact in relation to the storage temperature because it is a factor that influences the rate of growth of microorganisms: extended storage at low temperatures allows the growth of some psychrotrophic microorganisms. Further discussion on food safety and the role of quantification in microbial risk assessment will be given in Part IV of this book.

1.2.2 pH and acidity

The pH is a measure of acidity of a food that influences microbial growth and survival, as every microorganism possesses a minimum, an optimum, and a maximum pH for growth. Most bacteria exhibit an optimum pH near the neutral point (pH 7.0) although acetic and lactic acid bacteria are able to survive at reduced pH levels. Molds and yeasts are generally more

acid-tolerant than bacteria and therefore acidic foods are more susceptible to spoilage by these types of microorganisms.

Low pH values and associated high acid concentrations inhibit microbial growth and survival in foods due to the acid-induced denaturation of cell wall proteins. A decrease of pH also reduces the heat resistance of microorganisms. Moreover, the pH can interact with water activity, redox potential, salt, and preservatives to inhibit growth of food-borne pathogens and spoilage microorganisms. The undissociated form of weak acids shows antimicrobial activity because they pass freely through the cell membrane and then dissociate, as the cytoplasmic pH is usually higher than that of the growth medium. This leads to the release of protons, which in turn results in an acidification of the cytoplasm. Bacterial growth can be prevented by addition of weak organic acids alone or in combination with other preservatives as well as by production of lactic and acetic acids by fermentation.

Food products can essentially be divided into three types according to their pH: (i) low-acid foods where pH is greater than 4.6 and less than 7.0, (ii) acid foods that have a pH lower than 4.6, and (iii) acidified foods obtained by addition of acids into low-acid foods. This classification is based on the fact that pathogenic microorganisms generally cannot grow at pH values below 4.6. Low-acid foods can be fermented (fermented foods) by acid-producing bacteria that reduce the pH below 4.6. Foods can also be characterized by their buffering capacity, which is defined as the ability to resist changes in pH. The pH of foods with a low buffering capacity in the presence of acidic or alkaline compounds produced by microorganisms will change quickly, whereas foods with a high buffering capacity are more resistant to pH changes.

1.2.3 Water activity

Water is a requirement for growth and metabolic activities of microorganisms in a food product. However, microbes can only use water in an available form. Free water that is not in the bound state participates in many chemical and biochemical reactions, supports microbial growth, and acts as a transporting medium for compounds (sugars, salt, organic acids) in the food system. Water activity (a_w), defined as the free or available water in a food, is therefore a better indicator for microbial growth than the water content. In a food matrix, the requirements for moisture by microorganisms are expressed in terms of a_w (the a_w of pure water is 1.00 and the a_w of a completely dehydrated food is 0.00) and the lower limit for microbial growth in a food product will be determined by the a_w. Food products can thus be broadly classified by water activity into (i) high a_w (>0.92), (ii) intermediate a_w (0.85 to 0.92), and (iii) low a_w (<0.85). Fresh foods (meat, vegetables, and fruits) generally have a_w values higher than 0.97. By reducing water activity below 0.7, osmotic pressure is increased, thus inhibiting microbial growth and maximizing the shelf life of the food product. This reduction can be accomplished by adding sugars or salt, removing water by drying or baking, and binding the water to various macromolecular components such as cellulose, protein, or starch in the food.

Microorganisms exhibit optimum and minimum levels of a_w for growth, depending on a number of other ecophysiological factors (pH, temperature, oxido-reduction level, and nutrients). Bacteria are more sensitive than yeasts and molds, and Gram-positive bacteria are more resistant to lower values of a_w than Gram-negative bacteria. The growth of food-borne pathogens is inhibited below a_w 0.86, except that *Staphylococcus aureus* can grow down to a value of 0.83 and produces toxin below a_w 0.90. Growth of molds will be controlled at a_w 0.80 and mycotoxin production requires a higher a_w than that of growth.

1.2.4 Oxygen and redox potential

Based on their oxygen requirements and tolerance, microorganisms are classified into the following groups: (i) obligate aerobes are microorganisms that require oxygen for growth;

(ii) obligate anaerobes are microorganisms that do not need or use oxygen, which is toxic for them; and (iii) facultative aerobes (or facultative anaerobes) are microorganisms that can grow in the presence and absence of oxygen, switching to aerobic respiration in the presence of oxygen but under anaerobic conditions they grow by fermentation or anaerobic respiration.

The oxidation–reduction or redox potential is an intrinsic factor that influences the growth of microorganisms in foods. The redox potential of the food varies according to the physicochemical characteristics, partial pressure of oxygen, and the presence of other gases in the storage atmosphere (water vapor, nitrogen, CO_2). The presence of substances that are highly hydrogenated, that contain SH radicals, reducing sugars, or other compounds such as ascorbic acid (vitamin C) and tocopherols (vitamin E) in a food creates a reducing environment. When the redox potential (Eh) is negative in terms of millivolts, this means a reduced state, while the presence of oxygen at the surface or in the bulk has an oxidizing effect (an oxidized state). Aerobic organisms require a food environment with a positive redox potential (+500 to +300 mV) whereas anaerobes require a negative potential (+100 to less than −250 mV) and facultative anaerobes tolerate a range in potential between +300 and −100 mV.

1.2.5 Nutrient content

The nutritional needs of microorganisms can usually be met in foods due to the presence of water, carbohydrates (sources of carbon and energy), fats, proteins, vitamins, and minerals. However, these nutrients must be available in an easily digestible form, such as simple sugars and amino acids, for many microorganisms. Some microorganisms possess specific enzymes that allow them to degrade more complex structures such as proteins and fibers. Most spoilage microorganisms have no fastidious nutritional requirements and possess essential metabolic activities such as glycolysis and proteolysis. In this way, a complex microbial community capable of degrading the nutrients present will colonize any type of food. Therefore, it is practically impossible to predict the microbial ecology of a food based on its nutrient composition.

1.2.6 Physical structure and microenvironments

The physical barriers to food spoilage by microorganisms are: (i) the skin of fish and meats, (ii) the shell of nuts and eggs, (iii) the external layers of seeds, and (iv) the outer covering of fruits and vegetables such as the husk or rind. These protective biological structures are usually composed of macromolecules that are relatively resistant to penetration or degradation. They constitute hostile microenvironments for the growth of microorganisms by having a low water activity, a lack of readily available nutrients, and a presence of antimicrobial compounds such as short-chain fatty acids (on animal skin) or essential oils (on plant surfaces). During the preparation of foods, processes such as cutting, grinding, and heating break down the biological barriers and change microenvironments, thus favoring contamination and proliferation of microbes inside the food product. The impact of these unit operations on specific microorganisms will be further detailed in Part II of this book. Most microorganisms will grow in the majority of foods, as individual free-floating (planktonic) cells in the aqueous phase or as an association of microbial cells with a solid substrate either through entrapment, constrained growth, attachment, or a combination of these factors (Skandamis and Nychas, 2012).

1.2.7 Food preservation processes (antimicrobials, preservatives)

Food preservation mainly involves a prevention or exclusion of microbial activity. This may be achieved: (i) by inhibiting the growth or shortening the survival of microorganisms, (ii) by excluding or removing microorganisms, and (iii) by killing the microorganisms. Some plant- and animal-based foods contain natural antimicrobial compounds such as essential oils and lysozyme, respectively, that inhibit the growth of spoilage microorganisms. Some chemical

food additives such as salts, sugars, and organic acids are commonly applied for creating a hostile environment in food products. The presence of gases (carbon dioxide, ozone, and oxygen) is also able to inhibit the growth and proliferation of microorganisms by direct toxic effects and by indirect inhibitory effects, by modifying the gas composition and thus altering the ecology of the microbial environment. Various types of food processing such as heating, smoking, and fermentation are also used for the formation of antimicrobial substances in food. Part II of this book contains more information on specific food preservation operations, including fermentation. Food fermentation is one of the oldest food processing technologies that can suppress the growth and survival of spoilage microorganisms in food products. This process depends on the biological activity of microorganisms that produce a large range of metabolites (acids, alcohols, and carbon dioxide) by fermentation or oxidation of carbohydrates or derivatives. For example, among members of competitive microbiota, lactic acid bacteria (LAB) exhibit unique metabolic activities and are employed as starters for the fermentation of milk, meats, cereals, and vegetables and are used as probiotic cultures (Champagne *et al.*, 2005). In addition to the production of lactic, acetic, and propionic acids leading to an acidic environment appropriate for controlling the growth and metabolic activity of many pathogenic and spoilage microorganisms, these beneficial bacteria are also able to produce ethanol, hydrogen peroxide (H_2O_2), diacetyl, and bacteriocins. Bacteriocins are protein or peptide antimicrobial substances that inactivate other bacteria through depolarization of the target cell membrane or through inhibition of cell wall synthesis. In addition, LAB can also produce antifungal compounds, including reuterin, carboxylic acids, cyclic dipeptides, and fatty acids (Crowley *et al.*, 2013).

Antimicrobial substances produced by microorganisms provide an additional hurdle for keeping the natural population of microorganisms under control. Indeed, traditional food preservation has often been achieved by the combination and interactions of pH, a_w, atmosphere, numerous preservatives, and other inhibitory factors, referred to as the "hurdle effect". These preservative factors (hurdles) temporarily or permanently disturb the homeostasis of microorganisms, defined as the tendency to uniformity and stability in the internal status of an organism; microorganisms remain in the lag phase or even die before homeostasis is re-established (Leistner, 2000).

1.3 Understanding microbial growth, death, persistence, competition, antagonism and survival in food

1.3.1 Principles of microbial growth

In a food environment where nutrients are not limiting, microbial cells increase in number in a characteristic manner and at a specific rate as determined by their genetic traits. It is well known by microbiologists that the growth curve exhibits four different phases: (i) the lag phase in which microorganisms, by a series of biochemical activities, acclimate to their environment and initiate cell reproduction and growth; (ii) the exponential or log phase, where cell components are synthesized in order to allow cell replication at a logarithmic rate determined by their generation time and ability to assimilate the substrate; (iii) the stationary phase, which begins when a microbial population tends to stabilize due to accumulation of metabolic end-products and limitations in substrates necessary for growth, leading to reduction of the growth rate; and (iv) the decline or death phase, when microorganisms die and lyse (autolysis) due to nutrient depletion and the toxic effects of metabolic end-products.

1.3.2 Survival

Microbial populations in foods are subjected to stressful conditions such as low or high temperatures, acidity, low water activity, modified

atmospheres, or nutrient deprivation. By a variety of strategies, microorganisms attempt to resist and adapt to these hostile conditions, constantly switching between growth and merely surviving. The stress response, which results in a characteristic change in the pattern of gene expression, helps to restore cellular homeostasis and increase resistance to subsequent stressful conditions. Although death is an irreversible state, bacterial cells can be sublethally injured or enter a dormant state. These cells may repair the damage caused by the hostile environment and survive, even growing when conditions become favorable (Aertsen and Michiels, 2004; Wesche *et al*., 2009). The stress response in relation to microbial ecology of food will be discussed in Part IV of this book.

1.3.2.1 The viable but non-culturable state

Many stressed organisms may regain the characteristics of normal cells, but some severely injured cells remain metabolically active but cannot be resuscitated under routine laboratory conditions, entering a viable but non-culturable (VBNC) state (Wesche *et al.,* 2009). The VBNC state can be a significant means of survival if the cells have the ability to increase metabolic activity and become culturable once resuscitated (Oliver, 2005).

A large number of non-spore-forming bacteria are capable of entering the VBNC state. Although the exact role of this state in bacteria is yet to be elucidated, it can be induced by stressful conditions such as nutrient starvation, temperature, osmotic concentration, oxygen concentration, and food preservatives. Hence, the VBNC state might be an adaptive strategy for long-term survival of bacteria under unfavorable environmental conditions. In contrast to dead cells that have a damaged membrane, VBNC cells have an intact membrane retaining chromosomal and plasmid DNA and differ from "injured" bacteria that are unable to grow on selective media. VBNC cells do not grow on any medium, even if non-selective. However, VBNC bacteria have higher resistance to physical and chemical stresses than culturable cells and can resuscitate when environmental conditions become favorable (Li *et al*., 2014; Oliver, 2005, 2010).

1.3.3 Strategies for persistence

Many microorganisms associated with food survive treatments such as heat and disinfection, so they persist during storage and their numbers remain unchanged.

1.3.3.1 Sporulation

Some bacteria can form spores as a defense mechanism against unfavorable environmental conditions (e.g., Gram-positive bacteria such as *Bacillus* and *Clostridium*). Indeed, endospores are very resistant structures with no measurable metabolism but can confer a great advantage for these bacteria to persist for prolonged periods of time and endure extreme stress conditions (high temperatures and UV irradiation, extreme freezing, desiccation, chemical damage by disinfectants, and enzymatic destruction). Under favorable environmental conditions, the endospore can undergo activation and germination. Hence, metabolic activity is restored and the cell becomes vegetative.

1.3.3.2 Biofilm formation

Generally, bacteria do not live freely in suspension as planktonic cells and biofilms protect them from desiccation, bacteriophages, and sanitizing agents. Biofilm formation thus constitutes one of the survival strategies of microorganisms in hostile environments. The persistence of food-borne pathogens and spoilage microorganisms on foods and food contact surfaces often adversely affects the quality and safety of raw and minimally processed foods.

1.3.4 Competition

The composition of the microbial community of a food varies according to many ecophysiological factors that have been described so far. However, the ecosystem is also altered by the interactions among microbes themselves.

Mixed cultures in food fermentation processes represent some of the best examples of microbial interactions. Microbe–microbe interactions can be classified as positive (+), neutral (0), and negative (–). These interactions can be further subdivided into: (i) mutualism (+/+ interaction: both microbes involved benefit from the interaction, e.g., synergism or protocooperation among yogurt bacteria or mutualism between yeast and bacteria during sourdough fermentation); (ii) commensalism (+/0 interaction: one organism benefits from the interaction while the other is not affected, e.g., cultivation of propionic bacteria in the presence of LAB in Swiss-type cheese); (iii) amensalism (–/0 interaction: interspecies interaction in which one organism adversely affects the other without being affected itself, e.g., bacteriocin production by LAB and ethanol production by yeasts); (iv) parasitism (+/– interaction: one species benefits at the expense of another, e.g., bacteriophages in fermentations); and (v) competition (–/– interaction: two or more species, strains, or subpopulations of microbes compete for energy sources and nutrients) (Ivey *et al.,* 2013; Sieuwerts *et al.*, 2008; Smid and Lacroix, 2013).

Microorganisms compete for nutrients, adhesion/attachment sites, as well as by their ability to alter the environment by producing metabolites. Preservation methods combined with ecophysiological factors and the genetic characteristics of each microorganism (lag phase, growth rate, and total cell biomass yield) lead to selection of microbial associations of a particular food at any given point in time during production and storage. For example, psychrotrophic bacteria such as *Pseudomonas* spp. dominate proteinaceous foods (meat, poultry, milk, and fish) stored at refrigeration temperatures under aerobic conditions. In meat and fish products, a change in the atmosphere (e.g., vacuum packaging) promotes LAB at the expense of *Pseudomonas*. Microbial ecology of food provides a comprehensive overview of the dominance of an organism based on its origin, substrate composition, temperature, pH, a_w, and atmosphere, regardless of raw material and processing.

In addition to these conditions determining the association of microbiota in food, there are three aspects of microbial interaction that must be taken into consideration according to Gram *et al.* (2002), namely: (i) antagonism, (ii) metabiosis, and (iii) cell-to-cell communication.

1.3.4.1 Antagonism

In addition to changes in environmental conditions such as lowering pH by acid-producing microorganisms, antagonistic abilities include competition for nutrients. Scavenging growth-limiting compounds such as iron represents one type of nutritional competition. Microorganisms with higher metabolic activity may selectively consume required nutrients, resulting in growth inhibition of other organisms with lower activity. The growth rate of particular microorganisms may also be affected by an overgrowing microbiota in a phenomenon described as the "Jameson effect", which is essentially non-specific nutrient competition (Gram *et al*., 2002).

1.3.4.2 Metabiosis

The microbial profile of a food evolves over time because of the changes in environmental conditions caused by the action of the community on the supply of nutrients from limiting metabolic compounds. This refers to the term "metabiosis", which describes the interrelationships among microorganisms to produce a given environment (Gram *et al.*, 2002).

1.3.4.3 Cell-to-cell communication

The role of quorum sensing (QS) or cell-to-cell communication in food microbial ecology is now considered a microbial behavioral pattern that is correlated with the density of the microbial population and with the ability to regulate gene expression as a function of cell density (Gram *et al*., 2002; Skandamis and Nychas, 2012). In Part IV of this book, the quantitative aspects of quorum sensing will be applied to microbial ecology.

The food matrix is composed of interconnected microenvironments where the levels of intrinsic ecophysiological factors (oxygen, pH, a_w, nutrients, preservatives, and antimicrobial compounds) may change. A large variety of microorganisms proliferates as microcolonies or biofilms and reaches high densities (10^7–10^9 cfu/g) in these *in situ* environments. The growth and activity of any one species or strain will be determined by the presence of other species since we can assume that quorum or other sensing molecules are released (*in situ* cell-to-cell ecological interactions). Microorganisms interact and influence the growth of one another by synthesizing specific low-molecular-weight diffusible signaling molecules as a function of population density :(i) Gram-negative bacteria produce and utilize *N*-acyl homoserine lactones (AHLs) or autoinducer-1 (AI-1) for intraspecies communication; (ii) autoinducing peptides (AIPs) are produced and used by Gram-positive bacteria for intraspecies communication; and (iii) furanosyl borate diester derivatives or autoinducer-2 (AI-2) are produced by both Gram-positive and Gram-negative bacteria and seem to serve as a universal language for inter- and intraspecies communication (Bai and Rai, 2011).

1.4 Methods to study the microbial ecology of foods

The aims of microbial ecology studies are to determine changes in microbial populations by characterizing community structure, diversity, activity, and interactions in their natural environments. The three basic questions that detection methods must answer are: (i) "who is there?" by identifying the types of microorganisms such as food-borne pathogens, spoilage microorganisms, starter cultures, or potentially probiotic and beneficial microorganisms present in the specific food environment; (ii) "who is doing what?" by assigning functional roles to these microorganisms; and (iii) "how do the activities of these microorganisms contribute to specific ecosystem functions or processes" (Ndoye *et al.*, 2011; Ercolini, 2013).

Culture-independent methods can circumvent the limitations of traditional microbiological methods for the analysis of complex microbial ecosystems. These methods have been used in various types of foods, especially for cheese in recent years (Ndoye *et al.*, 2011). In contrast to conventional microbiological techniques based on cultivation of the microorganisms on media and phenotypic or genotypic characterization of a fraction of the community (culture-dependent methods), culture-independent techniques are based on direct analysis of DNA or RNA for efficient characterization of whole microbial communities, evaluation of *in situ* gene expression and determination of metabolic activities of microbial populations present in a particular food product. However, both culture-dependent and culture-independent methods have limitations and should be combined as much as possible through polyphasic approaches to undertake analysis of both the community and activity of natural microbiota and spoilage microorganisms (Cocolin *et al.*, 2013; Ercolini, 2013; Ndoye *et al.*, 2011).

1.4.1 Culture-independent analysis of microbial communities

The application of molecular techniques has modified our understanding of the microbial ecology of food, allowing significant insights into all aspects of microbial populations (identification of specific isolates, changes in microbial communities, nature of the functional groups). These techniques have been classified into two major categories: (i) partial community analysis approaches and (ii) whole community analysis approaches (Rastogi and Sani, 2011).

1.4.1.1 Partial community analysis approaches

These approaches are based on the direct extraction of total DNA or RNA from the food product. Then, the genetic materials extracted from food samples, either DNA or cDNA (after

reverse transcription of the total RNA), are amplified by polymerase chain reaction (PCR)-based methods. The application of the various molecular culture-independent tools allows: (i) direct identification of members of a community and assessment of their abundance; (ii) reliable fingerprinting of complex bacterial communities; (iii) analysis of the diversity and dynamics of the dominant microbial community; (iv) comparison of spatial and temporal changes in bacterial community structure; and (v) accurate quantification of target species (Ndoye *et al.*, 2011).

1.4.1.2 Whole community analysis approaches

Next-generation DNA sequencing (NGS) or high-throughput sequencing is a hundred times faster and cheaper than the conventional Sanger approach and is already considered as the most powerful culture-independent method for analysis of all the genetic information present in total DNA/RNA extracted from food samples or pure cultures. The NGS approach provides a more global perspective on food microbial communities, including molecular mechanisms of metabolically active microorganisms in food ecosystems. Metagenomics is defined as the investigation of the collective microbial genomes retrieved directly from environmental samples. When combined with other "omics" (functional genomics, transcriptomics, proteomics, and metabolomics), these data provide deeper insight into microbial diversity and the metabolic potential of microbial communities as well as predictive models of the contribution of individual microorganisms to the development of food quality and safety (Rastogi and Sani, 2011; Solieri *et al.*, 2012).

1.5 Perspectives on applying food ecosystem modeling

Predictive microbiology, which will be detailed in Chapter 2, was originally conceived for analyzing the behavior of pure cultures of food-borne pathogens, and then spoilage bacteria, in order to develop food processes that adequately control microbial growth throughout the shelf life of food products. For example, the growth boundary models for *L. monocytogenes* erroneously predict the growth of this pathogen, as it does not take into account the biofilm microbiota interactions (Guillier *et al.*, 2008). In addition, pH and a_w could not solely account for growth arrest in the stationary phase, without including non-specific competition for nutrients (Jameson effect). Considering factors that determine enzyme production has revealed crucial restraints on litter decomposition rates in soil (Allison *et al.*, 2012), so these metabolic factors have great potential for application to food products as well. Genome-scale metabolic models are becoming useful in analyzing interactions in multispecies microbial systems from a metabolic standpoint, requiring the integration of the ecological concept of trade-offs between individual and community fitness criteria (Zomorroddi *et al.*, 2012).

Advances in our understanding of microbial interactions will allow us to envisage more complex predictive models (Figure 1.2). Complex system science is a process of integrating a multiplicity of variables and knowledge from an array of disciplines (Perrot *et al.*, 2011). In the case of food, this means joining together the skills of mathematicians, physicists, and computer scientists with those of microbiologists to complete food science and engineering. First used in environmental ecosystem modeling, this approach is beginning to be applied in order to comprehend the Camembert cheese ripening process (Sicard *et al.*, 2012). Viability theory from complex science was employed to define an optimal trajectory for Camembert cheese ripening, which was validated through pilot studies by manipulating cheese size, relative humidity, and temperature controls. As a result, the cheese ripening process was shortened by four days without significant changes in the microorganism kinetics. The quality target was reached and the sensory properties of the cheeses produced were similar to those obtained under

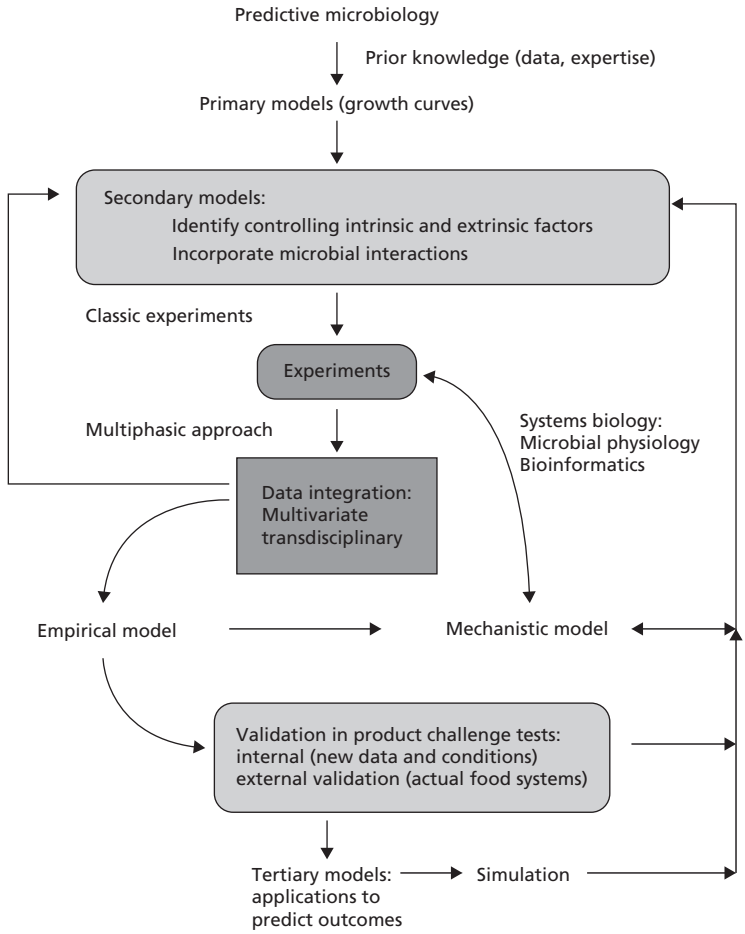

Figure 1.2 Towards developing models from predictive microbiology to quantitative microbial ecology and systems biology.

standard conditions (Sicard *et al.*, 2012). This is one example of how microbial community modeling can have a concrete impact on developing more efficient and less costly food processes.

Multiple species community modeling is still in its early stages and faces many challenges, especially applied to food. The next step, microbial community engineering, has even greater challenges and potential rewards in ensuring food quality. Continual innovation in analytical methods will contribute to improve the prospects of microbial community modeling in the future.

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