3

Microorganisms and Food Fermentation

Giorgio Giraffa and Domenico Carminati

CONTENTS

3.1 Introduction ....................................................................................................................... 49
3.2 Microorganisms in Fermented Foods .................................................................................. 50
  3.2.1 Starter Cultures ............................................................................................................. 50
3.3 Microbial Estimation: Ecological and Biochemical Considerations .................................. 51
3.4 Problems Related to Microbial Identification and Estimation in Fermented Foods .............. 51
  3.4.1 Survival Mechanisms of Microorganisms in Foods ....................................................... 51
  3.4.2 Microbial Communication and Quorum Sensing ........................................................ 52
3.5 Microbial Dynamics in Food ............................................................................................... 52
  3.5.1 Culture-Dependent Methods ......................................................................................... 52
  3.5.2 Culture-Independent Methods ....................................................................................... 53
  3.5.3 Present Trends ............................................................................................................... 54
    3.5.3.1 Phenomics ............................................................................................................... 54
    3.5.3.2 Quantitative PCR .................................................................................................... 54
    3.5.3.3 Chip, DNA Array-Based, Technology ................................................................. 55
    3.5.3.4 Functional Proteomics ............................................................................................. 55
    3.5.3.5 Data Analysis .......................................................................................................... 55
3.6 Final Remarks and Future Needs ......................................................................................... 56

References ................................................................................................................................. 56

3.1 Introduction

Fermentation is one of the oldest forms of food preservation technologies in the world. It is responsible for many properties of fermented foods, such as flavor, shelf life, texture, and health benefits. Food fermentation covers a wide range of microbial and enzymatic processing of food and ingredients to achieve desirable characteristics such as prolonged shelf life, improved safety, attractive flavor, nutritional enrichment, and promotion of health (Giraffa 2004; Sieuwerts et al. 2008). Fermentation is a relatively efficient, low-energy preservation process, which increases the shelf life and decreases the need for refrigeration or other forms of food preservation technology. Fermentation processes enhance food safety by reducing toxic compounds such as aflatoxins and cyanogens and producing antimicrobial factors such as lactic acid, bacteriocins, carbon dioxide, hydrogen peroxide, and ethanol, which facilitate inhibition or elimination of foodborne pathogens. Fermentation also improves the nutritional value of foods through the biosynthesis of vitamins, essential amino acids, and proteins, by improving protein and fiber digestibility, by enhancing micronutrient bioavailability, and by degrading antinutritional factors. It also provides a source of calories when used in the conversion into human foods or substrates that are unsuitable for human consumption. Therapeutic properties of fermented foods have also been reported. In addition to its nutritive, safety, and preservative effects, fermentation enriches the diet through the production of a diversity of flavors, textures, and aromas. It improves the shelf life of foods while reducing energy consumption required for their preparation. The production of fermented foods is also important in adding value to agricultural raw materials, thus providing income and generating employment (Giraffa 2004).
The consumption of fermented foods has increased greatly since the 1970s, and, today, fermented foods are among the most popular types of consumed foods. In recent years, there has been massive product diversification, and many prebiotic and probiotic products with a high added value have emerged. Simultaneously, artisanal products have gained popularity due to their particular flavor and aroma characteristics (Sieuwerts et al. 2008). A variety of fermented foods can be found throughout the world. They include common foods like dairy products (yogurt, cheeses, buttermilk), fermented sausages, sourdoughs, fermented alcoholic beverages, vegetables, fruits, and sauces, as well as ethnic foods such as kefir and koumiss. Collectively, sales of fermented foods on a global basis exceed $1 trillion, with an ever greater overall economic impact. Fermented dairy products represent 20% of the total economic value of fermented foods produced worldwide (O’Brien 2004; Hutkins 2006).

3.2 Microorganisms in Fermented Foods

Within the fermentation industry, microorganisms are used for the production of specific metabolites such as acids, alcohols, enzymes, antibiotics, and carbohydrates. Major fermentation microorganisms include lactic acid bacteria (LAB), molds, and yeasts. In particular, LAB are one of the most industrially important groups of bacteria and have a very long history of use in fermentation. These organisms are used in a variety of ways, including food production, health improvement, and production of macromolecules, enzymes, and metabolites. LAB are arguably second only to yeast in importance in their services to mankind. They have been used worldwide in the generation of safe, storable, and organoleptically pleasant foodstuffs for centuries. Today, LAB play a prominent role in the world food supply, performing the main bioconversions in fermented dairy products, meats, and vegetables. LAB are also essential for the production of wine, coffee, silage, cocoa, sourdough, and numerous indigenous food fermentations (Makarova et al. 2006).

Agricultural products of animal or vegetable origin are fermented by either the indigenous microflora or an added starter culture to improve or obtain shelf life, nutritional value, health benefit, flavor, or texture. Unlike the Western world, Asia has developed many foods based on vegetable proteins using fungi, often in a solid-state fermentation process. In Europe and the United States, the main focus of food fermentation has been on food preservation by means of acid fermentation, whereas properties such as taste, nutritional value, and health effect are more important in Asian (fungal) fermentation (Oyewole 1997; Caplice and Fitzgerald 1999; Wood 1998; Hansen 2002; Holzapfel 2002).

3.2.1 Starter Cultures

The basic role of starter cultures is to drive the fermentation process. Concomitantly, they contribute to the sensorial characteristics of the products and to their safety. In fermentation, the raw materials are converted by microorganisms (bacteria, yeasts, and molds) to products that have acceptable food qualities. Spontaneous (natural) fermentation, that is, a process initiated without the use of a starter inoculum, has been applied to food preservation for millennia. In a natural fermentation, the conditions are set so that the desirable microorganisms grow preferentially and produce metabolic by-products that give the unique characteristics of the product. The majority of small-scale fermentation in developing countries and even some industrial processes such as sauerkraut fermentation are still conducted as spontaneous processes. Various types of starter cultures are widely used in such fermentations, even in industrialized countries (Carminati et al. 2010).

However, spontaneous food fermentations are neither predictable nor controllable. The natural microflora of the raw material is either inefficient, uncontrollable, and unpredictable or is destroyed altogether by the heat treatments given to the food. Moreover, the industrialization of food production led to the optimization and upscaling of many fermentation processes. Similarly, industrially produced starter cultures have emerged, leading to improved and reproducible fermentation control and product quality. In a controlled fermentation, the fermentative microorganisms (usually LAB or yeasts) are isolated and characterized and then maintained for use. Because the starter industry relies on the use of selected strains
of given species with known metabolic properties, the introduction of commercial starter cultures has undoubtedly improved product quality and process standardization (Giraffa 2004; Sieuwerts et al. 2008).

### 3.3 Microbial Estimation: Ecological and Biochemical Considerations

Searching for the presence, numbers, and types of microorganisms in fermented foods is of paramount importance for the starter and food industry. Whatever the primary objective of these microbial analyses (e.g., control of food quality, food preservation, efficiency of starter cultures, monitoring of particular species/strains), the taxonomic level of the microbial discrimination needed may depend upon the sensitivity of the technique (either phenotypic or genotypic) used and may range from genus (or species) to subspecies or strain level (subtyping). While the above methods may describe microbial identity and composition at given spatial/temporal combinations, it must be considered that populations in a food microbial system are continuously evolving. The dynamics of growth, survival, and biochemical activity of microorganisms in foods are the result of stress reactions in response to changing physical and chemical conditions that occur in food microenvironments (e.g., pH, salt, temperature), the ability of microorganisms to colonize the food matrix and to grow into spatial heterogeneity (e.g., microcolonies and biofilms), and the in situ cell-to-cell ecological interactions that often take place in a solid phase (Fleet 1999).

To effectively manage the growth and activities of microorganisms in fermented foods, the following points should be raised: (1) information on diversity, taxonomic identity, growth cycle, quantitative changes, and spatial distribution of microbial species that ferment into the food at every stage of production; (2) biochemical and physiological data on the food colonization process; (3) impact of the intrinsic, extrinsic, and processing factors influencing microbial growth, survival, and biochemical activity; and (4) relationship between growth and activity of individual microorganisms and product quality and safety. Therefore, to evaluate microbial diversity in fermented food is problematic because of the concomitant action of different ecological and biochemical factors. This task is further complicated because of the difficulty to cultivate a portion of the viable bacteria (Fleet 1999; Giraffa 2004; Giraffa and Carminati 2008).

### 3.4 Problems Related to Microbial Identification and Estimation in Fermented Foods

In many microbial systems, previously unrecovered species may not easily be isolated. This is the case of the complex microflora colonizing the human gut or the microbial communities characterizing many traditional fermented foods. This made it necessary to apply more than a single method to identify and/or type bacteria (Giraffa and Neviani 2001; Ventura et al. 2004). Moreover, the viability of microorganisms can make it difficult to evaluate microbial composition in food systems. Determination of bacterial viability is a complex issue, as illustrated by the numerous scientific papers published on the topic (Kell et al. 1998; Colwell 2000; Nystrom 2001). Traditionally, plate counting has been the method of choice for viability assays. However, it is widely accepted that plate culturing techniques reveal little of the true microbial population in natural ecosystems. This phenomenon can be explained by the inability to detect novel microorganisms, which might not be cultivable using known media, and/or the inability to recover known microorganisms that either are stressed or enter a nonculturable state (Fleet 1999). The determination of bacterial viability in fermented foods, especially in probiotic products, is of economic, technological, and clinical significance (Lahtinen et al. 2005).

#### 3.4.1 Survival Mechanisms of Microorganisms in Foods

Stress is any change in the genome, proteome, or environment that imposes either reduced growth or survival potential. Such changes lead to attempts by a cell to restore a pattern of metabolism that fits it
either for survival or for faster growth. For any stress, the bacterial cell has a defined range within which the rate of increase of colony forming units is positive (growth), zero (survival), or negative (death). In the first two cases, that is, growth and survival, the cells are sublethally injured, whereas in the case of death (e.g., after a bacteriophage attack), the cells are lethally damaged and rapidly autolyze. The individual values at which the cell moves from one physiological state into the next are conditional on the degree of stress imposed by other environmental conditions (Booth 2002).

Stress can influence cell viability and culturability in different ways. Kell et al. (1998) have suggested four terms to describe different life stages of microorganisms: (1) viable (active and readily culturable), (2) dormant (inactive but ultimately culturable), (3) active but nonculturable (VBNC), and (4) dead (inactive and nonculturable). In microbial populations, viable cells are usually countable on both nonselective and selective media, whereas stressed cells are able to form colonies on nonselective media but are not countable on selective media. In foods, many adverse conditions such as nutrient depletion, low temperature, and other stresses can sublethally damage microorganisms. In a recent study, it has been shown that probiotic bacteria become dormant during storage (Lahtinen et al. 2005). The VBNC state is a kind of stress that induces healthy, cultivable cells to enter a phase in which they are still capable of metabolic activity but do not produce colonies on media (both nonselective and selective) that normally support their growth. Many food-associated bacteria, including a variety of important human pathogens, are known to respond to various environmental stresses by entry into a VBNC state (Giraffa and Carminati 2008; Oliver 2010).

3.4.2 Microbial Communication and Quorum Sensing

A wide range of communication mechanisms have been described so far within bacteria, such as production of bacteriocins, pheromones, and signaling molecules (e.g., acyl-L-homoserine lactones). In addition to releasing the signaling molecules, bacteria are also able to measure the number (concentration) of the molecules within a population. The term “quorum sensing” (QS) is applied to describe the phenomenon whereby the accumulation of signaling molecules allows a single cell to sense the number of bacteria (cell density), thus enabling microorganisms to coordinate their behavior (Konaklieva and Plotkin 2006). Mechanisms regulating the multitude of language signals that diffuse through different microbial (including food) communities are rapidly being elucidated (Di Cagno et al. 2011).

Little is still known on the role of QS in food ecosystems. It has been shown that this mechanism regulates the in situ phenotypic expression and population behavior of food spoilage bacteria (Gram et al. 2002). An implication of QS in the expression of genes that code for bacteriocins in LAB has recently been demonstrated (Diu et al. 2009). QS signal molecules released by probiotics may also interact with human epithelial cells from the intestine, thus modulating several physiological functions (Gobbetti et al. 2010).

3.5 Microbial Dynamics in Food

3.5.1 Culture-Dependent Methods

Culture-dependent methods include traditional cultivation methods in combination with phenotypic (physiological and biochemical) and genotypic [species-specific and randomly amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR)] identification and typing techniques of isolated strains. Generally, microbiota present during fermentation are enumerated by cultivating on media with the intention of selecting different groups of bacteria (e.g., total aerobic count on plate agar count medium, presumptive streptococcal and lactococcal counts on LM17 and LM17 plus cycloheximide, respectively, enterococci on Kanamycin Aesculin Azide (KAA) medium, leuconostocs on Mayeux, Sandine and Elliker (MSE) medium, mesophilic lactobacillus on Facultatively Heterofermentative (FH) medium, thermophilic lactobacillus on deMan, Rogosa and Sharpe (MRS) medium, etc.). Differences in oxygen tolerance, nutritional requirements, antibiotic susceptibility, and colony morphology and color constitute the bases of differentiation among these methods. Selectivity of enumeration, however, can be strongly
improved by altering incubation temperatures, incubation times, and the pH of the medium. Media allowing the separate (or simultaneous) enumeration of probiotic bacterial species are available today for quality control of fermented milks (Giraffa 2004).

Classical plating methodologies, therefore, only allow a rough measure of microbial groups or genera and rarely is their sensitivity down to the species level. As stated above, the isolation, species identification, and (facultative) typing of microbial isolates from agar plates are needed to increase sensitivity. For the latter two purposes, either phenotyping or genotyping (or both) is applied (Olive and Bean 1999; Giraffa and Neviani 2000). Concerning phenotyping, identification down to species level can be performed by API galleries or other phenotypic methods such as the Phene Plate System (Iversen et al. 2002; Lund et al. 2002). These techniques allow dynamic trends of cultivable, dominant microbial populations or microbial groups to be highlighted.

Species-specific PCR has been introduced as a molecular tool for microbial identification down to the species level. The online availability of DNA sequences of ribosomal RNA (rRNA) genes and rRNA gene spacers of practically all the known microbial species and the presence of taxa-specific oligonucleotide stretches within the ribosomal locus have enabled these genes (or portions of them) to be routinely PCR-amplified and examined for differences indicative of species identity. Several PCR amplification protocols are presently available for practically all food-associated LAB species (Giraffa and Carminati 2008). Among PCR-based typing techniques, RAPD-PCR is the most popular applied technique in studying the microbial ecology of fermented foods. In recent years, hundreds of articles reported the application of RAPD-PCR to identify the presence, succession, and persistence of microorganisms (both useful and pathogens) in both fermented food and industrial environments (Cocolin and Ercolini 2008).

Classical phenotyping is laborious and time consuming, especially when both spatial and temporal distribution of microbial populations are needed. Most importantly, culture-dependent (both phenotypic and genotypic) identification and/or typing methods are not effective if, for any reason, microorganisms are not cultivable.

3.5.2 Culture-Independent Methods

In the last two decades, culture-independent, nucleic acid-based, molecular approaches have undergone considerable development in microbial ecology. Compared to traditional culturing, these methods aim to obtain a picture of a microbial population without the need to isolate and culture its single components. This is possible because these techniques are based on a “community DNA/RNA isolation approach.” Although there are limitations to these methods, they can nevertheless be very useful once these limitations are taken into consideration (for a review, see Forney et al. 2004). Such limitations include technical problems, such as obtaining representative genomic DNA from food samples, to conceptual questions, such as using universally accepted and meaningful definitions of microbial species. Such techniques enable analyses of total microbial communities and greatly improve our understanding of their composition, dynamics, and activity (Wilmes and Bond 2009).

Culture-independent methods are being increasingly applied to detect, quantify, and study microbial populations in food or during food processes. Culture-independent techniques and their application to fermented food have been exhaustively reviewed in a recent book (Cocolin and Ercolini 2008). They can be generally grouped into in situ methods and PCR-based techniques. The common trait of the in situ methods is that morphologically intact cells (both cultivable and uncultivable) can be identified and counted directly, that is, in minimally disturbed samples. The fluorescence in situ hybridization (FISH) with rRNA targeted oligonucleotide probes is the most popular in situ method, and its applications in food microbiology have recently been reviewed (Bottari et al. 2009). FISH has been used to evaluate bacterial community structure and location in fermented foods, probiotics, and cheeses (Giraffa and Carminati 2008).

PCR-based, culture-independent techniques have been the subject of considerable focus in food microbiology. Although most of these methods are generally based on the amplification of only the variable regions or the totality of the 16S rRNA genes, amplified fragments can derive also from total RNA extracted from food and amplified by reverse transcriptase–PCR (RT-PCR). Because active bacteria have a higher number of ribosomes than dead cells, the use of RNA instead of DNA highlights the
metabolically active populations present in the ecosystem. PCR-based methods such as PCR-denaturing gradient gel electrophoresis (PCR-DGGE) are routinely used to quantify either pathogens or beneficial populations such as starter microbes or probiotics in fermented foods (Le Dréan et al. 2010; Giraffa and Carminati 2008; Malorni et al. 2008). It should be mentioned that by combining different methods (e.g., PCR-DGGE, cloning and sequencing of rRNA gene amplicons, and classical cultivation techniques) in a “polyphasic ecology” approach, it is now possible to profile time-dependent specific shifts in the composition of complex food microflora, to evaluate and quantify noncultivable food populations, and, among these latter, to monitor the metabolically active microbial groups (Giraffa 2004). For example, DGGE and other techniques have successfully been applied in polyphasic studies to monitor the microbial dynamics of food ecosystems (Ercolini 2004; Ercolini et al. 2004).

3.5.3 Present Trends

3.5.3.1 Phenomics

In recent years, a breakthrough technology, the Phenotype MicroArrays (PM), made it possible to quantitatively measure thousands of cellular phenotypes all at once (Bochner 2009). This technology, following on to proteomics and genomics technologies, can be categorized as "phenomics." The advantage of phenomic research is the ability to conduct simultaneous testing of numerous bacterial phenotypes by using an automated instrument to measure growth in the presence of various substrates. This instrument determines cell growth every 5 min by monitoring absorbance of substrate color change of an appropriate indicator. For substrate metabolism, 700–2000 different substrates located in microtiter well plates could be tested at one time.

The application of PM technology to food microbiology for a global phenotypic characterization of bacteria is still in its early stages and concerns the phenotypic analysis of human and animal pathogens (Guard-Bouldin et al. 2007; Viti et al. 2008; Tang et al. 2010). The information provided may be complementary to, and often more easily interpretable than, information provided by global molecular analytical methods such as gene chips and proteomics. Phenomics could help in discovering the physiology and the variability in the phenotypic expression of starter or probiotic bacteria in many different growth conditions, thus helping to predict their behavior in fermented food products.

3.5.3.2 Quantitative PCR

It is well known that standard PCR reactions are not quantitative. A promising tool for the advancement of studies on food-associated microbial populations, either cultivable or not cultivable, is the application of quantitative PCR (qPCR) to food systems. Typical qPCR utilizes approaches originally developed in clinical microbiology, with the 5'-fluorogenic exonuclease (TaqMan) assay representing the widest applied development. By using an internal probe, which is labeled with fluorescent dyes, in addition to standard PCR amplification primers, TaqMan chemistry provides in-tube, real-time detection of PCR product accumulation (real-time PCR) during each amplification cycle and at very early stages in the amplification process. Using DNA as starting material, qPCR offers the possibility to quantify microbial populations through measurement of gene numbers. Using RNA as a template combined with reverse transcription (RT), qPCR can also estimate transcript amounts, therefore providing data on microbial activity (Giraffa and Carminati 2008).

In the last years, (RT-)qPCR applications in food microbiology have strikingly developed. Real-time PCR is increasingly applied for specific detection and quantification of pathogens and spoiling agents in food (McGuinness et al. 2009; Juvonen et al. 2008) and for the enumeration of beneficial microbial populations in fermented milk products (Bleve et al. 2003; Furet et al. 2004; Masco et al. 2007; Zago et al. 2009; Le Dréan et al. 2010), dairy starters (Friedrich and Lenke 2006), and wine (Neeley et al. 2005). Innovation is moving toward simultaneous identification of several microbial species by applying multiplex amplification. By TaqMan chemistry, several species-specific DNA probes can be applied using different fluorophores, thus enabling different targets to be co-amplified and quantified within a single reaction tube (Smith and Osborn 2009).
3.5.3.3 Chip, DNA Array-Based, Technology

The DNA chip microarray technology is a direct result of the availability of genome sequence information. The technique involves very large (approximately 100,000) cDNA sequences or synthetic DNA oligomers being attached onto a glass slide (the chip) in known locations on a grid. An RNA sample is then labeled and hybridized to the grid, and relative amounts of RNA bound to each square in the grid are measured. Such DNA chips can be used for simultaneous monitoring of levels of expression of all of the genes in a cell in order to study whole genome expression patterns in various matrices during development. Moreover, because parallel hybridizations to hundreds or thousands of genes in a single experiment can be performed by high-throughput DNA microarrays, direct profiling of microbial populations is achievable. Rudi et al. (2002) combined the specificity obtained by enzymatic labeling of species-specific, oligonucleotide probes with the possibility of detecting several targets simultaneously by DNA array hybridization with 16S rRNA gene from pure cultures. By hybridization of chip-bound probes with bulk DNA extracted from food, this is a promising tool for microbial community analyses in foods. In one development of this basic technique, Bae et al. (2005) described genome-probing microarrays (GPMs), which consist of depositing hundreds of microbial genomes as labeled probes on a glass slide and hybridizing with bulk community DNAs. GPM enabled quantitative, high-throughput monitoring of LAB community dynamics during fermentation of kimchi, a traditional Korean food. Compared to currently used oligonucleotide microarrays, the specificity and sensitivity of GPM were remarkably increased (Bae et al. 2005).

3.5.3.4 Functional Proteomics

Because the level of bacterial mRNA does not always correlate with the amount of expressed protein in the cells and because it has a short average life span, alternatives to DNA microarrays for gene expression have started to emerge. Functional proteomics, coupled to protein microarrays, is considered one of the leading technologies for bacterial and gene identification. Proteins and antibodies can be attached to chemically pretreated slides to build a protein microarray chip that is able to identify bacteria, antibodies, and viruses. Unlike DNA, proteins are difficult to attach to a glass slide and to synthesize and are easily denatured. Therefore, building a protein array can be more challenging than building the DNA chip (Al-Khalidi and Mossoba 2004). Because of the amount of data we may uncover by proteomics, protein microarray could be helpful for identification and functional characterization of food microorganisms.

3.5.3.5 Data Analysis

Driven by automated DNA sequencing technology and the different genome projects, analysis of DNA and protein sequence data has spurred the dramatic growth of a new scientific discipline—bioinformatics—in the 1990s. Bioinformatics can be defined as the use of computers for the acquisition, management, and analysis of biological information. Bioinformatics combines in silico biological techniques with the DNA sequencing analysis approach. In silico biology combines statistical and mathematical algorithms with the need to manage and elaborate huge numbers of biological data. The development of bioinformatics has enabled improvement of the interpretation and elaboration of microbiological data. The acquisition of specialized, commercially available software packages, which are expensive and demand a high level of technical skill for their efficient use, is necessary so that the most important international microbial collections can manage, compare, and implement databases holding information on nucleic acid (or protein) sequences, electrophoretic profiles, and phenotypic data.

One of the advantages of bioinformatics in relation to studying bacterial taxonomy and diversity concerns the possibility of sharing databases. As stated above, diagnostic tools based on PCR have been developed for rapid inexpensive genotype assay. In addition, several microbial genomes have now been sequenced (for a review, see Klaenhammer et al. 2005), while large numbers of DNA sequences have been compiled and are available via the World Wide Web. There is an urgent need to process this mass of information into a useful classification tool, which will require further automation and software development in order to effectively link different databases. In addition, bioinformatics could allow advances in
functional genomics, that is, conversion of the mass of sequence data presently available in public data banks into knowledge, so that microbial diversity could be assessed not only at the molecular level but also at the functional level (Perego and Hoch 2001).

### 3.6 Final Remarks and Future Needs

Modern food microbiologists are fortunate in having a variety of tools that provide very advanced molecular differentiation of microorganisms and that can be tailored to fit the needs of both research laboratories and the food industry. Both cultivable and noncultivable bacteria can be analyzed, microbial populations can be quantified, and new microbial species can be isolated and characterized. Once efficiently integrated via advances in bioinformatics, molecular identification and fingerprinting techniques will provide more precise information on microbial taxonomy and functional diversity of a given food system at a particular time and space. Several of these molecular methods, once applied to the food industry, could enable the creation of large reference libraries of typed organisms to which new strains can be compared either within the same laboratory or across different laboratories. Aspects such as changes in microbial populations, identification of contamination sources, management of customer/supplier disputes, assessment of sanitation programs, authentication of starter cultures, and verification of laboratory culture integrity could then be efficiently monitored. The choice of a molecular typing method will depend upon the needs, level of skill, and resources of the laboratory concerned.

However, more specific problems arising in the analysis of microbial communities of food ecosystems need specific answers, for example, how we can increase our knowledge of cell physiology, cell-to-cell interactions, and in situ modification of the microbial metabolism in natural ecosystems, especially in response to adverse environmental conditions; how we can detect and possibly quantify nonculturable and/or nondominant species/strains; and whether there are nondestructive methods of sample preparation to better evaluate spatial distribution and colonization of microorganisms in heterogeneous food matrices.

Another key point to be addressed is that many of the methods reported above are PCR based. PCR is an excellent technique for examining mixed microbial communities. Amplification, however, often introduces bias that makes quantification of natural populations difficult. The ability to detect target rRNA sequences without amplification (i.e., direct profiling of rRNA sequences) would greatly improve our capacity in determining the relative number of sequences representing natural microbial populations. In this regard, high-throughput DNA microarrays, which provide for parallel hybridizations to hundreds or thousands of genes in a single experiment, may play a major role in the study of food systems. Furthermore, standard PCR reactions are not quantitative. Promising tools for the advancement of studies on food-associated microbial populations, either cultivable or not cultivable, include qPCR, which has the potential for accurate and highly sensitive enumeration of microorganisms.

Finally, more knowledge is needed on the physiological state (viability), the stress response, and the survival of microorganisms into foods, especially probiotic foods. Stressed, sublethally injured, or otherwise “viable but nonculturable” cells often go undetected when using traditional microbiological techniques. In this regard, flow cytometry (FCM) has been recently applied to analyze, in different proportions, subpopulations of variably stressed (or damaged) bacteria in probiotic products and dairy starters. The sensitivity of the FCM method was significantly higher than agar plate techniques (Budde and Rasch 2001; Bunthof and Abee 2002).

### REFERENCES


