Food Safety: What Can We Learn From Genomics?

Máire Begley[1] and Colin Hill[1,2,3]

[1]Food for Health Ireland, University College Cork, Cork, Ireland

[2]Department of Microbiology, University College Cork, Cork, Ireland

[3]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland; email: m.begley@ucc.ie, c.hill@ucc.ie

Annu. Rev. Food Sci. Technol. 2010. 1:341–61

First published online as a Review in Advance on December 7, 2009

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi:
10.1146/annurev.food.080708.100739

1941-1413/10/0410-0341$20.00

Key Words

food pathogen, comparative genomics, functional genomics, virulence, stress responses

Abstract

The entire genome sequences of numerous foodborne pathogens have been determined, and genome sequencing projects of many others are currently underway. The resulting sequence information will permit detailed bioinformatic analyses and provide direction for subsequent functional analyses. Genomics-driven studies will have many applications in food safety, such as assisting with the development of tools for the rapid detection and identification of pathogens and helping to provide insights into their evolution, biology, and ecological fitness. These studies will also aid in elucidating the mechanisms employed by pathogens as they adapt to the variety of conditions encountered throughout their life cycle, from the food-processing environment to in vivo during infection. It is anticipated that genomics will aid in the development of novel preventative and control strategies, which in turn will ultimately lead to a safer food supply.

INTRODUCTION

Morbidity and mortality resulting from the consumption of microbially contaminated foods remain significant problems in modern society. The Centers for Disease Control and Prevention (CDC) has estimated that foodborne diseases are responsible for approximately 76 million illnesses, 323,000 hospitalizations, and 5200 deaths in the United States annually (Mead et al. 1999). In addition to the significant burden in terms of human health, the hospitalization and treatment costs associated with foodborne illnesses, together with recalls of implicated food products, have enormous economic impact.

Ensuring a safe food supply is a major challenge to the food industry, and there are several factors that can impact the likelihood of contamination and subsequent disease. These factors include an increased consumer demand for minimally processed, ready-to-eat convenience foods and imported and ethnic foods, the globalization of the food industry, significant changes in the methods used to control microbial spoilage (e.g., minimally processed foods, modified atmosphere packaging, natural preservation methods such as bacteriocins), and changing population demographics (more elderly and immunocompromised individuals) (Abee et al. 2004, De Vos 2001, Kuipers 1999).

Significant advances in DNA sequencing technologies have led to the elucidation of the entire genome sequences of numerous foodborne pathogens and that information is now guiding future efforts to link genotype to phenotype. The impact that this research has on food safety is discussed below, concentrating on *Listeria monocytogenes* as a model to illustrate the impact of genomics on our understanding of this important pathogen. *L. monocytogenes* was chosen as it is one of the most thoroughly studied bacterial food pathogens, and the genome sequences of numerous strains have been determined. Significant advances in our knowledge of listerial growth, survival, and virulence mechanisms have been made possible by taking advantage of fundamental genomics studies.

"OMICS" TECHNOLOGIES

The relatively small size of bacterial genomes, together with improvements in large-scale sequencing methodologies, has meant that the number of bacterial genomes sequenced has exploded in recent years. Indeed, genome sequencing has become a relatively trivial task, often outsourced to dedicated service providers. The majority of sequenced genomes are freely available in public databases such as the National Center for Biotechnology Information Genome Web site (**http://www.ncbi.nlm.nih.gov/sites/entrez**). Individual or multiple strains of the most prevalent foodborne pathogens have been sequenced, including strains of *Bacillus cereus* (Han et al. 2006, Hoffmaster et al. 2004, Ivanova et al. 2003, Rasko et al. 2004), *Campylobacter jejuni* (Parkhill et al. 2000), *Clostridium botulinum* (Sebaihia et al. 2007), *Escherichia coli* (Hayashi et al. 2001, Perna et al. 2001), *L. monocytogenes* (Glaser et al. 2001), and *Salmonella enterica* serovar Typhimurium LT2 (McClelland et al. 2001). The genome sequencing status of foodborne pathogens is briefly summarized in **Table 1**.

Once an entire genome sequence has been determined, it can be analyzed using a variety of computer-aided approaches. Major advances in bioinformatics mean that genomes can be mined to generate a wealth of useful information (Bansal 2005, Bhagwat & Bhagwat 2008, Fratamico 2008). For example, in silico analyses can compile detailed metabolic pathways and identify genes involved in growth and survival in different environmental niches. The genome sequences of foodborne pathogens can be compared with each other and to nonpathogenic organisms in order to identify common and unique genes. Comparative genomics can have many potential applications, including developing tools for discriminating between pathogen and nonpathogenic species within a given genus and for identifying virulence genes (see below for specific examples).

Table 1 Food-poisoning microorganisms and their genome sequence availability

Bacterium	Relevant characteristics	Number of genome sequences available on NCBI genome database
Listeria monocytogenes	Gram positive	24
Yersinia enterocolitica	Gram negative	1
Aeromonas hydrophila	Gram negative, toxin producer	2
Clostridium botulinum	Gram positive, toxin producer, spore former	15
Bacillus subtilis	Gram positive, toxin producer, spore former	5
Bacillus licheniformis	Gram positive, toxin producer, spore former	2
Bacillus cereus	Gram positive, toxin producer	20
Salmonella	Gram negative	20
Vibrio parahaemolyticus	Gram negative, toxin producer	7
Escherichia coli	Gram negative	20
Staphylococcus aureus	Gram positive	20
Clostridium perfringens	Gram positive	9
Campylobacter jejuni	Gram negative	13

Information was obtained from the National Center for Biotechnology Information (NCBI) genome database (**http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome**) on July 16, 2009.

Although our ability to successfully and precisely annotate gene function remains a significant bottleneck, many useful sequence analysis tools and software programs are Web-based and freely available. Most are very user friendly, and only a basic knowledge of bioinformatics is required. For example, the KEGG PATHWAY database (**http://www.genome.jp/kegg/pathway.html**) generates pathway maps from genomes and draws wiring diagrams of molecular interactions, reactions, and relations. It is also common for the institutes that sequence genomes to design dedicated Web sites that allow easy and rapid analysis of the genomes. For example, ListiList (**http://genolist.pasteur.fr/ListiList/**), provided by the Institut Pasteur, allows the user to locate a gene in the *L. monocytogenes* genome by name or function, perform pattern and homology searches, and draw maps of chosen genomic regions.

Perhaps most importantly, the information obtained from genome sequence analyses can provide testable hypotheses that can be used to design functional genomics experiments such as the identification of genes for targeted mutagenesis. Information can also be employed to design experimental conditions for and analyze the results obtained from transcriptomic, proteomic, and metabolomic studies. Such experiments generate enormous amounts of data, and allied developments in high-throughput analytical methods including microarrays, 2D electrophoresis, and mass spectroscopy have resulted in "omics" technologies becoming increasingly more accessible, faster, and more affordable.

"Omic" technologies are summarized in **Figure 1** and definitions of "omics"-related terminologies and technologies are provided in **Table 2**.

FOOD SAFETY: WHAT CAN WE LEARN FROM GENOMICS AND "OMICS" TECHNOLOGIES?

The information obtained from genomics-driven studies will have many applications in food safety, including the development of tools for rapid detection and identification of pathogens,

Table 2 Explanations of key genomics-related terms

Term	Explanation
Bioinformatics	in silico biology: the use of computers to answer biological questions. It is the computational branch of molecular biology.
Genome	The full set of genes in an organism.
Genomics	The study of the total DNA content of cells (includes chromosome and plasmids).
Genome annotation	The process of identifying the coding regions of genes in a genome and predicting function by bioinformatic analyses.
Functional genomics	The examination of gene (and protein) functions and interactions.
Comparative genomics	The study of the relationship of genome composition and function across different species or strains. Comparative genomics is used to identify genetic differences across entire genomes and correlate those differences with biological function.
Transcriptomics	Global analysis of gene expression; the analysis of the activity of each genetic locus. Transcriptomics can be used to examine expression of each gene under various conditions and identify when genes are up- and down-regulated in response to changes in the environment of the cell.
Proteomics	The study of the complete set of proteins produced by a cell. Proteomics can be performed under various conditions to identify when protein expression is up- and down-regulated in response to changes in the environment.
Metabolomics	The study of small molecules or metabolites. It provides a snapshot of the amount of each metabolite in the cell at a given point.
Systems biology	The study of organisms as systems; i.e., the study of the interactions between all of the genes, proteins, and biochemical reactions and how these interactions contribute to the function and behavior of the organism. Involves the integration of biological data into mathematical-based models such that biological outcomes can be predicted with accuracy.

and provision of relevant information regarding the ability of pathogens to adapt to processing conditions and survive and grow in various environments. Some of these applications will now be discussed in more detail with specific examples from the literature.

Pathogen Detection, Identification, and Typing

The rapid and precise detection and identification of foodborne pathogens are critical for food safety. In addition, the identification of bacteria at the strain level, i.e. bacterial strain typing, is important for the purposes of surveillance and outbreak investigation. Genomics technologies can aid in the development of improved pathogen detection, identification, and typing tools.

Detection and identification of food pathogens. Traditional detection and identification methods are primarily phenotype based and characterize bacterial genes through the measurement of biochemical and antigenic properties (Liu 2006). For example, *L. monocytogenes* can be identified based on its ability to hemolyse horse or sheep red blood cells or by detection of surface proteins (somatic O or flagellar H antigens) with antibodies. Traditional approaches are usually laborious, time consuming, and costly. Furthermore, as the biochemical and antigenic properties of bacteria can vary with changing environmental conditions, with growth phase etc., traditional methods can give variable results and consequently may be unreliable (Liu 2006). In recent years, numerous molecular-based diagnostic methods have been developed, and as they are less affected by natural variation than phenotypic methods they are considered as being inherently more precise (Call 2005, Giannino et al. 2009, Liu 2006, Mozola 2006, Scaria et al. 2008, Uttamchandani et al.

2008, Volokhov et al. 2002, Yoo & Lee 2008, Wang et al. 2007, Woods et al. 2008). Four of the most widely used molecular techniques, namely polymerase chain reaction (PCR), microarray, nucleic acid sequence-based amplification (NASBA), and ligation detection reaction-universal arrays (LDR-UA), are described and compared in a recent article by Lauri & Mariani (2009). These molecular methods will benefit greatly from the availability of food pathogen genome sequences. Analysis of genomes will enable the identification of regions unique to individual pathogens and permit the design of primers and probes with increased specificity. For example, *Listeria* genome information has been used to develop microarray-based strategies that can differentiate between the six listerial species (Volokhov et al. 2002) and can discriminate different *L. monocytogenes* serovars (Borucki et al. 2003) and phylogenetic lineages (Call et al. 2003). Genome analyses will also aid in the development of assays that accurately allow the simultaneous detection of multiple pathogens. This application of genomics on food pathogen molecular diagnostics has been elegantly demonstrated by Kim et al. (2008). These researchers compared the entire genome sequences of 11 pathogens (*B. cereus*, *L. monocytogenes*, *S. aureus*, *C. botulinum*, *C. perfringens*, *C. jejuni*, *V. parahaemolyticus*, *Y. enterocolitica*, *E. coli* O157:H7, *S. typhi*, and *S. typhimurium*) and identified regions specific to each. Oligonucleotide probes were subsequently designed for these regions (10 probes for each pathogen) and used to develop DNA microarrays. These microarrays were shown to be highly specific for each pathogen, and the authors suggest that their method can be applied to the rapid and accurate detection of foodborne pathogens in the food industry (Kim et al. 2008).

PCR: polymerase chain reaction

Bacterial strain typing. Similar to bacterial species-specific identification, classical typing methods involve either analysis of bacterial phenotypes, i.e., phenotyping, or analysis of bacterial genetic content, i.e., genotyping, which is also referred to as DNA fingerprinting. Examples of phenotypic methods include serotyping and phage typing, whereas genetic methods include pulse-field gel electrophoresis (PFGE), ribotyping, and PCR-based techniques such as random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), PCR-restriction fragment length polymorphism (PCR-RFLP), and multiple-locus variable number tandem repeat analysis (MLVA) (Li et al. 2009, Liu 2006). In comparison to phenotypic typing, genetic approaches are highly sensitive, discriminatory, and reproducible. The main genotyping methods currently in use have recently been reviewed by Li et al. (2009).

Genome sequencing of food pathogens will provide detailed information of intraspecies diversity (Binnewies et al. 2006, Fraser-Liggett 2005) and will enable the rational selection of adequate targets for many genotyping methods (Li et al. 2009). For instance, MLVA will benefit from the availability of genome sequences as highly polymorphic variable number tandem repeats (VNTR) or intergenic spacers can be identified (Van Belkum 2007). This approach was recently used by Mullane et al. (2008), who analyzed the *Enterobacter sakazakii* ATCC BAA-894 genome for VNTR and used the results obtained to develop a multiple-locus VNTR analysis strategy for the molecular subtyping of the bacterium. Their MLVA method successfully discriminated a collection of 112 isolates, leading the authors to suggest that their method could potentially be used to rapidly monitor clonal outbreaks of the bacterium in food production facilities (Mullane et al. 2008). The availability of food pathogen genome sequences also facilitates the rational choice of primers for PCR-based genotyping methods and aids in probe design in DNA microarray–based genotyping (Zhou 2003). For example, Doumith et al. (2004a) developed a rapid multiplex PCR using four sets of primers to differentiate the major *L. monocytogenes* serovars isolated from food and patients (1/2a, 1/2b, 1/2c, and 4b). The specificity and reliability of this multiplex assay were validated by a multicenter study (Doumith et al. 2005).

Growth and Survival Strategies

Genomics-driven studies can provide information regarding the ability of pathogens to survive and adapt to various environmental conditions. These studies will enable the identification of genes linked to niche adaptation and aid in understanding the molecular basis of bacterial diversity. Genomics technologies will also be instrumental in deciphering the mechanisms employed by pathogens to resist antimicrobial agents.

Stress adaptation and cross-adaptation. Foodborne pathogens encounter many environmental insults during food production, preparation, and storage, including low pH, elevated osmolarity, exposure to preservatives, and temperature fluctuations. As it is becoming increasingly popular for food processors to use a combination of food preservation strategies (often referred to as hurdle technology), food pathogens may have to overcome many stressful conditions in succession (Hill et al. 2002, Leistner 2000). Research has shown that pre-exposure of bacteria to sublethal levels of a given stress allows cells to adapt and protect themselves against subsequent exposure to normally lethal levels of the same stress (stress adaptation). Pre-exposure to one stress may also confer protection against another (stress cross-adaptation). This phenomenon, in which exposure of cells to mild stresses allow them to be trained or prepared for exposure to harsher stresses, is termed the bacterial stress response, and it undoubtedly contributes to the growth and survival of pathogens in food. Understanding how bacteria sense and respond to stresses is therefore essential to designing optimal processing regimes combining maximum safety with consumer demands for fresh food.

Several of the systems employed by bacteria to protect themselves against the conditions used in food preservation were identified in the pregenome era, including low pH and salt survival mechanisms in *L. monocytogenes* (Hill et al. 2002). However, it is anticipated that research in this area will be accelerated by the availability of bacterial genome sequences. For example, potential stress sensing and stress management systems can be identified based on homologies to characterized systems in other bacteria, and comparative genomic analyses can be employed to identify strain- or species-specific stress tolerance mechanisms. We have employed both of these approaches in our laboratory to identify listerial stress survival mechanisms. *In silico* analysis of the *L. monocytogenes* EGDe genome revealed the presence of homologs of characterized arginine deiminase (*arc*) genes that were known to play a role in tolerating low pH in other bacterial genera (Ryan et al. 2009). Mutation of the listerial genes confirmed that they encode a functional arginine deiminase system that contributes to both growth and survival of the bacterium under acidic conditions. In a separate study, our comparative genome analyses of all of the publicly available *L. monocytogenes* genomes identified a group of five genes that were present in some strains (nonserotype 4 strains) but consistently absent in others (serotype 4 strains) (Ryan 2006). A deletion mutant lacking all five genes was created, and subsequent experiments revealed that this mutant was impaired in growth at low pH and at high salt concentrations and demonstrated decreased ability to survive and grow in a model food system (frankfurters). We suggest that this group of genes, which we term SSI-1 for stress survival islet, can contribute to increased environmental persistence of certain strains of *L. monocytogenes* (see **Figure 2** for an outline of the experimental approaches used to identify and characterize SSI-1).

Microarrays can be employed to look at the response of pathogens to food-related stress conditions and identify stress stimulons (Puttamreddy et al. 2008). For example, microarray technology has identified *L. monocytogenes* genes whose expression alters in response to exposure to alkali (Giotis et al. 2008), low temperature (Chan et al. 2007), and high hydrostatic pressure processing (Bowman et al. 2008). Microarrays have also been performed to explore strategies used by *L. monocytogenes* when growing in microbial consortia on ripening smear cheese, which is an important

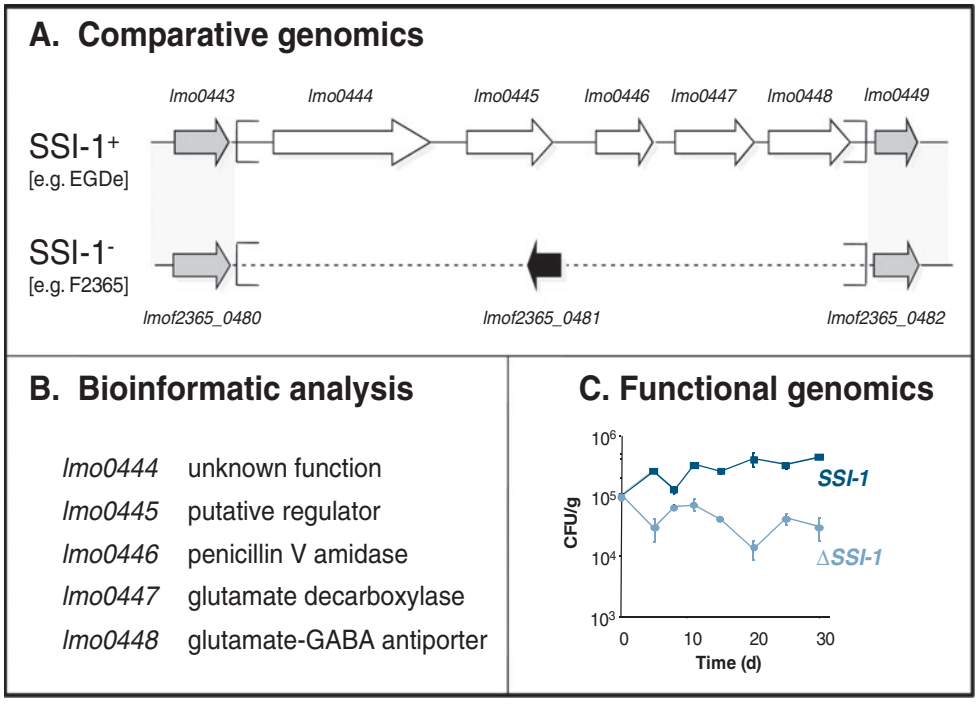

Figure 2

Correlating genes and functions: identification of a five gene islet in *Listeria monocytogenes*. (*a*) Comparative genomics revealed a five gene islet present in some strains of *L. monocytogenes* that is absent in others (replaced by a single gene at the same chromosomal location). (*b*) Bioinformatic analysis predicts that genes are involved in regulation, penicillin resistance, and pH homeostasis, suggesting that the islet may be involved in stress survival (stress survival islet −1, SSI-1). (*c*) Functional genomics: The five gene islet is excised from the chromosome and the behavior of the parent and mutant strains is compared in homogenized hotdog slurry at 4°C, confirming a role for SSI-1 under stressful conditions.

source of food poisoning strains (Hain et al. 2007). The consortium was grown on agar plates, and filter membranes were placed on top onto which *L. monocytogenes* was spread. *L. monocytogenes* gene expression at specific time points was examined by microarray analyses and it was found that nearly 400 genes are up- or down-regulated upon contact with the antilisterial consortium. Microarrays have recently been employed to examine *B. cereus* gene expression in response to oxygen limitations (van der Voort & Abee 2009) and salt (den Besten et al. 2009). Steil et al. (2003) utilized a transcriptional profiling approach to investigate the responses of *B. subtilis* following sudden exposure to low salinity (0.4 M NaCl) and also following prolonged growth at high salinity (1.2 M NaCl). Experiments revealed that initial adaptation to salt shock and continuous growth at high salinity share only a limited set of differentially expressed genes, suggesting that these two phases of adaptation require different strategies employed by *B. subtilis*. Budde et al. (2006) used a combined transcriptomic and proteomic approach to analyze the adaptational responses of *B. subtilis* at low temperature (minimal medium at 15°C). Propagation of *B. subtilis* under these conditions triggered induction of 279 genes and the repression of 301 genes in comparison to growth at 37°C. Microarrays have been used to examine alterations in *B. subtilis* gene expression during competence development and initiation of sporulation (Veening et al. 2006). Allen et al. (2008) employed microarrays to examine the response of *E. coli* O157:H7 to acid stress and low temperature.

Alternative stress sigma factor: a sigma factor that controls expression of genes required to tolerate suboptimal environmental conditions

Regulon: a set of genes that are regulated by the same regulatory protein

Microarrays can be performed on strains that are sensitive and resistant to a particular environmental condition to provide more in-depth analyses of microbial stress responses. For instance, Malone et al. (2006) exposed high pressure–resistant and pressure-sensitive strains of *E. coli* to a sublethal pressure (100 MPa for 15 mins at 23°C) and identified genes associated with decreased resistance to pressure (e.g., genes encoding Fe-S cluster assembly proteins) and others associated with barotolerance (e.g., a gene encoding a trehalose synthesis protein, OtsA).

Although technically challenging, the ultimate aim is to perform global transcriptional gene expression profiling of pathogens in real food matrices and under actual processing conditions. One such study by Liu & Ream (2008) monitored the gene expression profiles of *L. monocytogenes* strain F2365 in ultrahigh-temperature-processed (UHT) skim milk.

An enormous amount of data can be gleaned from microarray experiments. However, it is important to remember that microarrays indicate only alterations in transcription levels of genes and subsequent functional experiments are essential in order to confirm the role of specific gene products in growth and survival in food. Proteomics can be employed to examine bacterial protein expression in foods. Lippolis et al. (2009) recently examined proteomic changes in *E. coli* in milk, and Mujahid et al. (2008) explored *L. monocytogenes* proteins expressed in meat. Future studies should focus on combining various approaches (transcriptomics, proteomics, mutant analysis) in order to obtain a detailed picture of the mechanisms that are central to pathogen survival in foods.

Environmental sensing and coordination of stress responses. In order to survive at various stages of the food chain, pathogens must have the ability to assimilate information about their environment and process this information quickly to adapt to their surroundings by expressing genes that assist in survival and repressing those that are unnecessary. Genomics will aid in discovering the molecular mechanisms involved in stress sensing and coordination of stress responses.

Stress may be sensed by two-component systems (TCSs) consisting of a membrane-associated histidine kinase and a cytoplasmic response regulator. The histidine kinase senses alterations in an environmental parameter, and the associated response regulator effects changes in cellular physiology, often via regulation of gene expression. Williams et al. (2005) identified 16 TCSs in the *L. monocytogenes* EGDe genome and successfully mutated 15 of the response regulators. Characterization of the resultant mutants demonstrated that only the deletion of *degU* had a significant effect on the in vitro and in vivo growth of the bacterium (Williams et al. 2005).

A common strategy that bacteria use to counter stressful conditions is to activate a specific alternative stress sigma factor, which leads to transcription of a set of genes (regulon), the products of which protect the cell against the adverse conditions. In Gram-positive bacteria (*L. monocytogenes*, *B. subtilis*, *S. aureus*), the alternative sigma factor SigB is the key sigma factor controlling the stress response, whereas in Gram-negative bacteria (*E. coli*, *Salmonella*, *Vibrio*), stress responses are controlled by the alternative sigma factor Sigma S (RpoS). These regulators have been shown to be involved in tolerance of low pH, elevated osmolarity, temperature, ethanol, bacteriocins, antibiotics, and prolonged starvation and have also shown to play a role in biofilm formation and sporulation (Abram et al. 2008a, 2008b; Becker et al. 1998; Begley et al. 2005, 2006; Cebrián et al. 2009; Dodd & Aldsworth 2002; Dong & Schellhorn 2009; Hengge 2008; Klauck et al. 2007; Pané-Farré et al. 2006; Raengpradub et al. 2008; Senn et al. 2005; van Schaik & Abee 2005; van Schaik et al. 2007). Genome sequence analyses and whole genome microarrays can be employed to identify genes under the control of stress sigma factors. For example, Hain et al. (2008) used whole genome–based transcriptional profiling to identify *L. monocytogenes* SigB-dependent genes at different growth phases. This study identified 105 positively regulated genes and 111 genes that appear to be under negative control of SigB. It was concluded that the SigB regulon includes approximately 7.6% of all genes in the *L. monocytogenes* genome (Hain et al. 2008). Functional

genomic experiments can confirm the regulation of genes by SigB. For example, the bile salt hydrolase gene *bsh* was shown to be regulated by SigB at the transcriptional level (Begley et al. 2005, Hain et al. 2008, Kazmierczak et al. 2003, Sue et al. 2003). This finding was subsequently confirmed by bile hydrolysis assays with a *sigB* mutant as the ability of the mutant to hydrolyze bile was significantly reduced compared with the wild type (Begley et al. 2005, Sue et al. 2003).

Microarray analyses can also aid in uncovering the interplay between regulators and help elucidate the complex regulatory networks governing the response of a bacterium to its environment. For instance, microarrays have revealed that *L. monocytogenes* SigB can interact with regulators CtsR (Hu et al. 2007b), HrcA (Hu et al. 2007a), and PrfA (Milohanic et al. 2003, Ollinger et al. 2009).

The science of genomics has been recently advanced significantly by Toledo-Arana et al. (2009), who used tiling microarrays to determine the transcriptional profile of *L. monocytogenes* under a variety of conditions. This landmark study revealed the presence of 50 small RNAs that are expected to play a variety of regulatory roles under different environmental conditions. This discovery layers an additional level of complexity on an already poorly understood network of regulatory factors at work within the cell and will have to be factored into future systems biology approaches to understanding bacterial behavior in food and in vivo.

Strain-Specific Genes with Roles in Niche Adaptation

It has been widely demonstrated that strains within a given species can show significant diversity in terms of their general physiology, but in most instances a definitive explanation for this heterogeneity has not been established. It is anticipated that the data generated from genome sequencing projects will be crucial to our understanding of the molecular basis of this strain variation.

L. monocytogenes strains show major differences in their ability to grow and survive under various environmental conditions (e.g., foods, soil, intestine, etc.). For example, serotype 4 strains are less frequently detected in foods than other serotypes but are responsible for more outbreaks of listeriosis (Gilbreth et al. 2005, Gilot et al. 1996). This may suggest that serotype 4 strains are better adapted to surviving environmental conditions encountered during infection of the host than they are to surviving food environments. Analysis of the complete genome sequences of several *L. monocytogenes* strains (of different serotypes) has revealed high levels of similarity in both gene content and organization. This indicates that genomic compositions of different strains of *Listeria* are highly stable, and consequently it is the relatively small number of distinct regions that may play significant roles in the capability to adapt to specific niches (Buchreiser et al. 2003, Doumith et al. 2004b, Nelson et al. 2004). Nelson et al. (2004) compared the genomes of three strains of *L. monocytogenes* that have been associated with foodborne illness in the USA: F2365 (serotype 4b, cheese isolate), F6854 (serotype 1/2a, frankfurter isolate), and H7858 (serotype 4b, meat isolate). Their whole genome comparative analyses identified numerous strain- and serotype-specific genes that may contribute to the ability of strains to grow in their respective environmental niches, a hypothesis that can be verified by future functional genomics experiments.

Analysis of an *L. monocytogenes gadD1T1* mutant demonstrated that these genes, which encode a glutamate decarboxylase and associated transporter, contribute to growth of the bacterium at mildly acidic pH (Cotter et al. 2005b). Subsequent *L. monocytogenes* genome analyses revealed the strain variable nature of *gadD1T1* genes, and it was observed that the ability of *L. monocytogenes* strains to grow at mildly acidic pH could be correlated with the presence or absence of *gadD1T1* genes, in that strains that possessed these genes were better able to grow at low pH (Cotter et al. 2005b, Van der Veen et al. 2008).

The aforementioned *L. monocytogenes* studies demonstrate the use of genomics in identifying genes linked to niche adaptation. Similar studies have been carried out or are underway for other

food pathogens. An excellent example is the study by Mols et al. (2007), which assessed the growth performance of two *B. cereus* strains for nearly 2000 phenotypes and analyzed both genomes for strain-specific genes. Several food-relevant phenotypic differences were found between the strains and these differences could be linked with corresponding genetic features (Mols et al. 2007). The authors concluded that their results provided detailed insights into the metabolic capacity of the two strains and may provide indicators for their performance in different ecological niches.

Biofilm Formation

Biofilms are surface-associated communities of bacteria embedded in an organized, self-produced polymeric matrix (Jefferson 2004). The formation of biofilms is thought to be a microbial survival strategy that allows cells to survive hostile conditions (Hall Stoodley et al. 2004, 2005). Biofilm formation has been demonstrated by a wide variety of bacteria including several foodborne pathogens, e.g., *L. monocytogenes* (Yang et al. 2009), *E. coli* O157:H7 (Silagyi et al. 2009), *Cronobacter sakazakii* (Beuchat et al. 2009), and *S. enterica* (Mangalappalli-Illathu et al. 2008). Biofilms can occur on food-handling and food-processing surfaces, conveyer belts, stainless steel equipment, floors, and drains. Biofilms are a major concern in the food industry as they provide a means of bacterial persistence and transmission. Bacteria can be shed from biofilms to contaminate new surfaces; for example, the surviving microflora from meat carcasses can contaminate surfaces of equipment, and this in turn can lead to contamination of products placed on these surfaces. Biofilms can also be transferred to food products postprocessing, leading to reduced shelf life of the product (Kumar & Anand 1998).

It is clear that biofilms are undesirable in the food industry environment. However, as biofilms are more resistant to disinfectants and sanitizing agents than planktonic cells, their elimination from food-processing facilities presents a significant challenge and more effective measures are required for their prevention and removal. In order to develop such strategies, there is a need to fully understand the factors affecting bacterial attachment and biofilm formation, and this will be greatly aided by bacterial "omics" technologies. Considerable insights have been obtained on *E. coli* biofilm formation from whole-transcriptome profiling in combination with phenotypic assays, in vivo DNA binding studies and analysis of specific mutants (Wood 2009). For example, very recent experiments by Amini et al. (2009) revealed that lipopolysaccharides are crucial to the formation of *E. coli* biofilms and multiple pathways, including acid tolerance, capsule biosynthesis, and regulation of cell morphology, modulate this phenotype.

It has also been suggested that biofilms may contribute to the survival of pathogens in the human gastrointestinal tract (MacFarlane & Dillon 2006), and exposure to bile has been shown to improve biofilm formation of several pathogens (Begley et al. 2009b). Biofilm may protect bacteria against host defenses and the action of antimicrobial agents, but also planktonic cells may be shed from biofilms to reinfect the same host or be transmitted. Future studies on food pathogens may lead to a greater understanding of bacterial biofilm formation in food, food-processing environments, and in vivo and may lead to the identification of potential targets for the development of antibiofilm agents. In addition, "omics" technologies can be employed to fully investigate the mode of action of antibiofilm agents as recently demonstrated by Attila et al. (2009). As strains of a species differ greatly in their ability to form biofilms, it will be important to include a large collection of strains in all future experiments.

Resistance to antimicrobial agents. Foodborne pathogens may come into contact with antimicrobial agents at various stages of their life cycle, including exposure to bacteriocins in food products, sanitizers and detergents in food-processing environments, and antibiotics during infection.

Bacteriocins. Bacteriocins are microbially produced, gene-encoded peptides that have a bactericidal or bacteriostatic effect on other species. They have been shown to have many potential applications in the preservation of various foods, including extension of the shelf life of products by inhibiting spoilage organisms and generating safer foods by targeting food pathogens (Cotter et al. 2005a). Bacteriocins can be used either alone or in combination with other preservation techniques as part of hurdle technologies that can help reduce or eliminate the need for chemical preservatives as well as diminish the intensity of heat treatments (Galvez et al. 2007, 2008). The use of bacteriocin-based strategies for food preservation is therefore viewed as an attractive alternative by the food industry to satisfy consumer demands for more naturally preserved, minimally processed foods. However, one concern with the use of bacteriocins is the development of resistant foodborne pathogens. Genomics-related studies (microarrays, targeted mutagenesis, proteomics, etc.) should aid in understanding the development of bacteriocin-resistant pathogen derivatives in food and help in developing strategies to mitigate their emergence. In addition, although numerous bacteriocins have been identified and characterized, the need still exists to identify novel bacteriocins with more desirable properties such as enhanced potency, target specificity, or physicochemical properties. In silico analyses of bacterial genomes can be considered an attractive alternative to traditional functional assays for the identification of novel bacteriocin-producing strains and can identify excellent candidate strains for further investigation (Begley et al. 2009a, de Jong et al. 2006, Pfeiler & Klaenhammer 2007).

Sanitizers and disinfectants. In food-processing facilities, product contact surfaces may typically be cleaned and sanitized several times per day, whereas environmental surfaces may be cleaned on a weekly basis. The emergence and spread of resistance among foodborne organisms to the microbiocides used in these cleaning and sanitizing treatments are becoming concerns as such resistance may explain the persistence of organisms in food-processing facilities (Poole 2002). In addition, it has been suggested that pathogens that develop resistance to microbiocides may also become resistant to clinically important antibiotics. It is therefore of utmost importance to uncover the mechanisms involved in resistance. This can be achieved through genomics-related experiments such as transcriptional profiling and mutant analyses. Results may identify generalized resistance mechanisms and may aid in choosing targets and cleaning formulations for optimal safety and effectiveness.

Antibiotics. The prevalence of antimicrobial resistance among foodborne pathogens has proven to be a problem in the treatment of humans with antibiotics. Experiments similar to those mentioned for determining bacteriocin and detergent resistance could also be employed to reveal molecular mechanisms underlying antibiotic resistance. For example, efflux pumps are thought to be important determinants for intrinsic and/or acquired resistance to antimicrobials (Poole 2007). The availability of pathogen genomes means that efflux pump genes can now be identified and targeted.

HOST-PATHOGEN INTERACTIONS

Tolerance of Physiologically Relevant Stress Conditions

As similar types of stresses can be encountered both in food and in vivo, genes necessary for survival of suboptimal food-processing conditions may also be required for efficient colonization of the host during infection. The genomics-related experiments described in the previous section used to uncover stress adaptation systems may also identify systems that contribute to the virulence

of foodborne pathogens. Although lowering the pH, the addition of salt, or the limitation of oxygen levels are approaches often used in the preservation of foods, pathogens will also encounter low pH in the stomach and intracellularly in macrophages and be exposed to varying levels of osmolarity and oxygen tensions during gastrointestinal transit. Therefore, acid-, salt-, and low oxygen-tolerance loci identified through genomics experiments may be important to the growth and survival of pathogens both outside and within the host. Recent experiments by Bo Andersen et al. (2007) with *L. monocytogenes* support the hypothesis that conditions that may be encountered in food-processing environments may influence the virulence of pathogens. Their study revealed that oxygen deprivation increases the infective potential of the bacterium in vitro in Caco-2 cells and in vivo in guinea pigs (Bo Andersen et al. 2007). Furthermore, the listerial general stress regulator SigB has been shown to contribute to survival within the host by regulating expression of genes involved in intestinal survival and by interacting with PrfA, the global regulator of virulence (Garner et al. 2006, Ollinger et al. 2009).

Therefore, as it is becoming increasingly evident that the environmental conditions to which pathogens are exposed prior to ingestion may influence the outcome of infection, the concept of stress adaptation or cross-adaptation is highly relevant for safety assessment. The number of pathogenic bacteria present in a given food item is important for food safety, but their physiological condition and the food environment can also affect the likelihood of disease (Bo Andersen et al. 2007). Consequently, it is of critical importance to fully understand how preservation regimes can affect survival of pathogens within the host, and genomics-related experiments will be instrumental in achieving this goal.

Identification of Virulence Factors

The availability of genome sequences of food pathogens can greatly increase our understanding of host-pathogen interactions as virulence factors can be identified through the use of bioinformatics, targeted mutant construction, and microarrays (Burrack & Higgins 2007, Hamon et al. 2006, Raskin et al. 2006). These different approaches have been very successfully applied to decipher the genetic basis of virulence in *L. monocytogenes*, and in this review we have highlighted specific examples from the literature to illustrate these approaches.

Genes that are present in the genome of a pathogenic species but absent from closely-related but nonpathogenic species can be identified and analyzed. Comparison of *Listeria* genomes revealed that a bile salt hydrolase (*bsh*) gene is present in the pathogenic *L. monocytogenes* but absent from the nonpathogenic *L. innocua*. Creation and analysis of an *L. monocytogenes bsh* mutant revealed that BSH contributes to bile tolerance and intestinal survival (Begley et al. 2005, Dussurget et al. 2002). In another example, postgenomic analysis has also revealed that although bacteria usually possess one pathway of isoprenoid biosynthesis, either the classical mevalonate pathway or the alternative nonmevalonate 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, *L. monocytogenes* unusually possesses the genes for both pathways (Begley et al. 2004). Creation and analyses of mutants confirmed that both pathways are functional and that the MEP pathway has an intermediate, HMB-PP, which stimulates human Vγ9/Vδ2 T cells and also contributes to virulence in mice (Begley et al. 2008). Examination of the *L. innocua* genome revealed that this nonpathogenic species naturally lacks the last two genes of the MEP pathway (and thus does not produce HMB-PP), and bioinformatic analysis strongly suggests that the genes have been lost through evolution (Begley et al. 2008).

Genes can be selected from the genome for functional analyses based on their homology to previously characterized virulence factors in other organisms. Cotter et al. (2008) performed in silico analyses of bacterial genomes to search for homologs of Streptolysin S (SLS), a hemolytic

and cytotoxic virulence factor of Group A *Streptococcus*. Potential SLS-related gene clusters were identified in the food pathogens *L. monocytogenes*, *S. aureus*, and *Cl. botulinum*. Comparative analyses of sequenced *L. monocytogenes* genomes revealed that the gene cluster (designated *Listeria* pathogenicity island 3 LIPI-3) is found predominantly among those strains responsible for the majority of listeriosis outbreaks (i.e., lineage I strains). More detailed functional analyses in *L. monocytogenes* strain F2365 revealed that expression of these genes results in the production of a hemolytic and cytotoxic factor that contributes to virulence of the pathogen (Cotter et al. 2008). This work represents the first identification of a virulence factor that is unique to certain epidemic strains of *L. monocytogenes*. In the future, studies such as this may permit greater discrimination by food regulators between strains with high and low virulence potential, such as is already the case between verotoxic *E. coli* and commensal strains that do not produce the toxin.

General stress response: a stress response induced by a set of diverse environmental stimuli

Whole genome DNA arrays can be used to screen large collections of strains of a pathogenic species and can be used to identify genes that are present in and specific to all virulent strains. Comparative genomics of a large collection of *L. monocytogenes* strains by genomic hybridization revealed important differences in gene content among different isolates, giving some clues as to how differences in virulence potential might have evolved (Doumith et al. 2004b). Subsequent comparative transcriptomic analyses of strains of the two major *L. monocytogenes* lineages by Severino et al. (2007) revealed differences in expression of genes encoding proteins involved in cell wall synthesis, the stress-related SigB regulon, and virulence. The authors suggest that these findings indicate different patterns of interaction with host cells and the environment as well as key factors for host colonization and survival in the environment.

Bacterial Gene Expression During Infection

Genes expressed by a pathogen within the host milieu can be identified by microarray experiments. This approach has provided valuable insights into the behavior of *L. monocytogenes* during infection. Chatterjee et al. (2006) infected a murine macrophage cell line (P288D1) with *L. monocytogenes* strain EGDe and examined the bacterial gene expression profile inside the vacuolar and cytosolic environments of the host cell using whole genome microarray followed by analyses of selected mutants. They found that approximately 17% of the total genome was mobilized to enable adaptation for intracellular growth. Adaptative intracellular gene expression involved genes associated with virulence and general stress response, cell division, and changes in cell wall structure and numerous other genes with unknown functions. Joseph et al. (2006) used microarray analyses to investigate the transcriptional profile of intracellular *L. monocytogenes* following infection of epithelial cells (Caco-2). Approximately 19% of genes were differently expressed relative to their level of transcription in brain-heart infusion medium, including genes encoding transporters, stress proteins, and transcriptional factors. These studies provide critical insights into the strategy of intracellular survival and the measures taken by *L. monocytogenes* to escape the host cell responses. Camejo et al. (2009) utilized DNA macroarray technology to profile the transcriptome of *Listeria* during murine infection. They compared the expression profiles of *L. monocytogenes* grown in standard culture medium in the exponential phase to bacteria recovered from mouse spleens 24, 48, and 72 hours after intravenous infection. Their results demonstrate that during infection *L. monocytogenes* shifts the expression of its entire genome to promote virulence, subverting host defenses and adapting to host conditions. Toledo-Arana et al. (2009) analyzed the transcription of the entire *L. monocytogenes* genome in vitro (brain-heart infusion broth), in vivo (cells extracted from mice intestines), and ex vivo (growth in human blood). Their data elegantly illustrate successive and coordinated global transcriptional changes in these different environmental locations.

CONCLUSIONS

Predictive microbiology: the prediction of microbial growth, survival or inactivation as a function of environmental factors

It is evident that information obtained from genomics-driven studies can have many applications to the field of microbial food safety. In addition to providing important insights into the evolution, biology, and ecological fitness of food pathogens, genomics studies will assist in the development of tools for their rapid and reliable detection and identification. Furthermore, genomics-based studies will permit analysis of bacterial behavior across the entire food production process from the raw material to the consumer, "from farm to fork," and will provide more accurate and precise data on the inactivation kinetics and growth inhibition of pathogens in food products and in the food-processing environment. The examination of the responses of pathogens to various environmental conditions will contribute to the field of predictive microbiology by allowing the construction of models that predict more accurately whether they are likely to grow, survive, or die in products or upon product processing in the food industry (Brul et al. 2002a, 2002b, 2006, 2008). Knowledge gained from stress-related experiments could potentially be incorporated into concepts that can be used for food preservation. For example, the activation of SigB may lead to a considerable increase in stress resistance and so SigB could serve as an important biomarker to assess the resistance of strains to preservation (Van Schaik & Abee 2005). In addition, the knowledge gained on SigB could be used to support the design of a process with sequential preservation steps in which the stress response is not activated or even repressed, thereby sensitizing the bacteria to subsequent preservation and inactivation treatments. Altogether, genomics and other "omics" technologies will permit the precise design of effective preservation strategies and hence make it possible to attain a balance between ensuring product safety and maintaining product quality. Genomics-related studies will also be instrumental in deciphering the mechanisms employed by pathogens to survive within the host. It is hoped that results obtained will aid in identifying the Achilles heel of pathogens and aid in the development of prevention and control strategies (Tang & Moxon 2001).

In conclusion, it is evident that any new technology, such as the "omics" technologies, that provides new biological insights can be applied to the science of food safety. The ability of food scientists to recognize the opportunities presented by these new technologies, and the willingness of regulators to respond to new information regarding the level of threat presented by individual strains or novel processing conditions, will determine the extent of the benefits for the food industry and consumers that can be derived from these exciting advances in basic science.

SUMMARY POINTS

1. Foodborne pathogens represent a serious threat to food safety and public health. The main challenges faced by food processors in ensuring a safe food supply include changing population demographics, changes in eating habits, alterations in the methods used to control microbial spoilage, and the development of novel products.
2. The entire genome sequences of several foodborne pathogens have been determined, and the genomes of many more are currently being sequenced. The analyses of these genome sequences and the results of subsequent experiments with "omic" technologies will significantly impact our ability to ensure food safety.
3. Genomics technologies can (*a*) aid in the development of pathogen detection and identification tools, (*b*) identify mechanisms used by pathogens to survive the various conditions encountered in the food-processing environment, e.g., low pH, preservatives, or salt (identify pathogen stress response mechanisms), (*c*) provide insights into the physiology and evolution of pathogens, and (*d*) identify systems important for pathogen survival in vivo during infection (virulence factors).

4. Genomics and related technologies may help food processors develop effective preservation strategies by using a knowledge-based combination of preservation hurdles [predictive modeling, hazard analysis and critical control points (HACCP)].

FUTURE ISSUES

1. Given the significant microbial diversity within species the genome sequences of a large collection of strains should be determined. This will allow more accurate conclusions to be drawn from in silico and comparative genome analyses.
2. As a consequence of microbial diversity, it is important that conclusions on the physiological properties of a pathogen not be based on a single strain of a species. Large collections of strains should be included in all experiments.
3. The functions of a large proportion of the genes identified in bacterial genomes are unknown and cannot be predicted from computational and homology analyses. Future efforts should focus on the mutation of these loci and analysis of the phenotypes of the resultant mutants.
4. Expression studies should be performed in real life situations including actual food matrices and not just under standard laboratory conditions. However, the technical challenges associated with accurately measuring bacterial gene expression in complex matrices will need to be overcome to realize this goal.
5. The knowledge gained from experiments on bacterial stress responses will have to be incorporated into practical concepts that can be used for food preservation. Results from these experiments should allow the development of a knowledge-based combination rather than an empirical combination of appropriate preservation hurdles.
6. Novel methods of food pathogen detection need to be tailored to the practical needs of the food industry.

Hazard analysis and critical control points (HACCP): involves identifying potential food safety hazards so that key actions can be taken to reduce or eliminate the risk of the hazards being realized

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to all of those whose work we have not cited because of space constraints. Our research is funded by Science Foundation Ireland, the Irish Government through a grant to the Alimentary Pharmabiotic Centre, and by Enterprise Ireland through a grant to Food for Health Ireland.

LITERATURE CITED

Abee T, van Schaik W, Siezen RJ. 2004. Impact of genomics on microbial food safety. *Trends Biotechnol.* 22(12):653–60

Abram F, Starr E, Karatzas KA, Matlawska-Wasowska K, Boyd A, et al. 2008a. Identification of components of the sigmaB regulon in *Listeria monocytogenes* that contribute to acid and salt tolerance. *Appl. Environ. Microbiol.* 74:6848–58

Abram F, Su WL, Wiedmann M, Boor KJ, Coote P, et al. 2008b. Proteomic analyses of a *Listeria monocytogenes* mutant lacking sigmaB identify new components of the sigmaB regulon and highlight a role for sigmaB in the utilization of glycerol. *Appl. Environ. Microbiol.* 74:594–604

Allen KJ, Lepp D, McKellar RC, Griffiths MW. 2008. Examination of stress and virulence gene expression in *Escherichia coli* O157:H7 using targeted microarray analysis. *Foodborne Pathog. Dis.* 5:437–47

Amini S, Goodarzi H, Tavazoie S. 2009. Genetic dissection of an exogenously induced biofilm in laboratory and clinical isolates of *E. coli*. *PLoS Pathog.* 5(5):e1000432

Attila C, Ueda A, Wood TK. 2009. 5-fluorouracil reduces biofilm formation in *Escherichia coli* K-12 through global regulator AriR as an antivirulence compound. *Appl. Microbiol. Biotechnol.* 82:525–33

Bansal AK. 2005. Bioinformatics in microbial biotechnology—a mini review. *Microb. Cell Fact.* 4:19

Becker LA, Cetin MS, Hutkins RW, Benson AK. 1998. Identification of the gene encoding the alternative sigma factor sigma B from *Listeria monocytogenes* and its role in osmotolerance. *J. Bacteriol.* 180:4547–54

Begley M, Bron PA, Heuston S, Casey PG, Englehert N, et al. 2008. Analysis of the isoprenoid biosynthesis pathways in *Listeria monocytogenes* reveals a role for the alternative 2-C-methyl-D-erytritol 4-phosphate pathway in murine infection. *Infect. Immun.* 76:5392–401

Begley M, Cotter PD, Hill C, Ross RP. 2009a. Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for LanM proteins. *Appl. Environ. Microbiol.* 75:5451–60

Begley M, Gahan CGM, Kollas AK, Hintz M, Hill C, et al. 2004. The interplay between classical and alternative biosynthesis controls γδ T cell bioactivity of *Listeria moncytogenes*. *FEBS Lett.* 561:99–104

Begley M, Hill C, Ross RP. 2006. Tolerance of *Listeria monocytogenes* to cell-envelope acting antimicrobial agents is dependent on SigB. *Appl. Environ. Microbiol.* 72:2231–34

Begley M, Kerr C, Hill C. 2009b. Exposure to bile influences biofilm formation by *Listeria monocytogenes*. *Gut Pathog.* 1:11

Begley M, Sleator RD, Gahan CGM, Hill C. 2005. Contribution of three bile associated loci, *bsh*, *pva* and *btlB* to gastrointestinal persistence and bile tolerance of *Listeria monocytogenes*. *Infect. Immun.* 73:894–904

Beuchat LR, Kim H, Gurtler JB, Lin L-C, Ryu J-H, Richards GM. 2009. *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. *Int. J. Food Microbiol.* 136:204–13

Bhagwat AA, Bhagwat M. 2008. Methods and tools for comparative genomics of foodborne pathogens. *Foodborne Pathog. Dis.* 5:487–97

Binnewies TT, Motro Y, Hallin PF, Lund O, Dunn D, et al. 2006. Ten years of bacterial genome sequencing: comparative-genomics-based discoveries. *Funct. Integr. Genomics* 6:165–85

Bo Andersen J, Roldgaard BB, Christensen BB, Licht TR. 2007. Oxygen restriction increases the infective potential of *Listeria monocytogenes* in vitro in Caco2 cells and in vivo in guinea pigs. *BMC Microbiol.* 7:55

Borucki MK, Krug MJ, Muraoka WT, Call DR. 2003. Discrimination among *Listeria monocytogenes* isolates using a mixed genome DNA microarray. *Vet. Microbiol.* 92:351–62

Bowman JP, Bittencourt CR, Ross T. 2008. Differential gene expression of *Listeria monocytogenes* during high hydrostatic pressure processing. *Microbiology* 154:462–75

Brul S, Coote P, Oomes S, Mensonides F, Hellingwerf K, Klis F. 2002a. Physiological actions of preservative agents: prospective of use of modern microbiological techniques in assessing microbial behaviour in food preservation. *Int. J. Food Microbiol.* 79:55–64

Brul S, Klis FM, Oomes SJCM, Montijn RC, Schuren FHJ, et al. 2002b. Detailed process design based on genomics of survivors of food preservation processes. *Trends Food Sci. Technol.* 13:325–33

Brul S, Mensonides FIC, Hellingwerf KJ, Teixeira de Mattos M. 2008. Microbial systems biology: new frontiers open to predictive microbiology. *Int. J. Food Microbiol.* 128:16–21

Brul S, Schuren F, Montijn R, Keijser BJF, Van Der Spek H, Oomes SJCM. 2006. The impact of functional genomics on microbiological food quality and safety. *Int. J. Food Microbiol.* 112:195–99

Buchreiser C, Rusniok C, The Listeria Consortium, Kunst F, Cossart P, Glaser P. 2003. Comparison of the genome sequences of *Listeria monocytogenes* and *Listeria innocua*: clues for evolution and pathogenicity. *FEMS Immunol. Med. Microbiol.* 35:201–13

Budde I, Steil L, Scharf C, Völker U, Bremer E. 2006. Adaptation of *Bacillus subtilis* to growth at low temperature: a combined transcriptomic and proteomic appraisal. *Microbiology* 152:831–53

Burrack LS, Higgins DE. 2007. Genomic approaches to understanding bacterial virulence. *Curr. Opin. Microbiol.* 10:4–9

Call DR. 2005. Challenges and opportunities for pathogen detection using DNA microarrays. *Crit. Rev. Microbiol.* 31:91–99

Call DR, Borucki MK, Besser TE. 2003. Mixed-genome microarrays reveal multiple serotype and lineage-specific differences among strains of *Listeria monocytogenes*. *J. Clin. Microbiol.* 41:632–39

Camejo A, Buchrieser C, Couvé E, Carvalho F, Reis O, et al. 2009. In vivo transcriptional profiling of *Listeria monocytogenes* and mutagenesis identify new virulence factors involved in infection. *PLOS Pathog.* 5(5):e1000449

Cebrián G, Sagarzazu N, Aertsen A, Pagán R, Condón S, Mañas P. 2009. Role of the alternative sigma factor sigma B on *Staphylococcus aureus* resistance to stresses of relevance of food preservation. *J. Appl. Microbiol.* 107:187–96

Chan YC, Raengpradub S, Boor KJ, Wiedmann M. 2007. Microarray-based characterization of the *Listeria monocytogenes* cold regulon in log- and stationary-phase cells. *Appl. Environ. Microbiol.* 73:6484–98

Chatterjee SS, Hossain H, Otten S, Kuenne C, Kuchmina K, et al. 2006. Intracellular gene expression profile of *Listeria monocytogenes*. *Infect. Immun.* 74:1323–38

Cotter PD, Draper LA, Lawton EM, Daly KM, Groeger DS, et al. 2008. Listerolysin S, a novel peptide haemolysin associated with a subset of lineage I *Listeria monocytogenes*. *PLoS Pathog.* 4(9):e1000144

Cotter PD, Hill C, Ross RP. 2005a. Bacteriocins: developing innate immunity for food. *Nature Rev. Microbiol.* 3:777–88

Cotter PD, Ryan S, Gahan CGM, Hill C. 2005b. Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. *Appl. Environ. Microbiol.* 71:2832–39

Den Besten HM, Mols M, Moezelaar R, Zwietering MH, Abee T. 2009. Phenotypic and transcriptomic analyses of mildly and severely salt-stressed *Bacillus cereus* ATCC 14579 cells. *Appl. Environ. Microbiol.* 75:4111–19

de Jong A, van Hijum SA, Bijlsma JJ, Kok J, Kuipers OP. 2006. BAGEL: a web-based bacteriocin genome mining tool. *Nucleic Acids Res.* 34:W273–79

De Vos WM. 2001. Advances in genomics for microbial food fermentations and safety. *Curr. Opin. Biotechnol.* 12:483–98

Dodd CE, Aldsworth TG. 2002. The importance of RpoS in the survival of bacteria through food processing. *Int. J. Food Microbiol.* 74:189–94

Dong T, Schellhorn HE. 2009. Global effect of RpoS on gene expression in pathogenic *Escherichia coli* O157:H7 strain EDL933. *BMC Genomics* 10:349

Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. 2004a. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819–22

Doumith M, Cazalet C, Simoes N, Frangeul L, Jacquet C, et al. 2004b. New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. *Infect. Immun.* 72:1072–83

Doumith M, Jacquet C, Gerner-Smidt P, Graves LM, Loncarevic S, et al. 2005. Multicenter validation of a multiplex PCR assay for differentiating the major *Listeria monocytogenes* serovars 1/2a, 1/2b, 1/2c and 4b: toward an international standard. *J. Food Prot.* 68:2648–50

Dussurget O, Cabanes D, Dehoux P, Lecuit M, Buchreiser C, et al. 2002. Listeria monocytogenes bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. *Mol. Microbiol.* 45:1095–106

Fraser-Liggett CM. 2005. Insights on biology and evolution from microbial genome sequencing. *Genome Res.* 15:1603–10

Fratamico PM. 2008. The application of "omics" technologies for food safety research. *Foodborne Pathog. Dis.* 5(4):5369–70

Gálvez A, Abriouel H, López RL, Ben Omar N. 2007. Bacteriocin-based strategies for food preservation. *Int. J. Food Microbiol.* 120:51–70

Galvez A, Lopez RL, Abriouel H, Valdivia E, Omar NB. 2008. Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Crit. Rev. Biotechnol.* 28:125–52

Garner MR, Njaa BL, Wiedmann M, Boor KJ. 2006. Sigma B contributes to *Listeria monocytogenes* gastrointestinal infection but not to systemic spread in the guinea pig infection model. *Infect. Immun.* 74:876–86

Giannino ML, Aliprandi M, Feligini M, Vanoni L, Brasca M, Fracchetti F. 2009. A DNA array based assay for the characterization of microbial community in raw milk. *J. Microbiol. Methods* 78:181–88

Gilbreth SE, Call JE, Wallace FM, Scott VN, Chen Y, Luchansky JB. 2005. Relatedness of *Listeria monocytogenes* isolates recovered from selected ready-to-eat foods and listeriosis patients in the United States. *Appl. Environ. Microbiol.* 71:8115–22

Gilot P, Genicot A, André P. 1996. Serotyping and esterase typing for analysis of *Listeria monocytogenes* populations recovered from foodstuffs and from human patients with listeriosis in Belgium. *J. Clin. Microbiol.* 34:1007–10

Giotis ES, Muthaiyan A, Blair IS, Wilkinson BJ, McDowell DA. 2008. Genomic and proteomic analysis of the alkali-tolerance response (AITR) in *Listeria monocytogenes* 10403S. *BMC Microbiol.* 8:102

Glaser P, Frangeul L, Buchrieser C, Rusnoik C, Amend A, et al. 2001. Comparative genomics of Listeria species. *Science* 294:849–52

Hain T, Chatterjee SS, Ghai R, Kuenne CT, Billion A, et al. 2007. Pathogenomics of Listeria sp. *Int. J. Med. Microbiol.* 297:541–57

Hain T, Hossain H, Chatterjee SS, Machata S, Volke U, et al. 2008. Temporal transcriptomic analysis of the *Listeria monocytogenes* EGDe σ^B regulon. *BMC Microbiol.* 8:20

Hall Stoodley L, Costerton J, Stoodley P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *Nature Rev. Microbiol.* 2:95–108

Hall Stoodley L, Stoodley P. 2005. Biofilm formation and dispersal and the transmission of human pathogens. *Trends Microbiol.* 13:7–10

Hamon M, Bierne H, Cossart P. 2006. *Listeria monocytogenes*: a multifaceted model. *Nature Rev. Microbiol.* 4:424–34

Han CS, Xie G, Challacombe JF, Altherr MR, Bhotika SS, et al. 2006. Pathogenomic sequence analysis of *Bacillus cereus* and *Bacillus thuringiensis* isolates closely related to *Bacillus anthracis*. *J. Bacteriol.* 188:3382–90

Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, et al. 2001. Complete genome sequence of enterohemorragic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res.* 8:11–22

Hengge R. 2008. The two-component network and the general stress sigma factor RpoS (sigma S) in *Escherichia coli*. *Adv. Exp. Med. Biol.* 631:40–53

Hill C, Cotter PD, Sleator RD, Gahan CGM. 2002. Bacterial stress response in *Listeria monocytogenes*: jumping the hurdles imposed by minimal processing. *Int. Dairy J.* 12:273–83

Hoffmaster AR, Ravel J, Rasko DA, Chapman GD, Chute MD, et al. 2004. Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax. *Proc. Natl. Acad. Sci. USA* 101:8449–54

Hu Y, Oliver HF, Raengpradub S, Palmer ME, Orsi RH, et al. 2007a. Transcriptomic and phenotypic analyses suggest a network between the transcriptional regulators HrcA and sigmaB in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 73:7981–91

Hu Y, Raengpradub S, Schwab U, Loss C, Orsi RH, et al. 2007b. Phenotypic and transcriptomic analyses demonstrate interactions between the transcriptional regulators CtsR and Sigma B in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 73:7967–80

Ivanova N, Sorokin A, Anderson I, Galloran N, Candelson B, et al. 2003. Genome sequence of *Bacillus cereus* and comparative analysis of *Bacillus anthracis*. *Nature* 423:97–91

Jefferson KK. 2004. What drives bacteria to produce a biofilm? *FEMS Microbiol. Lett.* 236:163–73

Joseph B, Przybilla K, Stühler C, Schauer K, Slaghuis J, et al. 2006. Identification of *Listeria monocytogenes* genes contributing to intracellular replication by expression profiling and mutant screening. *J. Bacteriol.* 188:556–68

Kazmierczak MJ, Mithoe SC, Boor KJ, Wiedmann M. 2003. *Listeria monocytogenes* sigmaB regulates stress response and virulence functions. *J. Bacteriol.* 185:5722–34

Kim H-J, Park S-H, Lee T-H, Nahm B-H, Kim Y-R, Kim H-Y. 2008. Microarray detection of food-borne pathogens using specific probes prepared by comparative genomics. *Biosens. Bioelectron.* 24:238–46

Klauck E, Typas A, Hengge R. 2007. The sigmaS subunit of RNA polymerase as a signal integrator and network master regulator in the general stress response in *Escherichia coli*. *Sci. Prog.* 90:103–27

Kuipers OP. 1999. Genomics for food biotechnology: prospects of the use of high-throughput technologies for the improvement of food micro-organisms. *Curr. Opin. Biotechnol.* 10:511–16

Kumar CG, Anand SK. 1998. Significance of microbial biofilms in food industry: a review. *Int. J. Food Microbiol.* 42:9–27

Lauri A, Mariani PO. 2009. Potentials and limitations of molecular diagnostic method in food safety. *Genes Nutr.* 4:1–12

Leistner L. 2000. Basic aspects of food preservation by hurdle technology. *Int. J. Food Microbiol.* 55:181–86

Li W, Raoult D, Fournier P. 2009. Bacterial strain typing in the genomic era. *FEMS Microbiol Rev.* 33:892–916

Lippolis JD, Bayles DO, Reinhardt TA. 2009. Proteomic changes in *Escherichia coli* when grown in fresh milk versus laboratory media. *J. Proteome Res.* 8:149–58

Liu D. 2006. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J. Med. Microbiol.* 55:645–59

Liu Y, Ream A. 2008. Gene expression profiling of *Listeria monocytogenes* strain F2365 during growth in ultrahigh-temperature-processed skim milk. *Appl. Environ. Microbiol.* 74:6859–66

MacFarlane S, Dillon JF. 2006. Microbial biofilm in the human gastrointestinal tract. *J. Appl. Microbiol.* 102:1187–96

Malone AS, Chung Y-K, Yousef AE. 2006. Genes of *Escherichia coli* O157:H7 that are involved in high-pressure resistance. *Appl. Environ. Microbiol.* 72:2661–71

Mangalappalli-Illathu AK, Vidović S, Korber DR. 2008. Differential adaptive response and survival of *Salmonella enterica* serovar Enteritidis planktonic and biofilm cells exposed to benzalkonium chloride. *Antimicrob. Agents Chemother.* 52:3669–80

McClelland M, Sanderson KE, Spieth J, Clifton SW, Latrielle P, et al. 2001. Complete genome sequence of *Salmonella enterica* serovar Typhumurium LT2. *Nature* 413:852–56

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, et al. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–25

Milohanic E, Glaser P, Coppée JY, Frangeul L, Vega Y, et al. 2003. Transcriptomic analysis of *Listeria monocytogenes* identifies three groups of genes differently regulated by PrfA. *Mol. Micro.* 47:1613–25

Mols M, de Been M, Zwietering MH, Moezelaar R, Abee T. 2007. Metabolic capacity of *Bacillus cereus* strains ATCC 14579 and ATCC 10987 interlinked with comparative genomics. *Environ. Microbiol.* 9:2933–44

Mozola MA. 2006. Genetics-based methods for detection of Salmonella spp. in foods. *J. AOAC Int.* 89:517–29

Mujahid S, Pechan T, Wang C. 2008. Protein expression by *Listeria monocytogenes* grown on a RTE-meat matrix. *Int. J. Food Microbiol.* 128:203–11

Mullane NR, Ryan M, Iversen C, Murphy M, O'Gaora P, et al. 2008. Development of multiple-locus variable-number tandem-repeat analysis for the molecular subtyping of *Enterobacter sakazakii*. *Appl. Environ. Microbiol.* 74:1223–31

Nelson KE, Fouts DE, Mongodin EF, Ravel J, DeBoy RT, et al. 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res.* 32:2386–95

Ollinger J, Bowen B, Wiedmann M, Boor KJ, Bergholz TM. 2009. *Listeria monocytogenes* σ^B modulates PrfA-mediated virulence factor expression. *Infect. Immun.* 77:2113–24

Pané-Farré J, Jonas B, Förstner K, Engelmann S, Hecker M. 2006. The sigmaB regulon in *Staphylococcus aureus* and its regulation. *Int. J. Med. Microbiol.* 296:237–58

Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, et al. 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 103:665–68

Perna NT, Plunkett G 3rd, Burland V, Mau B, Glasner JD, et al. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* 409:529–33

Pfeiler EA, Klaenhammer TR. 2007. The genomics of lactic acid bacteria. *Trends Microbiol.* 15:546–53

Poole K. 2002. Mechanisms of bacterial biocide and antibiotic resistance. *J. Appl. Microbiol.* 92(Suppl.):55S–64S

Poole K. 2007. Efflux pumps as antimicrobial mechanisms. *Ann. Med.* 39:162–76

Puttamreddy S, Carruthers MD, Madsen ML, Minion FC. 2008. Transcriptome analysis of organisms with food safety relevance. *Foodborne Pathog. Dis.* 5:517–29

Raengpradub S, Wiedmann M, Boor KJ. 2008. Comparative analysis of the σ^B-dependent stress responses in *Listeria monocytogenes* and *Listeria innocua* strains exposed to selected stress conditions. *Appl. Environ. Microbiol.* 74:158–71

Raskin DM, Seshadri R, Pukatzki SU, Mekalanos JJ. 2006. Bacterial genomics and pathogen evolution. *Cell* 124:703–14

Rasko DA, Ravel J, Okstad OA, Helgason E, Cer RZ, et al. 2004. The genome of *Bacillus cereus* ATCC 10987 reveals metabolic adaptations and a large plasmid related to *Bacillus anthracis* pXO1. *Nucleic Acids Res.* 32:977–88

Ryan S. 2006. Molecular and phenotypic characterisation of selected acid resistance systems of *Listeria monocytogenes*. PhD thesis. University College Cork. 238 pp.

Ryan S, Begley M, Gahan CGM, Hill C. 2009. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. *Environ. Microbiol.* 11:432–45

Scaria J, Palaniappan RUM, Chiu D, Phan JA, Ponnala L, et al. 2008. Microarray for molecular typing of *Salmonella enterica* serovars. *Mol. Cell. Probes* 22:238–43

Sebaihia M, Peck MW, Minton NP, Thompson NR, Holden MTG, et al. 2007. Genome sequence of a proteolytic (Group I) *Clostridium botulinum* strain Hall A and comparative analysis of the clostridial genomes. *Genome Res.* 17:1082–92

Senn MM, Giachino P, Homerova D, Steinhuber A, Strassner J, et al. 2005. Molecular analysis and organization of the sigma B operon in *Staphylococcus aureus*. *J. Bacteriol.* 187:8006–19

Severino P, Dussurget O, Vêncio RZN, Dumas E, Garrido P, et al. 2007. Comparative transcriptome analysis of *Listeria monocytogenes* strains of the two major lineages reveals differences in virulence, cell wall, and stress responses. *Appl. Environ. Microbiol.* 73:6078–88

Silagyi K, Kim SH, Martin Lo Y, Wei C. 2009. Production of biofilm and quorum sensing by *Escherichia coli* 0157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. *Food Microbiol.* 26:514–19

Steil L, Hoffmann T, Budde I, Völker U, Bremer E. 2003. Genome-wide transcriptional profiling analysis of adaptation of *Bacillus subtilis* to high salinity. *J. Bacteriol.* 185:6358–70

Sue D, Boor KJ, Wiedmann M. 2003. SigmaB-dependent expression patterns of compatible solute transporter genes *opuCA* and *lmo1421* and the conjugated bile salt hydrolase gene *bsh* in *Listeria monocytogenes*. *Microbiology* 149:3247–56

Tang CM, Moxon ER. 2001. The impact of microbial genomics on antimicrobial drug development. *Annu. Rev. Genomics Hum. Genet.* 2:259–69

Toledo-Arana A, Dussurget O, Nikitas G, Sesto N, et al. 2009. The *Listeria* transcriptional landscape from saprophytism to virulence. *Nature* 459:950–56

Uttamchandani M, Neo JL, Ong BNZ, Moochhala S. 2008. Applications of microarrays in pathogen detection and biodefence. *Trends Biotechnol.* 27:53–61

Van Belkum A. 2007. Tracing isolates of bacterial species by multilocus variable number of tandem repeat analysis (MLVA). *FEMS Immunol. Med. Microbiol.* 49:22–27

Van Der Veen S, Moezelaar R, Abee T, Wells-Bennik MJH. 2008. The growth limits of a large number of *Listeria monocytogenes* strains at combinations of stresses show serotype- and niche-specific traits. *J. Appl. Microbiol.* 105:1246–58

Van Der Voort M, Abee T. 2009. Transcriptional regulation of metabolic pathways, alternative respiration and enterotoxin genes in anaerobic growth of *Bacillus cereus* ATCC14579. *J. Appl. Microbiol.* 107:795–804

Van Schaik W, Abee T. 2005. The role of σ^B in the stress response of gram-positive bacteria—targets for food preservation and safety. *Curr. Opin. Biotechnol.* 16:218–24

Van Schaik W, Van Der Voort M, Molenaar D, Moezelaar R, de Vos WM, Abee T. 2007. Identification of the σB regulon of *Bacillus cereus* and conservation of σ^B-regulated genes in low-GC-content gram-positive bacteria. *J. Bacteriol.* 189:4384–90

Veening JW, Smits WK, Hamoen LW, Kuipers OP. 2006. Single cell analysis of gene expression patterns of competence development and initiation of sporulation in *Bacillus subtilis* grown on chemically defined media. *J. Appl. Microbiol.* 101:531–41

Volokhov D, Rasooly A, Chumakov K, Chizhikov V. 2002. Identification of *Listeria* species by microarray-based assay. *J. Clin. Microbiol.* 40:4720–28

Wang XW, Zhang L, Jin LQ, Jin M, Shen ZQ, et al. 2007. Development and application of an oligonucleotide microarray for the detection of foodborne bacterial pathogens. *Appl. Microbiol. Biotechnol.* 76:225–33

Williams T, Bauer S, Beier D, Kuhn M. 2005. Construction and characterization of *Listeria monocytogenes* mutants with in-frame deletions in the response regulator genes identified in the genome sequence. *Infect. Immun.* 73:3152–59

Wood TK. 2009. Insights on *Escherichia coli* biofilm formation and inhibition from whole-transcriptome profiling. *Environ. Microbiol.* 11:1–15

Woods D, Reen FJ, Gilroy D, Buckley J, Frye JG, Boyd EF. 2008. Rapid multiplex PCR and real-time TaqMan PCR assay for detection of *Salmonella enteric* and the highly virulent serovars Choleraseis and Paratyphi C. *J. Clin. Microbiol.* 46:4018–22

Yang H, Kendall PA, Medeiros LC, Sofos JN. 2009. Efficacy of sanitizing agents against *Listeria monocytogenes* biofilms on high-density polyethylene cutting board surfaces. *J. Food Prot.* 72:990–98

Yoo SM, Lee SY. 2008. Diagnosis of pathogens using DNA microarray. *Recent Pat. Biotechnol.* 2:124–29

Zhou J. 2003. Microarrays for bacterial detection and microbial community analysis. *Curr. Opin. Microbiol.* 6:288–94

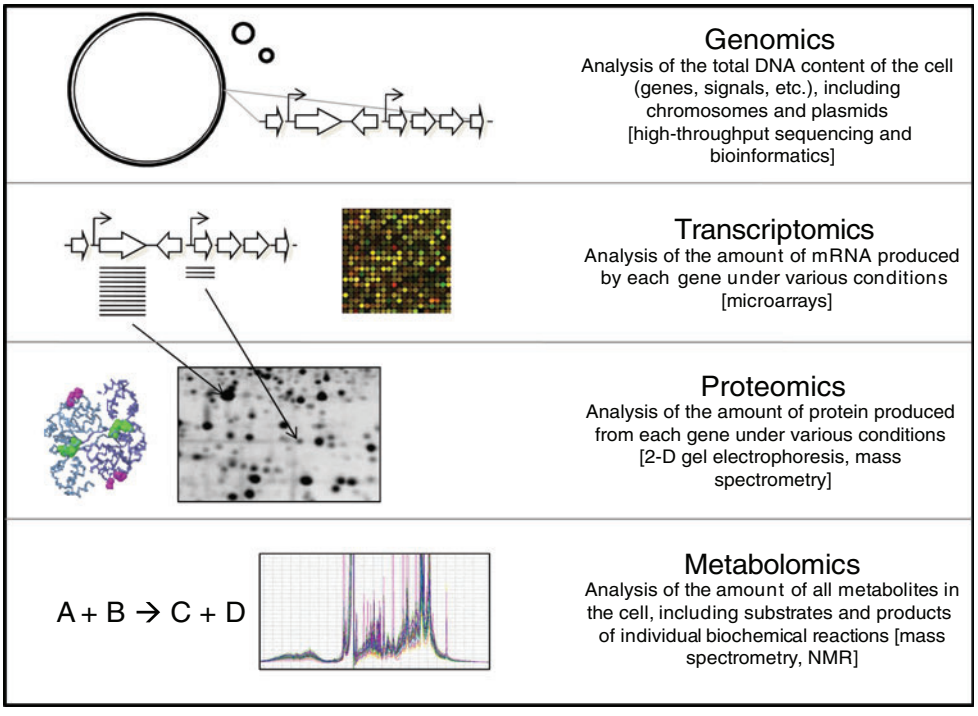

Figure 1

"omic" technologies. The methods used in each "omic" technology are indicated in square brackets.

Annual Review
of Food Science
and Technology

Volume 1, 2010

Contents

Errata

An online log of corrections to *Annual Review of Food Science and Technology* articles may be found at http://food.annualreviews.org