OMICS, MICROBIAL MODELING *and* TECHNOLOGIES *for* FOODBORNE PATHOGENS

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Table of Contents

Preface

Omics, Microbial Modeling, and Technologies in Food-borne Pathogens

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FOODBORNE diseases are common occurrences throughout the world and can result in serious consequences, including death. Furthermore, foodborne illness has an enormous impact on the global economy in terms of medical costs, loss of income, and loss of human potential. Over the past three decades, there have been many technological changes that have enhanced the ability to study foodborne pathogens. The advances in information technology, genomics, and innovative technology-driven "omics" including genomic sequencing, transcriptomics, metabolomics, and proteomics, along with microbial modeling-based risk assessment technologies and predictive microbiology, have led to revolutionary changes in food microbiology, food safety, public health, and epidemiology. A clear understanding of the characteristics of pathogens and the public health risks, as well as improved methods for surveillance and control of foodborne pathogen outbreaks, are necessary. These factors are dependent upon continuous systematic data collection by analytical processes achieved through mathematical modeling and molecular and functional characterization of foodborne pathogens. There have been no coordinated efforts to integrate traditional knowledge and methods with modern "omics" and information technologies.

The purpose of the book is to provide a comprehensive source of "omics", microbial modeling, and technologies that can be utilized in investigating foodborne pathogens. The book is divided into three parts: Part I (Chapters 1–12) details the use of "omics" technologies to study major foodborne pathogens; Part II (Chapters 13–15) covers microbial growth, modeling, and risk assessment; and Part III (Chapters 16–21) presents the creation of a new food safety information infrastructure, which can be used to evaluate the consistency and accuracy of various informational sources about foodborne pathogens. In addition, there is a systematic presentation of advances in current technologies, including next generation sequencers, microarray-based techniques, biometric methods, and wireless technologies in Part III. Overall, the book is a comprehensive introduction to the applications of omic technologies and microbial modeling necessary for understanding, detecting and controlling foodborne pathogens. The book also demonstrates how risk assessment can be carried out and how it can be used as a tool in preventing foodborne illness. Various foodborne pathogens are discussed in terms of their ecology, evolution, and adaptability to a variety of conditions, as well as their importance in food safety, public health, and government regulations.

We hope that the book may prove useful as a textbook in courses on functional genomics of foodborne pathogens or as a practical reference for research microbiologists, food processors, and government regulators. In addition, we hope that the book will fill a void in the scientific literature and will stimulate future research.

We are grateful for the opportunity to work with the authors of the various chapters and we are thankful that they were willing to donate their time and knowledge in the preparation of the chapters comprised in this book. We are also grateful for the assistance and patience of the staff at DEStech Publications, Inc.

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Part I
Omics of Food-borne Pathogens

CHAPTER 1

Omics, Microbial Modeling, and Food Safety Information Infrastructure: A Food Safety Perspective

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INTRODUCTION

THE U.S. Public Health Service has identified more than 250 different foodborne diseases, and most are caused by microorganisms. Scallan *et al.* (2011) estimate that 31 pathogens, including *Salmonella* spp., *Listeria monocytogenes, Campylobacter,* and *Toxoplasma* cause 9.4 million cases of foodborne illness, 55,961 hospitalizations, and 1,351 deaths per year in the United States. During the past three decades, over 1,500 microbial genomes have been sequenced completely, and sequencing of over 1,800 microbial genome sequences is in progress (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi?view=1). Also, advances in information and communication technologies (ICTs), "omics" technologies, microbial modeling, next generation sequencing (NGS), biometrics, mass spectrometry, and NMR spectroscopy have been well documented and are vastly expanding the research capability in many fields of food science, including food microbiology, food engineering, food safety risk assessment/management, pathogen detection, and foodborne pathogen-related food safety information infrastructure (FSII) development. Needless to say, the combination and integration of a variety of cutting-edge "-omics" technologies, biometrics, molecular and mathematical modeling, and ICTs have provided the ability to rapidly determine and interpret the mechanisms underlying pathogenesis and survival of human foodborne pathogens. To better understand

bacterial communities in foods and the public health risk associated with them, and to develop better surveillance and control methods, ongoing systematic data collection and analytic processes achieved through molecular and mathematical modeling, as well as functional characterization of foodborne pathogens are needed. However, there are no coordinated efforts to integrate traditional knowledge and methods with modern "omics" and information technologies. In this chapter, we briefly review the most important types of "omics" technologies, the key recent advances in biotechnology, and their applications for food safety. Next, we consider how techniques such as traditional dynamic mathematical modeling can provide information derived from omics-based statistical and molecular modeling and how the kinetics and molecular changes (e.g., gene and protein expression profiling) through molecular modeling can be an indicator of pathogen growth, survival, and stress resistance in food. Finally, from a food safety perspective, we describe the creation of a new FSII to address the consistency, completeness, and accuracy of distributed information resources (e.g., PulseNet, FoodNet, OutbreakNet, PubMed, NCBI, EMBL, and other online genetic databases and information) and eventually, to integrate pathogen profiling data, PMP, Combase, Foodrisk.org (http://www.foodrisk.org), PulseNet, FoodNet, OutbreakNet, and other relevant information into a user-friendly "homogeneous" information system (Yan *et al.*, 2011).

OMICS IN FOOD SAFETY

In this chapter, the term 'omics' (Kandpal *et al.*, 2009) represents a method to study foodborne pathogens, as well as the systematic collection of information from molecules, pathways, biomarkers, virulence markers, genotyping and serotyping, molecular simulation, and mathematical modeling of biological systems of foodborne pathogens by transcriptomics, proteomics, metabolomics, pathogenomics, interactomics, fluxomics, and metagenomics. We divide the "omics" technologies into the following areas:

Pathogenomics

Pathogenomics (Pallen and Wren, 2007) is the genome-wide study of microbial genetic diversity, virulence factors, antibiotic resistance profiling, and the functional interactions between the host and foodborne pathogens. There is a broad range of research directions and new

perspectives to be explored related to foodborne pathogen pathogenomic studies. From a food safety standpoint, we list a number of these specific topics: (1) development of novel intervention technologies for replacing or supplementing current antimicrobial treatments based on the knowledge of host/pathogen interactions (Fang, 1997); (2) development of alternative intervention strategies to control foodborne diseases in animals by the use of cytokines as an adjuvant with vaccines to enhance a protective immune response (Fasina *et al.*, 2008); (3) use of probiotic bacteria as biological control agents (Verschuere *et al.*, 2000); (4) inhibition of foodborne pathogens by bacteriocins, antimicrobial peptides, or bacteriophage (Joerger, 2003); and (5) development of diagnostic tools for rapid detection of known pathogens, as well as emerging pathogens, and identification of novel virulence factors and their evolutionary patterns (Malorny *et al.*, 2003).

Genomics, Transcriptomics, and Proteomics

Genomics, transcriptomics, and proteomics (Anderson and Anderson, 1998; Blackstock and Weir, 1999; Hoheisel, 2006; Corbin *et al.*, 2003) are three approaches for the genome-wide analyses and comparative genomics/proteomics/gene-expression profiling studies of foodborne pathogens that have been widely used (Abee *et al.*, 2004). Chapter 2 to Chapter 12 in the present book have systematically summarized and discussed the current and emerging applications of genomics, transcriptomics, and proteomics that have contributed to the basic understanding of the key aspects of foodborne pathogens including microbial evolution, genetic diversity, molecular epidemiology, and dynamic gene and protein expression patterns. These studies have provided important new insights on the causes of many foodborne diseases and pathogen virulence factors and have led to the development of rapid molecular detection and identification methods for established and emerging foodborne pathogens (Doumith *et al.*, 2004; Fratamico, 2007). In Chapter 18, Dr. Liu discusses RNA-Seq (Ozsolak *et al.*, 2009), also known as "whole transcriptome shotgun sequencing", as a promising new replacement of the current microarray technology for accurately measuring gene expression levels, and it is defined as "a revolutionary tool for transcriptomics". RNA-Seq profiling has compelling advantages and provides an extra dimension over microarrays for gene expression analysis not only because of the sequence depth that is obtained but also because less sample and materials are needed (Oliver *et al.*, 2009).

Metabolomics

Metabolomics (Harrigan and Goodacre, 2003; Nicholson and Lindon, 2008; Mashego *et al.*, 2007) is the study of small molecule metabolites in an organism. These small molecules include metabolic intermediates such as sugars, organic acids, essential amino acids, peptides, extra- and intra-cellular signaling molecules, and secondary metabolites. Technically, metabolomics has revolutionized the application of traditional biochemical and biomedical experiments. While unlike the situation with genomics and transcriptomics, metabolomics is not as nearly developed for food safety research. Nevertheless, mass spectrometry-based metabolomics will provide a unique opportunity to explore a systematic genotype-phenotyping relationship and develop an indicative phenotyping and envirotype modeling program due to the specificity, sensitivity, and predictive value of these small molecule metabolites (Ideker *et al.*, 2001; Ishii and Tomita, 2009; Jurgen *et al.*, 2001; Cascante and Marin, 2008). However, a key limitation of metabolomics for food safety research is the fact that foodborne pathogen metabolomes (Dunn *et al.*, 2005) are not at all well characterized nor studied due to the difficulty in the detection and identification of all of the metabolites and the genotypic diversity of foodborne pathogens. To give an example, there has been a recent explosion of knowledge on the DNA sequence of the swine and cow genomes, and thus there is now a need to begin swine and cow metabolomics research. This research could allow genomic characterization of "systems" of proteins and their applications at the metabolite level, which could be related, for example, to cell signaling, energy transfer, and cell-to-cell communication (Schmidt, 2004).

Interactomics

According to Kiemer and Cesareni (2007), interactomics is "a discipline at the intersection of bioinformatics and biology that deals with studying both the interactions and the consequences of those interactions between and among proteins and other molecules within a cell" or tissue. To some extent, interactomics is a combination and extension of proteomics and metabolomics that could go beyond the cellular and molecular level. Obviously, the integration of pathogenomics, genomics, transcriptomics, proteomics, and metabolomics, with interactomics will provide systematic and comprehensive insights on foodborne

pathogens (Díaz-Mejía *et al.*, 2009; Parrish *et al.*, 2007; Boesten and de Vos, 2008). From a mathematical biology, or predictive microbial modeling and informatics viewpoint, a foodborne pathogen interactome network could constitute various propositional semantic networks (Yan, 2011), which contribute to the most important interactions pertinent to the normal/stress physiological functions of foodborne pathogens. Comparison of the interactomic networks of various human pathogens is particularly suitable for foodborne pathogen outbreak prediction, detection, and analysis (Kint *et al.*, 2010).

Fluxomics

Fluxomics, also called in vivo NMR, the study of dynamic changes of cellular molecules over time, may offer the best and most direct measure of time-dependent metabolic fluxes by applying computational methods, such as molecular simulation, optimization, and parameter estimation for modelling of complex biological systems. It is a real-time metabolotyping technique (Fukuda *et al.*, 2009), and it could be used to combine metabolomics, lipidomics, bioenergenics, and dynamic molecular modeling to evaluate and determine the effects of productivity and diversity on the bacterial community with each responding compound associating with its own dynamic profile and metabolic relationships with other compounds (Sekiyama and Kikuchi, 2007). To the best of our knowledge, the use of fluxomics for measuring real-time metabolic fluxes has not been extensively reported in food science; however, this technique has been used to identify novel glucose transporters in *Lactococcus lactis* MG1363 for strain improvement and industrial application (Pool *et al.*, 2006), as an example.

Metagenomics

Metagenomics is commonly referred to as environmental and community genomics. The importance of viable but non-culturable forms of food- and water-borne bacteria has not been well studied. Metagenomics is a relatively new and ever expanding field of research that was specifically designed for the study of metagenomes of non-culturable microbes, which cannot be cultured on conventional laboratory growth media, in the natural niches of the microorganisms (Marco, 2010). This technique has tremendous scope for microbial detection, identification, and taxonomy research. It can be used for the discovery of genes in-

volved in survival and persistence in the gastrointestinal tract, as well as for the assessment of bacterial diversity in cattle, sheep, goats, swine, rodents, rabbits, humans, poultry, and food products by applying species-specific molecular signature sequences or 16S rRNA applications. Metagenomics approaches for food safety can also be applied to define bacterial stress responses, the production of acetic acid, ethanol, antimicrobials (such as bacteriocins, exopolysaccharides and their derived products), and enzymes, as well as protein-protein, protein-DNA, protein-small molecule complexes, protein-small polypeptides, and microbe-oligosaccharide interactions. This type of research is very useful, as future attempts for genetic and cellular modifications of complex microbial communities present in the GI tract (through genetic or cellular re-engineering-based intervention strategies), as well as studies on prebiotic strains, could become a major source of information for the enhancement of food safety research. To date, discovery, clustering, and classification of microbial communities in complex and contaminated food matrices (such as ground meats) by using a metagenomic approach combined with sequencing of reference hosts and foodborne pathogens, which are components of the community or communities, are gaining more and more attention from the food science/safety research community (Wommack *et al.*, 2008).

MICROBIAL MODELING

Predictive microbiology (Juneja *et al.*, 2009) continues to be an important research area within the field of food microbiology. The theory of predictive microbiology is based on the fact that microbial growth, survival, and inactivation are affected by environmental factors. It is also based on the assumption that the responses of microorganisms to these factors are reproducible and can be characterized and quantified. The microbial response to environmental factors can be described mathematically (Chapter 13) and molecularly in terms of a series of mathematical modeling strategies and identification of gene networks. In other words, microbiological modeling is an attempt to define the response of a microorganism to its environment in terms of either mathematical equations or molecular interaction networks, molecular diagnostic testing (by measuring dynamic responses of specific molecular biomarkers), or gene profiling (reflecting the genetic changes in response to food environmental stresses). This information should provide comprehensive knowledge on dynamic changes, complexity,

and static signals by using computational molecular modeling methods to determine dynamic molecular signatures that are indicative of what occurs in actual foods and environmental samples. While static models describe the kinetic parameters of microorganisms under fixed, nonchanging, environmental conditions, dynamic models attempt to relate changing environmental conditions to the kinetic parameters of microorganisms (Bovill *et al.*, 2000). The latter models usually have the form of a set of differential equations. Most available models are empirical models (Gompertz, logistic, etc.) based on experimental data. On the other hand, mechanistic or deterministic models (Baranyi *et al.*, 1995; Huang, 2004a, 2005) are built upon a theoretical understanding of microbial behavior and have the potential to give accurate predictions. Although microbial modeling can be a powerful tool for foodborne pathogen risk assessment (Chapters 14 and 15) and management, and various microbial models have been developed for quantitative analysis of pathogens in foods under isothermal and dynamic temperature conditions (Huang, 2004b, 2009; Solomon *et al.*, 2006), such models are not particularly suitable for making food safety decisions, as these models cannot provide real-time analysis of food safety risk, are usually independently operated, and require special training for their use. Traditional microbial modeling only takes into consideration certain factors, like temperature, pH, water activity, the presence of preservatives and antimicrobials, and the composition of the atmosphere. Limitations of primary microbial models in predictive microbiology could be overcome through the following inclusive approaches. First, microbial models should be amenable to computer simulations with the continuous advances in ICT; second, microbial modeling should be able to integrate and extend the information obtained from various "omics" technologies, which will allow researchers to obtain molecular information at the DNA, RNA, protein, or metabolite level; third, microbial modeling is not limited by wet experimental constraints. The recent advances in molecular microbiology, in conjunction with the "omics" revolution, have enabled researchers to obtain insight into mechanisms underlying the phenomena that are unveiled by approaches employed in microbial modeling. It is now feasible to develop innovative predictive models for the responses of microbial pathogens in select food matrices and then validate their robustness from a biological perspective. In other words, "omics" technologies open a new door to predictive microbiology (Torres-García *et al.*, 2009). There is a considerable amount of empirical knowledge on the growth kinetics and substrate utiliza-

tion, as well as the biochemistry and molecular biology of microorganisms. However, relationships of microbial growth and inactivation with respect to food constitution and environmental conditions, along with cell variability and the physiology of microorganisms are not currently thoroughly understood. The major challenges and driving forces for future research both in food microbiology and in the determination of the precision of predictive models are the integration of omics technologies and advances in ICTs. As an example, genomics can play a significant role in food manufacturing by guiding predictive modeling of the behavior of microorganisms by providing a molecular mechanistic basis (a molecular "fingerprint") of the events that take place. Use of hurdle principles or combined preservation treatments in a mechanistic knowledge-based manner can lead to increased robustness of the predictive models (Webb-Robertson *et al.*, 2009; Brul *et al.*, 2008).

In this book, we will systematically show how knowledge from various omics technologies can provide information on pathogen profiling, as well as contribute to traditional microbiology/food microbiology research to help in understanding bacterial stress response mechanisms and in the development of methods for pathogen detection and phenotypic classification (Fratamico *et al.*, 2005; Fratamico, 2008; Campbell and Ghazal, 2004; and Mansmann, 2005). All of this information coupled with an increased knowledge of "systems biology" approaches (Brul *et al.*, 2008) and the application of "state of the art" new technologies (Chapter 18, 19, 20, and 21) for pathogen profiling and predictive modeling will allow a better understanding of foodborne pathogens by the scientific research community.

FOOD SAFETY INFORMATION INFRASTRUCTURE

Over the last three decades, the remarkable advances in ICTs, proteomics, and genomics have provided the ability to rapidly determine and interpret the mechanisms of survival and pathogenesis of foodborne pathogens. Therefore, public health risk assessment and pathogen surveillance and control are addressed by the ongoing, systematic collection, analysis, and interpretation of data related to agent/hazard, risk factor, exposure, illness, etc. Data collection, analysis, and the timely dissemination of these data are essential for the planning, implementation, and evaluation of public health practices. Currently, there are over 112 listed mechanisms (Uniform Resource Locators [URLs]) for data sharing and accessing of food safety information, which cover micro-

bial and chemical contamination, pathogen characteristics, and predictive microbiology, public health surveillance, risk assessment and risk analysis, food consumption, inspection, management and regulation, recalls, violations, prevention and control, and others (Taylor and Batz, 2008). However, there are no coordinated efforts or centralized information systems in the U.S. to handle the complex and isolated food safety information resources. In particular, comprehensive network analysis and semantic information describing the content of these resources and information is solely lacking, as is the integration of this information with data in public repositories.

There are many challenges associated with establishing a centralized Food Safety Information Reporting System (FSIRS). One of the largest challenges when creating a FSIRS is accurate and reliable prediction of the combined effects of complex multi-factorial factors on the growth and inactivation of foodborne pathogens. Data sharing and accessibility, collaboration, standards, format, and integration are other large challenges facing the development of a FSIRS. Also, data mining and large scale statistical data analysis are time-consuming processes. Most importantly, the presentation of foodborne pathogen surveillance and prevention systems (Chapter 16 and 17) must be accurate and be ready to detect changes in complex heterogeneous data systems very quickly. This requires advanced algorithms, data structures, and dynamic communication tools (e.g., the Internet) for detection and prediction of transmission patterns of foodborne pathogens. Advances in algorithms, data structures, and artificial intelligence allow for practical applications of data-driven outbreak detection methods, which can handle the complexity of the task at hand by learning from examples in historical data and from real or simulated recorded outbreaks. Besides the above challenges, there are some critical challenges that need to be addressed from both surveillance and prevention points of view, as well:

- *Heterogeneous data representation:* There is a tremendous amount of online references, regulations, and pathogen profiling data; however, there is no standard language to represent semantics and heterogeneities of mined knowledge in the semantically heterogeneous scenario. Although manual translation is possible, it is time-consuming and error-prone if the amount of information is large.
- *The correctness and accuracy of knowledge prediction:* The key problems associated with the correctness and accuracy of knowledge prediction is lack of a unified data annotation ontology standard,

corrected applied algorithms, and timely entry of public health data. Therefore, there is a need to set up an ontology standard and to develop a dynamic user interface by using semantic web technology.

- *Timing of food safety emergency responses:* An important part of foodborne pathogen surveillance and prevention is the timing of food safety emergency responses. In some cases, determining the geographical scope of a food safety emergency and/or investigating the course of the food safety "crisis" is difficult. There is a need to compile multivariate data very quickly (ideally, in real time) through neural network analysis to maintain the data correlation ability of prediction hypotheses, the regulatory threshold, and the individual cases. Advances in algorithms, data ontology analysis, and neural networks allow for practical application of data-driven outbreak investigation methods.

SUMMARY POINTS

Emerging biotechnologies and the rapid accumulation of a wide range of omics data at various levels will continue to play a large role in food safety research, and applying these advances will provide a better understanding of foodborne pathogens and a focus on stimulating ideas and applying these fundamentals to real food safety issues. The efficient use of microarray technology, NGS (Chapter 18) and optical imaging technology for foodborne pathogen differentiation and detection (Chapter 19) will continue to generate a tremendous amount of heterogeneous raw data, and the ability to exploit a great amount of data will be an important factor for the improvement of public health and food safety. Moreover, an integrated FSII system would allow researchers to combine and integrate multiple omics data sets and molecular and mathematical modeling (Fiehn *et al.*, 2001; Hood *et al.*, 2004; De Keersmaecker *et al.*, 2006) to generate a homogeneous computational system for data sharing, data integration, and communication among researchers. This will facilitate the control of foodborne pathogens and reduce public health costs. Mathematical models are useful tools for exploring the risks and dangers of foodborne pathogens during food processing, storage, and consumption, as well as for providing information on disease prevalence in populations (Fu *et al.*, 1991; Mogilevskaya *et al.*, 2009). The more risk factors that are included, the more accurate the model will be. There are a number of factors that lead to contamination or growth of pathogens in food resulting in cases and outbreaks of food-

CHAPTER 21

RFID Technologies for Inspection of Imported Foods

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ABSTRACT: Foodborne illnesses typically occur due to contamination of food products with Escherichia coli, Salmonella spp., Listeria monocytogenes and other pathogens. Unfortunately, it takes several weeks to identify the source of such contamination, possibly due to lack of a central database system that is capable of tracking food products and a real-time food safety decision tool. In addition, the volume of imported foods to the U.S. has been increasing at an alarming rate since 1994, which makes inspection at port-of-entry a daunting task. It is important to develop an information infrastructure for food safety using a sustainable Radio-Frequency Identification (RFID) system. The system consists of: a) a passive RFID interface for sensing of shipped items; b) a portable hand-held system for automatic data logging and alerts; and c) a central database system for food safety data and coordination. This system can facilitate automatic data logging and real-time data reporting, networking, and coordination among the various users of food safety information, from production to consumption. Successful implementation of the system can be expanded in the future to a comprehensive food safety and risk assessment system for tracking all food products within the U.S.

INTRODUCTION

PREVENTION of foodborne illnesses requires safe handling, cooking, and storage of food products. Safe steps during food handling are important not only in consumers' homes, but also in the producers' factories or agricultural farms, and during shipping by suppliers. TV newscasts frequently report on outbreaks of *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* due to contamination of food products. According to the *Centers for Disease Control* (CDC) in June 2008, a Salmonella outbreak due to imported tomatoes (NewsInferno.com, 2008) sickened at least 613 people in 33 states and the District of Columbia, and 69 victims were hospitalized. Unfortunately, it takes several weeks to identify the source of such contamination, possibly because

of a lack of a central database system that is capable of tracking shipments of food products from source to consumers, and a real-time food safety decision tool in the event of temperature abuse. In addition, the United States imports about $80 billion worth of food products every year from foreign countries, and the volume of the import of foreign foods has been increasing at an alarming rate since 1994, which makes inspection at port-of-entry a daunting task. However, less than 2% of imported goods to the U.S. are inspected at the port-of-entry, which results in incomplete or inconclusive determination of the safety of imported foods.

On July 28, 2009, Ms. Lori Wallach, Director of Public Citizen's Global Trade Watch, testified (Wallach, 2009) before the House Appropriations Subcommittee on Agriculture, Rural Development, and FDA, and outlined six key issues regarding imported food safety. According to her testimony, the volume of imported food in the United States is increasing at a staggering rate, and safety inspection is unable to keep up with this rapid influx. She also stated that computerized records are not consistent, regulation and food safety standards are contradictory and full of confusion, and inspection methods at the port-of-entry are outdated and obsolete. There are lengthy delays in responding to requests from field officers on determination of 'equivalency' and in translation of documents from a foreign language to English. With the rapid growth of imported foods and limitations of inspection methods, U.S. consumers are increasingly forced to rely on foreign governments for the quality of foods consumed. "Relying on foreign governments and their food safety systems to protect Americans' health is a recipe for disaster—and must be changed" (Wallach, 2009).

To address these problems, it is important to develop an automatic data logger and tracking system using sustainable Radio-Frequency Identification (RFID) and wireless sensing technologies for better sharing and coordination of food safety information. The data logger and tracking system will facilitate data logging and real-time data reporting, networking, and coordination among the various users of food safety information, from production to consumption. It will also minimize the over-aggressive safety recall of many meat and vegetable products, saving consumers from possible foodborne diseases, and saving producers from devastating economic losses.

BACKGROUND

Microbial pathogen growth may occur at every level, from produc-

tion of food products to transportation and distribution, storage, and post-cooking phases prior to consumption. Microbial growth is one of the major sources of food spoilage, and over the years producers and decision makers have routinely relied upon prediction of microbial growth in food products. It should be emphasized that a complete microbial analysis of food products often requires several days, which makes it necessary to predict pathogen growth using analytical models. Various mathematical models have been developed for quantitative analysis of pathogens in foods under isothermal and dynamic temperature conditions (Arnout *et al.*, 2007; Artés *et al.*, 2007; Coleman *et al.*, 2003; Juneja *et al.*, 2009; Noriega *et al.*, 2008; Shimoni and Labuza, 2000). Such models are not particularly suitable for making food safety decisions, as these models cannot provide real-time analysis of food safety risk, and usually are independently operated and require special training for using the models. We need to provide a solution to the disconnection between the food risks and food safety decisions.

Frozen and refrigerated foods are often transported in large refrigerated containers at distances of thousands of miles. These transportation containers are expected to maintain the temperature of the food within close limits so as to ensure safety and quality. James *et al.* (2006) presents a review of modeling of food temperature, microbial growth, and other parameters during transport. Note that food temperature modeling often involves heat transfer analysis between the environment and the container. Food temperature modeling offers some quick estimates or clues for food contamination levels. However, pathogen testing is still an essential and accurate component for food safety, but unfortunately it is an expensive and time-consuming process that may require several weeks before a conclusion can be made. For this reason, there is a growing demand for biosensors that could minimize the testing time. A survey of biosensors and their market study can be found in Alocilja and Radke (2003).

Although microbial growth models have been widely investigated in the literature (Bernaerts *et al.*, 2004; Cassin *et al.*, 1998; McAvoy *et al.*, 1998; Ross and McMeekin, 2003; Vereecken *et al.*, 2000), there are limitations on our understanding of factors leading to the effects of various environmental conditions, such as temperature, pH, presence of organic acids and other compounds in food products. Most often it is necessary to use a combination of mathematical models and statistical models in predicting food safety (López *et al.*, 2004; Stelling, 2004). Probabilistic models (Baker and Genigeorgis, 1990; Meng and Genige-

orgis, 1993; Roberts, 1995) provide an alternate approach to estimation of toxin production in various fish and poultry products. Probabilistic risk analysis (Albert *et al.*, 2005; Marks *et al.*, 1998; Ross and McMeekin, 2003) usually involves identification of variability and uncertainty of affecting parameters and Monte Carlo analysis using probabilistic models of pathogen generation.

Other than various government regulatory agencies, such as the USDA, FDA, and CDC, one of the organizations that has been a key constituent in food safety research is the Food Safety Research Consortium (FSRC). The FSRC is a multi-disciplinary collaboration of several research institutions, which focuses on developing analytical and decision making tools aw well as a framework for food safety; this organization has made several recommendations (Taylor and Batz, 2008) for improving the nation's 'food safety information infrastructure'. The FSRC has also made nineteen specific recommendations (Taylor and David, 2009) for strengthening state and local roles and building an integrated national food safety system to prevent foodborne illnesses.

Traditional methods for tracking food products from origin to consumers are based on the use of bar codes, and can be used by retailers and/or stakeholders for tracking food products (Gorny, 2001; Suslow, 2003); however, it is not sufficient to keep pace with the ever-increasing importing and supply of food products in the United States. Wireless communication and computer technologies have revolutionized modern communication systems in recent years, and have entered into every level of today's society. For example, we take wireless technologies for granted when we drive through EZpass lanes using active RFID technologies. Many U.S. transportation authorities have restructured their electronic fare collection systems using high frequency, passive RFID technologies. Large corporations, like Walmart, use passive RFID technologies to track their inventories in stores, and have modernized supplier-driven productions as customer-driven production markets. The Environmental Protection Agency has recently successfully tested environmentally friendly and sustainable RFID technologies (Dindal *et al.*, 2009; McKernan and Varner, 2009) for tracking transport of hazardous material across international borders. Needless to say, wireless technology has touched the life of each and every one of us, with or without wireless background. Using the global communication network, we can communicate anywhere, anytime and with anyone, gather information efficiently, and transmit it anywhere instantaneously. Engineers and scientists have even successfully integrated RFID technologies into super-

market shopping carts, contactless smartcards in public transit systems and have even implemented RFID tags in humans. Surprisingly, few wireless technologies have been introduced for food tracking and inspection.

FOOD SAFETY INFORMATION SYSTEM

There is a need to develop new wireless sensing and computerized data logger technologies that address the concerns of food safety. The system needs to incorporate passive RFID sensors and long distance readers (>3 meters), as well as a real-time data logger through cellular communication networks. Successful development of the sensing and data logging system can make the inspection and tracking of food products more efficient and reliable. The goal is to aggregate food shipment and storage information and to determine possible contamination or mishandling of food based on guidelines of the U.S. Government. The sensing and data logging system consists of three subsystems:

1. sustainable passive RFID tagging for sensing of shipped items and for measuring temperature;
2. a portable handheld device for real time alerts and data logging; and
3. a central secure database system for food safety data and coordination.

Figure 23.1 shows a vision of the *Integrated Food Safety Information Management* (IFSIM) system that can be used by all stakeholders in food safety, from producers to suppliers to consumers. The central unit of the system is the Imported Food Safety Database that contains detailed information of all food products produced in the U.S. or imported from foreign countries. The data logging part of the system consists of three subsystems: (1) handheld RFID interface; (2) handheld barcode interface; and (3) wireless sensor interface. These three modules can be used for automatic logging of data into the central database. The RFID and barcode interfaces deal with food product data, whereas the wireless sensor interface handles the environmental data, such as GPS geographical location information of where the food products were processed. In addition, the wireless sensor module can also be used for data logging of pathogen data obtained from biosensors or other sensors. The Field Inspector interface provides the field officers access to the central database for ‘equivalency’ and safety policies of

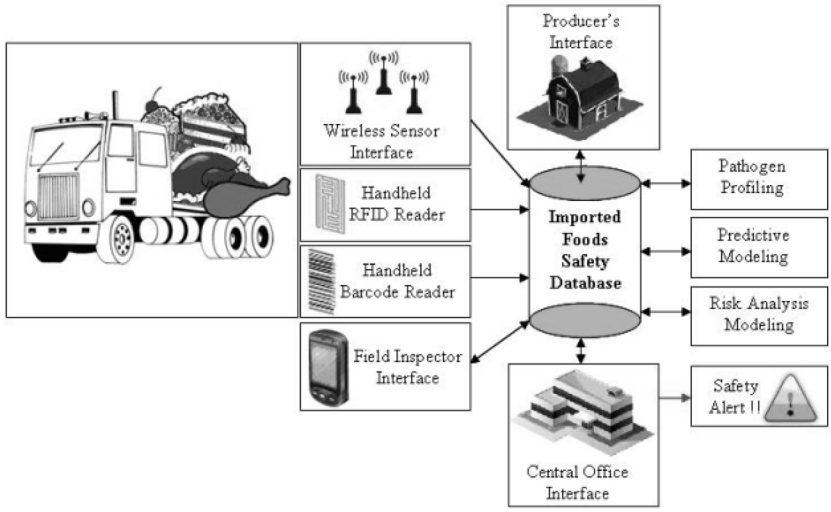

FIGURE 21.1. *A Conceptual Schematic of Food Safety Information System.*

food products. Figure 21.1 also shows pathogen profiling, predictive modeling, and risk analysis modeling that can be used by the central office for automatic generation of food safety alerts that are traditionally issued by regulatory agencies.

A centralized database system can be expanded in future research to a comprehensive food safety and risk assessment system. In particular, we are concerned primarily with the development of a food safety database and management system for imported foods, and the same system could also be used for tracking food products produced within the U.S. In addition, the centralized database could be interfaced with the *Pathogen Modeling Program* (PMP) (McMeekin *et al.*, 2006; McMeekin *et al.*, 2006; Ross *et al.*, 1999; Tamplin *et al.*, 2003) and the *Predictive Microbiology Information Portal* (PMIP) (Juneja *et al.*, 2009) developed at the USDA. It is possible to develop an Expert System capable of automatic tracking and decision making in the event of violations of safety regulations or an outbreak of illness due to bacterial contamination in food products.

It is important to develop a prototype Integrated Food Safety Information Management system with automatic wireless sensing and data logging capabilities. In particular, the system can concentrate on the most challenging parts of the complete system, such as RFID, barcode, and wireless interfaces for automatic data logging, and secure database development for tracking and safety alerts.

Sustainable Passive RFID Tagging With Low-Power Sensing

Wireless tagging systems can be developed either as actively-operated processes using batteries, or passively-operated processes through *radio frequency* (RF) signals. There are several differences, mainly due to range of distance in reading/writing. In general, active RFID systems have a longer distance for reading/writing; however, they are not suitable in an environment where a large number of tags are required to be read. The active RFID tags must be powered by batteries, which must be disposed of from time to time—an environmental problem. Using passive RFID technologies, we can develop tagging systems to identify a large number of goods without the concern of powering the tags using batteries. Passive RFID tags are powered by a RF signal from RFID readers, and these are inexpensive; currently, passive RFID tags cost less than 20 U.S. cents. There are different standards of RFID based on distance: (1) 125 KHz (low frequency); (2) 13.65 MHz (high frequency); and (3) 800 MHz–900 MHz (ultra high frequency). The UHF RFID tags are programmed with an *Electronic Product Code* (EPC), which consists of 96 bits of data and some CRC bits, and they can be read from a distance of 10 meters or more. Consequently, they are perfect for use in processing imported food containers, since the *Serial Shipping Container Code* (SSCC) complies with EPC codes. Although passive RFID technologies may be used to track shipping items, standard RFID's are not capable of handling other sensing information, such as temperature, pressure, or humidity.

Recently, Kitayoshi and Sawaya (2005) developed a long-distance, passive RFID system with temperature sensing for 900 MHz and 2.45 GHz (non-standard RFID) for 10 meter- and 30 meter-reading distance ranges. Intel research (Yeager *et al.*, 2008) has also proposed passive and reusable RFID tags with sensing technologies, such as WISP (wireless identification and sensing platform). Also, they have successfully implemented and demonstrated the RFID tagging and reported temperature reading of dairy products that requires a current as low as 50 μA.

RFID tagging is also routinely used by electronics industries and entertainment industries as an anti-theft measure by hiding RFID tags inside packages of CD/DVD or small electronic products. It is clear that Ultra-High Frequency (UHF) RFID tag technology can be used for tracking or inspecting imported foods in large containers with minimum changes in the shipping and packaging processes. We can concentrate on developing the necessary interface for data logging using

UHF RFID. An electronic reader can quickly record items shipped and measure the container temperature. If imported foods are not properly handled at the appropriate temperature, the system will automatically and quickly alert the food inspector about the mishandled foods.

As discussed earlier, data logging of imported food items is a costly and time-consuming process. For efficiency, data logging must be fully automated using a secure, computerized database system. A data logging system can be developed by using a portable handheld device that the food inspectors could use to record EPC and other pertinent information to a central computerized database.

Sustainable Passive RFID Tagging With Low-Power Sensing

This author envisions the implementing a portable system for field use by food inspectors. The system would have a front-end mobile phone unit with an integrated UHF RFID reader functionality and communication capability, as shown in Figure 21.2.

An inspector can validate food container information using EPC on passive RFID, and send authenticated messages to the back-end system, which is a central processing center, for validation. The intelligent back-end process can extract the origin of the imported foods and the food safety standard used in the originating country, or determine whether the food item meets U.S. safety standards, and then communicate the information to the food inspector at the port of entry. The complete data logging process can be done in real time over wireless networks, and is secure, fully automated, and efficient.

A Centralized Secure Database System for Data Logging and Coordination

To properly inspect and validate import data at the port-of-entry, we need to develop a centralized, secure database system for data logging and coordination. There are two inherent research problems that need to be addressed to create such a centralized database:

1. Security related issues, such as RFID data security and making it tamper-proof for data communication, etc.
2. Information management and transmission-related issues, such as how to store and manage shipment information for different inspection locations and time.

FIGURE 21.2. *Portable UHF Reader.*

The first problem is to protect wireless communication between mobile RFID readers and the centralized database system, and this can be addressed by using popular cryptographic protocols (Trappe *et al.*, 2007), such as AES, PKI or PGP. An easier way is to use a public-key infrastructure (PKI) method to encrypt data from mobile units and transmit cipher texts to the back-end database system. The PKI method is a relatively strong encryption method that prevents hackers from spoofing data, manipulating information, and man-in-the-middle attacks by replaying previously spoofed data through the network. We can also use the Diffie-Hellman key exchange method to establish trust between mobile units and the back-end database to reduce possibilities of man-in-the-middle attacks. In our opinion, the RFID tags do not reveal much valuable or critical food safety information, but nevertheless it is necessary to safeguard it.

The second problem is more challenging, as described by Derakhshan *et al.* (2007), and it is focused on fundamentally how to store and manage information efficiently. For a large shipping container, there may be several thousand RFID tags, and it may be impractical and expensive to transfer a large amount of RFID data from handheld units, and then transmit through cellular networks and store them in a relational database system entry-by-entry. A logical way to describe the shipment is to describe it in a semantic way. Instead of storing each RFID tag, we might just want to store a range of RFID tags. One approach is to use

semantic wrapper (Rishe *et al.*, 2000) for the centralized relational database. The mobile units only send and receive semantic messages from the database. The wrapper will understand and expand the semantic messages as SQL queries for updating/downloading data to/from the centralized database system.

Currently, various portable RFID data logging systems have been developed, and they can be extended for use in food inspection to create a fully functional Integrated Food Safety Information Management (IFSIM) system.

CONCLUSION

A centralized food system information system can significantly enhance national food safety surveillance, risk safety decisions, communication, and the monitoring of events of food safety violations. The embedded smart RFID devices, handheld devices, and central database system can provide a real-time framework to track food products. Pathogen growth models and pathogen profiling on specific models can be integrated in the central database to provide real-time growth potential and to calculate potential food safety risks, as well as issue alerts and recalls whenever necessary. The new system will significantly reduce the response time and improve the accuracy of food safety decisions.

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Index