



Latest developments in foodborne pathogens modeling

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Predicting survival, growth or inactivation of pathogens in foods has been one of the most productive activities within Food Microbiology research the last two decades. Aiming at the wider applicability of predictive models, software development has become an active area in foodborne pathogens modeling, with numerous applications for data storage, analysis, visualization and retrieval. At the same time, the need for describing variability in microbial models used in risk assessment motivated the development of stochastic models, i.e. models with a probabilistic structure that take into account the variability of factors affecting microbial behavior. Current and future advances in systems biology and metabolic network modeling are expected to allow for the joined utilization in predictive models of both phenotypic and molecular data, for the purpose of unraveling the underlying mechanisms of microbial behavior.

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Introduction

Predictive microbiology has been defined as the knowledge of microbial responses to environmental conditions, synthesized in mathematical models, which enables objective evaluation of processing, distribution and storage operations on the microbiological safety and quality of foods [1]. The discipline of predictive microbiology was initially based on three main principles: firstly, growth, survival and inactivation of microorganisms are considered

as reproducible responses; secondly, microbial behavior is mainly dictated by a limited number of environmental factors; and thirdly, by quantifying the combined effect of these factors it is possible to predict the responses of microorganisms. The above concept was first used in food microbiology in the early 20th century to describe the inactivation kinetics of foodborne pathogens during thermal processing of foods [2–4]. Although the later applications can be considered as the starting point of Predictive Microbiology it was the ‘Red Book’, published by McMeekin *et al.* [1], which acted as the spark that ignited an explosion in the development of microbial models. After this publication, predicting survival, growth or inactivation of microorganisms in foods has been one of the most productive research activities within food microbiology.

Despite the development of an increased number of microbial models, their use in food industry, research and teaching remained limited for many years. It was soon recognized that wider use of predictive models requires the availability of user-friendly application software providing quantitative information on microbial responses in a rapid and convenient way to different users without the need of a detailed mathematical background. As a result, the last decade, software development has become an active area in predictive microbiology with numerous applications for data storage, analysis, visualization, and retrieval.

The introduction of risk analysis in food safety management was another landmark in predictive microbiology history. According to Codex, microbiological risk assessment should explicitly consider the dynamics of pathogens’ growth, survival, and inactivation in foods. Predictive models are the most effective tools in estimating the changes in microbial levels in foods as the product moves through the farm-to-fork chain, and they have been recognized as important components of the risk assessment process. The use of predictive models in risk assessment, however, has different demands than ‘traditional’ predictive microbiology [5]. The Codex guidelines for the conduct of microbiological risk assessment clearly stress the importance of uncertainty and variability [6]. In this case, the use of deterministic models providing point estimates of microbial concentrations is problematic [7,8]. Such a deficiency highlighted the need for the development of models capable of expressing populations of pathogens in terms of probability (i.e. predict the probability distribution of the microbial concentration at the time of consumption), and motivated the commencement of the so-called ‘stochastic predictive microbiology’ [7].

This article presents an overview of the last developments in predictive modeling of foodborne pathogens. The available predictive software tools that can assist food safety decision-making are reviewed. Emphasis is given in the progress on the development of stochastic models for pathogens that are able to describe the variability and can be used in risk-based food safety management. Future challenges for the use of predictive models in conjunction with systems biology, in order to describe complex microbial behavior based on the underlying molecular mechanisms, are also discussed.

Predictive modeling software

The increased number of the microbial models produced during the last 25 years, led to the development of user-friendly information technology solutions in order to transfer knowledge between academics/researchers and stakeholders of the food sector [9^{*}]. In Table 1, 18 software tools are presented in terms of utilities, availability and modeling approach used for their development. The available tools offer a variety of utilities for the majority of foodborne pathogens including databases, fitting tools, predictions for growth, growth/no growth and inactivation, probabilistic models, and risk assessment modules. This allows for a wide range of applications such as HACCP system support, product and process design, shelf life assessment, compliance with microbiological criteria, exposure assessment, sampling plan and experimental design and education. The most important benefit for the users, however, is that software can assist decision-making in a short time frame and allow practices to be actioned almost in real time.

Wider use of predictive modeling software tools depends on the development of new features and the improvement of user interfaces through visualization of outputs targeting also less advanced users. Another important issue is the transparency in input data and used methodologies, which can improve confidence in predictive microbiology and expand the applicability of the tools to cover additional needs of food safety authorities and food business operators.

Stochastic modeling

In the context of a risk analysis framework, currently applied at the European and international levels for food safety management, the importance of variability of biological and natural phenomena is widely recognized [6]. The importance of describing variability in microbial models used in risk assessment was stressed by Nauta [10], who illustrated the effect of ignoring variability in management decisions. There are numerous sources of variability in microbial behavior that should be taken into account in risk-based approaches. Figure 1 presents an example of how the variations in various factors related to microbial growth can affect exposure assessment.

The state of the art of microbial ecology and predictive microbiology allowed the undertaking of the next important

step, that is, the development of stochastic models with a probabilistic structure that take into account the variability of factors affecting microbial behavior and food safety. Stochastic modeling can take into account all possible circumstances with their associated probabilities to quantitatively assess the probability (risk) that a food is unsafe. Once the mathematical model describing the relationship between environmental factors and pathogen behavior is constructed and the variability of the input factors is described, methods like Monte Carlo simulation can be used to assess the distribution of the output for different values of the input factors taken from their respective probability distributions [11–13]. The later probabilistic approach enables decision-making based on the ‘acceptable level of risk’, and can provide structured information on the effect of potential interventions, allowing decision-makers of public health authorities or the food industry to compare various interventions and identify those that can lead to effective and economic reduction of safety risks.

Several stochastic predictive modeling approaches, aiming at quantifying and integrating different types of variability, have been described the last decade [14–18]. Among the various variability sources, the so-called ‘biological variability’, which includes the inherent differences in microbial behavior among identically treated strains of the same species as well as the heterogeneity in individual cell behavior, has received particular attention [19,20]. The recent technological, methodological and computational developments allowed monitoring of biological variability and its quantitative description with stochastic models. The following paragraphs present some examples of the development of stochastic models for describing strain variability and individual cell heterogeneity of foodborne pathogens.

Modeling strain variability

Behavioral differences among strains of the same microbial species constitute an important source of variability in microbiological studies [20,21]. Recent studies have shown that, depending on the foodborne pathogen of concern, the variability in growth dynamics may constitute one of the most important factors affecting food safety risk [14,22]. An increasing interest in incorporating growth variability among strains of foodborne pathogens in predictive models has been observed during the last decade [11,15,17,18,20,23]. In the risk assessment of *Listeria monocytogenes* in ready-to-eat foods, undertaken by the U.S. Food and Drug Administration and the U.S. Department of Agriculture Food Safety and Inspection Service [24], the ‘relative rate’ approach was used to describe strain variability in the growth rate of the pathogen [5]. In this approach’s framework, a relative rate relationship based on the square-root model [25] for temperature was used, with a probability distribution being assigned to the growth rate at a reference temperature (μ_{ref}) included in the secondary model, while the

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