Concepts in Predictive Microbiology

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Introduction

One can surmise that few people spend much time during their normal day thinking about mathematical modeling. However, each day a wide range of activities and decisions that directly impact millions of individuals in this country are based on the results of models that were developed to describe and predict complex processes and events. Weather forecasts, mutual funds, airline schedules, and canned foods are just four of the myriad of examples of products and services that rely heavily on the use of mathematical models.

In that context, it is not surprising that predictive food microbiology and the use of mathematical and computer modeling to describe and predict the behavior of foodborne microorganisms is playing an ever-increasing role in food microbiology. However, it is important to note that, while the term predictive microbiology is relatively new, the use of mathematical models has always been an integral part of food microbiology. For example, the entire thermal processing industry is based on a series of simple first-order kinetics models. Food engineers and microbiologists have successfully used concepts such as D-values and Z-values to produce safe foods for well over half a century.

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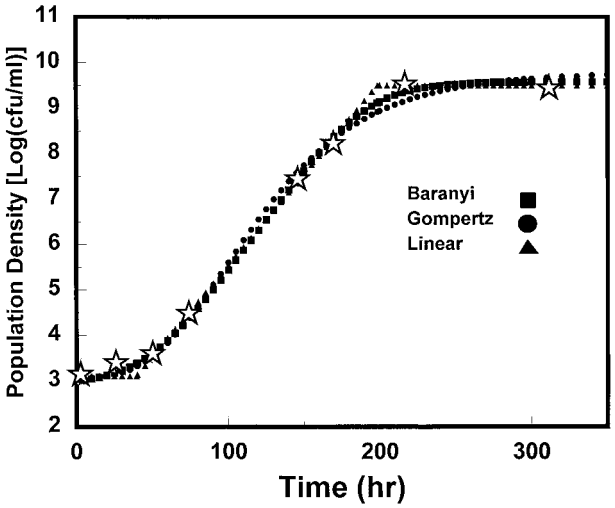
What is a Model?

A predictive food microbiology model is a mathematical expression that describes the growth, survival, inactivation, or metabolic activity of a foodborne microorganism of interest. Within that broad definition, models can be classified according to several different schemes. First, models can be classified based on the biological response being modeled, i.e., growth, survival, or inactivation. Alternatively, models are referred to as being mechanistic or empirical. In the former, the models are based on an underlying mechanism within the cells that control its metabolism. For example, Monad-type models relating substrate concentration to growth rate are considered mechanistic models. Empirical models are generally more statistical in nature. When sufficient experimental data are acquired relating bacterial growth or survival to an environmental variable, the data are fitted to an appropriate mathematical relationship with no attempt to infer a mechanistic rationale. While mechanistic models have the advantage of allowing greater latitude in extrapolating beyond the limits of available experimental data, most predictive food microbiology models available currently are empirical or semi-mechanistic.

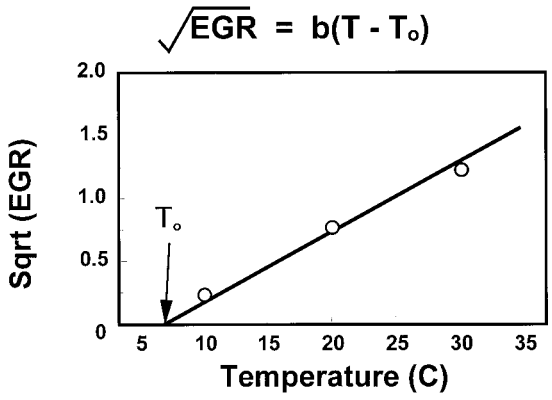
Another approach for differentiating classes of models that we have found very useful is based on a three-tier classification (Whiting and Buchanan, 1993). At the base are **primary models** that are used to describe how a population of bacteria changes with time when placed into a single environment. In the case of bacterial growth, this would be

FIGURE 1.



Example of the relative fits of growth curves generated using the Gompertz, Baranyi, and three-phase linear models for a set of data for Escherichia coli O157:H7. *(Adapted from Buchanan et al., 1997a).*

FIGURE 2.



Example of the "square root" model, a simple secondary model for describing the effect of temperature on the specific growth rate of bacteria.

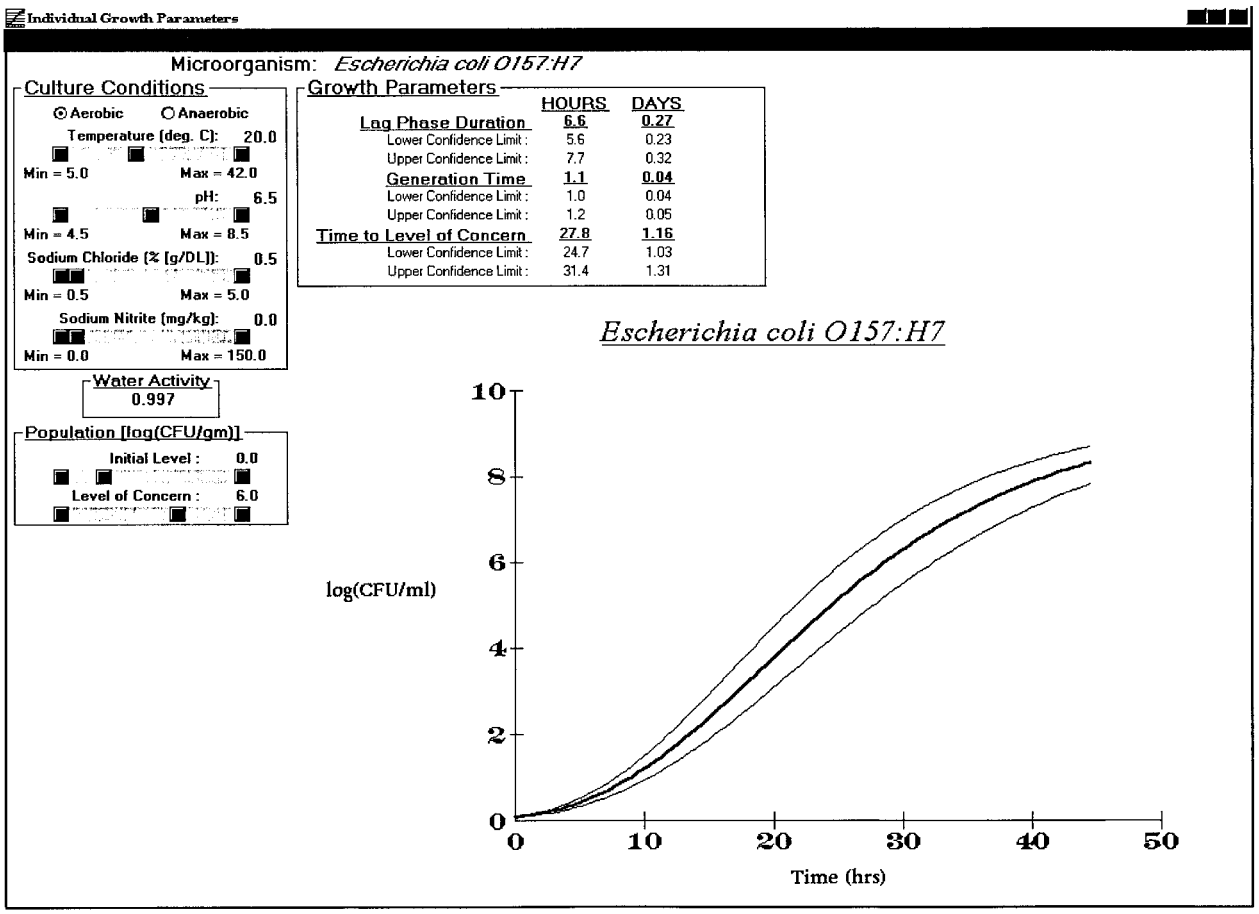
a mathematical expression that describes a growth curve. Several are available. Probably the most widely used has been the Gompertz model (Gibson et al., 1988; Buchanan and Phillips, 1990; Zwietering et al., 1990; Garthright, 1991), which is a sigmoidal function. More recently, there has been increased use of a differential equation developed by Dr. Joseph Baranyi and co-workers (Baranyi and Roberts, 1994; Baranyi et al., 1995). Alternatively, we have found in many cases that relatively simple models such as the three-phase linear model developed in our laboratory can be highly effective (Buchanan et al., 1997a). Each of these models are used in the same manner. The growth data are fitted to the model using curve-fitting software. The software iteratively tries different values for the model's parameters, measuring the "goodness of fit" between the model and the experimental data. This process continues until the computer finds the combination of values for the model's parameters that provides the best fit achievable. While each primary model has different strengths and weaknesses, the general agreement among the primary models for growth is generally excellent (Figure 1). Three parameters commonly derived either directly or indirectly from primary models are the specific growth rate (μ), lag phase duration ( ), and maximum population density (MPD).

A series of primary models have also been developed to describe bacterial survival and inactivation. The best known is the D-value. Underlying the D-value is the assumption that bacterial inactivation follows first-order kinetics, i.e., during inactivation at any set temperature, the log number of bacteria declines linearly as a function of time. We have also developed a series of primary models to describe the survival of foodborne bacteria in adverse environments such as low pH (Whiting and Buchanan, 1992; Whiting, 1993; Buchanan et al., 1994, 1995, 1997b).

When the parameter values have been determined for a sufficient number of different environmental conditions, we can then use them to develop **secondary models**. Secondary models describe how the parameters of a primary model (e.g., μ,) vary as a function of the environmental or cultural variables such as storage temperature, pH, or water activity. For single variable, secondary models can be relatively simple. For example, the effect of temperature on μ for temperatures below a bacterium's optimum can be described using the "square root model" (Figure 2; Ratkowsky et al., 1982). However, multiple variables are being considered, particularly when two or more of the variables interact, more complex models are required (Gibson et al., 1987; Buchanan and Phillips, 1990). Response surface analysis based on quadratic or cubic polynomials has been used extensively to simultaneously consider effects and interactions of three to six different independent variables. Typically, these types of models are developed iteratively. An initial data set is used to develop an initial model, which is then validated against new data to identify any "weak areas". New data can then be acquired and incorporated, and an updated model generated. This ability to continuously augment the initial set allows models to improve over time or be expanded by including additional variables. For example, we have been accumulating data on the growth of *Listeria monocytogenes* for almost nine years, and just released a set of updated models that are based on over 1200 individual growth curves (Buchanan et al., 1997c).

Microbiological modelers are well aware of the fact that most people avoid using mathematics. However, the wide-

FIGURE 3.



Example of the format used in Release 5.0 of the Pathogen Modeling Program.

spread availability of personal computers has allowed predictive microbiologists to overcome this propensity through the development of **tertiary models**. This term was coined to describe "user-friendly" software, expert systems, and other computer applications that allow a set of models to be used readily. Two of the largest tertiary models are **Food Micromodel**, a commercially available program developed by scientists in the United Kingdom, and **USDA Pathogen Modeling Program** (PMP), which was developed by ARS scientists at our Wyndmoor laboratories.

PMP 5.0 is the latest of a series of increasingly sophisticated versions of the software. It provides the user with an easy means of accessing the knowledge gained through experimentation conducted by a large team of scientists, technicians, and students. This Windows-based software includes growth models for eight foodborne pathogens (*Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella flexneri*, *Staphylococcus aureus*, *Yersinia enterocolitica*), survival models for four organisms (*E.coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and *S. aureus)*, and "time-to-toxin" probability models for both proteolytic and non-proteolytic *Clostridium botulinum*. A single format is used with all of the models. Using a series of sliding bars (Fig 3), the user selects values for the different independent variables covered for that microorganism (e.g., temperature, pH, sodium chloride content). Once supplied, these values are used to solve the mathematical models that are at the heart of software. The program automatically performs all needed calculations for the user, providing the user with the predicted growth or survival kinetics, confidence intervals, graphs, etc.

The PMP 5.0 is provided free-of-charge as a public service. It can be obtained by two means. The fastest and easiest is via the Internet. It can be downloaded from the ERRC homepage at http://www.arserrc.gov. Alternatively, disk copies of the program can be obtained by contacting the developers (R. Buchanan or R. Whiting).

Using Predictive Models

When employing microbiological models, there are several concepts that one must keep in mind to use the models effectively and correctly. Available models were generally

developed using microbiological media as a means of acquiring *estimates* of how the microorganisms are likely to behave in foods. Extensive experience has proven that this underlying assumption is valid. In general, the use of data based on microbiological media provide for reasonably conservative models. However, if the user is going to rely on models to make decisions concerning a specific food, then the model should be validated against that food. Validation is a process wherein the user conducts a series of inoculated pack studies to determine how well the model predicts the microorganism's behavior in the specific food of interest. In interpreting a model's predictions, the user must keep in mind that the models are statistical in nature. A predicted value is not an absolute; both the prediction and the confidence intervals around that prediction must be considered.

The role of predictive food microbiology has been steadily increasing as members of industry, government, and academia become familiar with its strengths and limitations. One application that we use routinely in our laboratories is in the design of experiments, particularly planning sampling schedules when conducting inoculated pack studies. Similarly, the models can be used to estimate minimum enrichment times needed for the isolation of various pathogens from foods. Another area where microbiological models are having a significant impact is in the assessment of food formulations or storage conditions in relation to the potential growth or survival of pathogens. This allows foods with increased risks to be rapidly identified so that additional evaluations can be conducted to further characterize a potential food safety problem. This is particularly beneficial in the development of new products that rely on multiple barriers to control microbial concerns. Formulations that do not provide the needed level of control can be eliminated before conducting expensive inoculated pack studies. A third area where models are being used extensively is in teaching. They provide an excellent means for demonstrating how variables interact and the basis for hurdle technologies.

The capabilities that predictive food microbiology provides appear to be a particularly important tool in relation to the development and implementation of HACCP (Hazard Analysis Critical Control Point) programs. As a food safety risk management system, HACCP is most effective when it can deal with microbial growth and survival in a quantitative manner. Mathematical models can help the developer fulfill four of the seven HACCP principles, i.e., conduct hazard analysis, identify CCPs, establish critical limits, and establish corrective actions. During a hazard analysis, the ability to assess rapidly the growth or survival of various pathogens aids in the identification of the pathogens that are of greatest concern under the conditions that the food product provides. Likewise, the ability to evaluate how different conditions during the manufacturing process are likely to impact the pathogens of concern are important for identifying CCPs and subsequently setting critical limits. This is particularly important in being able to link the chemical or physical attribute being controlled with the microbiological behavior and is the underlying reason for the CCP (Buchanan, 1995). For example, models provide a means of relating a requirement such as the chilling of a product to a temperature below 45°F within a specified number of hours with the potential extent of growth of the foodborne pathogens of concern. Likewise, the models can help provide a more scientific basis for the corrective actions that are specified when a process deviation does occur. For example, would exposure of a refrigerated food product to a temperature abuse condition of 75°F for 2 hours result in an unacceptable increase in the levels of *Salmonella*? 60°F for 6 hours? 51°F for 18 hours? Without knowledge of the relationship between temperature and the growth kinetics of this microorganism, it is not possible to provide sound scientific advice on the appropriate disposition of product exposed to these conditions.

A final application that would be virtually impossible without the recent advances in predictive food microbiology is the emerging field of quantitative microbial risk assessment. Evaluation of potential food safety options has always been hampered by our inability to link the impact of our efforts with their effects on public health. In large part, this was due to an inability to conduct meaningful exposure assessments as a result of the drastic changes that could occur in pathogen levels during production, distribution, marketing, and consumption. However, by coupling microbiological modeling with risk assessment techniques, it is possible to achieve dynamic risk assessment models that can deal realistically with the factors that influence the level of a foodborne pathogen actually ingested by the consuming population (Buchanan and Whiting, 1996; Whiting and Buchanan, 1997).

Concluding Remarks

Through concerted efforts of several research teams over a period of almost a decade, it has been possible to develop a series of microbiologial models based on an extensive set of systematically collected data. However, this does not mean that the job is done. A number of the groups have turned to the goal of developing a second generation of models. Their objective is to be able to account for factors such as the effects of acidulant and humectant identity, prior growth conditions and stress responses, and the variability of biological responses among strains. These all represent significant challenges to future modeling efforts. Further, there is an ever-increasing effort to couple the model development with the latest research into the physiology of foodborne pathogens. With this background, one of the easiest predictions in predictive food microbiology is that it will continue to be an increasingly important tool in modern food microbiology. For individuals who are interested in learning more about predictive food microbiology, several excellent texts and reviews are available (Buchanan et al., 1993; Farber, 1986; McMeekin et al., 1993; Whiting and Buchanan, 1994, 1997a).

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