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# Predictive food microbiology

Robert L. Buchanan

The need to assure the microbiological safety and quality of increasingly complex food products has stimulated interest in the use of mathematical modeling to quantify and predict microbial behavior. During the past several years there has been substantial advancement in both the concepts and methods used in predictive microbiology. Coupled with 'user-friendly' applications software and the development of expert systems, these models are providing powerful new tools for rapidly estimating the effects of formulation and storage factors on the microbiological relations in foods.

During the past 5–8 years, there has been a dramatic increase in research on the development of mathematical models that describe how microorganisms behave in foods. Activity and interest in this area, which has been termed 'predictive microbiology', has been so widespread that it is now one of the most rapidly advancing of the sub-specialties in food microbiology. This interest has been worldwide, with scientists establishing international collaborative efforts to share the ideas, concepts, mathematical techniques and databases that are needed to generate and validate new, more effective models. The scope of this effort was readily apparent at the recent 'International workshop on the application of predictive microbiology and computer modeling to the food industry' organized under the auspices of the Society for Industrial Microbiology (12–15 April 1992, Tampa, FL, USA). This conference, which brought together microbiologists, food scientists, mathematicians, engineers and statisticians from 15 countries, demonstrated the rate at which this part of food microbiology is maturing and gaining acceptance. The purpose of this Review is to provide an overview of the major thrusts and applications of predictive microbiology, and to emphasize the need for continuing international collaboration.

Historically, food microbiology has always been an active area for mathematical modeling, though often food microbiologists do not fully appreciate that a number of the techniques they routinely use are a form of predictive microbiology. A pertinent example is the

calculation of thermal resistances and process times. These mathematical techniques have become so ingrained that few food microbiologists give a second thought to the fact that when they calculate or discuss D-values, Z-values and F-values, they are employing a linear mathematical model to describe the exponential inactivation of bacteria.

There are a number of reasons why there has been a recent re-emergence of mathematical modeling of microbiological relations in foods, with three being particularly pertinent.

(1) The first is the ready availability of powerful micro-computers. The presence of such computers on most scientists' desks, and the accompanying increased awareness of practical mathematics that comes from working with software has stimulated exploration into potential microbiological applications.

(2) The second has been the consumers' preference for 'fresher, less processed' foods. This has resulted in the development of sophisticated multiple-barrier food preservation systems in which a combination of factors, no one of which is sufficient by itself, is used to delay microbiological spoilage. Therefore, there is a need to quantify the effects of each of the factors contributing to the total microbiological integrity of the product. The use of hazard analysis and critical control point (HACCP) as a safety management tool, with its requirement for setting critical limits for key stages of product handling, has reinforced the need for effective models. Without such models, it becomes difficult to deal quantitatively with interactions among multiple factors.

(3) The third is the realization that it would be virtually impossible, both scientifically and economically, to have quantitative microbiological information on the hundreds of different variations of each of the thousands of different foods and food ingredients that are present in international commerce. However, it is precisely this quantitative data on the behavior of pathogenic microorganisms that is needed to make informed decisions about the safety of food products. This limitation is being offset by the realization that there are a limited number of key factors that account for most of the behavior of microorganisms in food systems. Through systematic quantification and understanding of the impact of these factors in model systems and prototype products, it is possible to generate effective models that can estimate microbial behavior in a range of products. These models can subsequently provide industry with an important means of making objective initial assessments to establish priorities in relation to both product design and evaluation. Likewise, such priority-setting techniques are critical if regulatory agencies are to adopt risk-based inspection systems.

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Robert L. Buchanan is with the Microbial Food Safety Research Unit, Eastern Regional Research Center, US Department of Agriculture, Agricultural Research Service, Philadelphia, PA 19118, USA.

Within predictive microbiology, there are several means of classifying models based on either the microbiological event studied, the modeling approach

employed, or the number or type of variables considered. Models can be classified broadly on the basis of whether they describe microbial growth or inactivation; with the exception of models of thermal inactivation, models for microbial growth are generally more advanced than those for inactivation. Models are also categorized based on the mathematical approach used, with the two primary types being probability-based and kinetics-based models. Models can also be differentiated on the basis of whether they are mechanistic or empirical. While it is generally considered that mechanistic models are inherently superior, most of the successful models currently available are empirical.

Recently, we proposed an additional classification of models as primary, secondary or tertiary, based on the types of variables being described<sup>1</sup>. Primary models are mathematical expressions that define growth or survivor curves describing the response of an organism over time to a specific set of cultural conditions. Secondary models describe the impact of cultural and environmental variables on organism growth or survival characteristics. Using thermal processing as an example, a survivor curve used to calculate D-values is a primary model, while a thermal death-time curve used to calculate Z-values would be a secondary model. The term tertiary model is used to describe the incorporation of primary, secondary, or combinations of primary and secondary models into application programs and expert systems. Examples of each of the different types of models are introduced below.

### Mathematical modeling of microbial growth curves

One of the key breakthroughs that has allowed predictive food microbiology to progress rapidly during the past several years has been the identification of effective primary models for describing microbial growth curves in model systems and foods. These models have permitted growth curves to be described objectively as mathematical expressions, an attribute that is critical to the development of secondary models of microbial growth kinetics. Of particular importance has been the use of various sigmoidal relationships, such as the logistics and Gompertz curves. While these relationships have been used previously to describe other biological processes, it wasn't until the late 1980s that a research team from the AFRC Institute of Food Research in the UK introduced food microbiologists to their potential as a means of describing microbial growth<sup>2</sup>. Since their introduction, these equations have rapidly changed the way food microbiologists analyse quantitative growth kinetics data.

The Gompertz equation is a four-parameter double-exponential function that describes an asymmetrical sigmoidal curve:

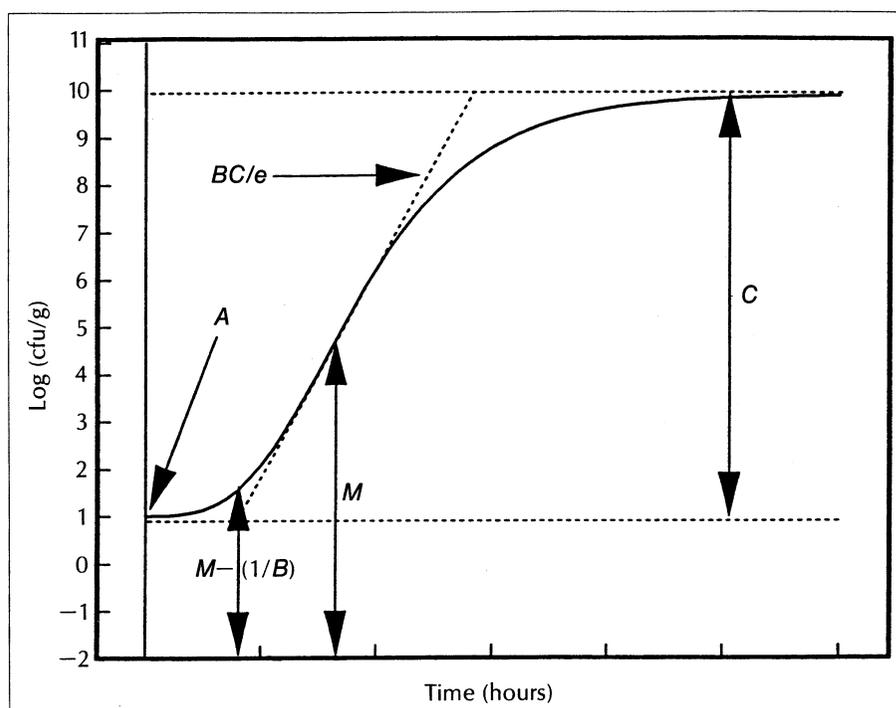


Fig. 1

Parameters associated with the Gompertz equation. See text for definitions of parameters.

$$L_t = A + C \exp\{-\exp[-B(t - M)]\}$$

where  $L_t$  is  $\log_{10}$  of the count of bacteria (number of colony-forming units; cfu) at time  $t$  (in hours);  $A$  is the asymptotic log count as time decreases indefinitely (approximately equivalent to the log of the initial level of bacteria);  $C$  is the asymptotic log count as time increases indefinitely (approximately equivalent to the log of the maximum population density during the stationary growth phase minus the log of the initial count);  $M$  is the time at which the absolute growth rate is maximal; and  $B$  is the relative growth rate at time  $M$  (Fig. 1). It has been the most widely used of the sigmoidal functions, with characteristics particularly useful to food microbiologists<sup>3,4</sup>.

Upon first glance, the equation seems complex. However, the four parameters can readily be related mathematically to cultural characteristics familiar to microbiologists:

$$\begin{aligned} \mu &= BC/\exp(1) \\ GT &= [\log_{10}(2)][\exp(1)/BC] \\ \lambda &= M - (1/B) \\ MPD &= A + C \end{aligned}$$

where  $\mu$  is the exponential growth rate  $\{[\log(\text{cfu/g})]/\text{h}\}$ ;  $GT$  is the generation time (h);  $\lambda$  is the lag phase duration (h) and  $MPD$  is the log of the maximum population density  $[\log(\text{cfu/g})]$ . The Gompertz equation can also be reparameterized to use data provided in the form of growth rates and lag phase durations<sup>3</sup>.

Coupled with good curve-fitting software, the Gompertz equation is easy to use. For example, our laboratory employs an iterative, nonlinear regression program in conjunction with a minicomputer to fit experimental data. While some experience and judgement are needed to optimize curve-fitting routines, we have found

that our technical staff quickly adapted to this approach. Once trained, an investigator takes ~30 s to generate a growth curve from a set of experimental data.

In addition to the specific applications of sigmoidal models for describing growth relations, the introduction of these equations has stimulated a great deal of activity in relation to defining or describing microbial relations. For example, it was a direct result of the availability of these sigmoidal kinetics that stimulated us to postulate a mathematical approach for defining and calculating the duration of the lag and exponential growth phases<sup>5,6</sup>. Likewise, there has been renewed interest in the development of new approaches for describing growth under constant or changing conditions<sup>7-9</sup>.

### Modeling the effects of cultural and environmental conditions on growth

The growth of microorganisms in food systems is dependent on the effects of (and at times interactions among) multiple variables. Examples of factors that influence microbial growth kinetics include temperature, pH, acidulant identity, water activity, humectant identity, absorption and desorption isotherms, oxygen availability, carbon dioxide levels, redox potential, nutrient content and availability, and the presence of antimicrobials. Traditional food preservation techniques typically involve manipulating one of these parameters so that it is outside the range that supports the growth of most foodborne species. For example, increasing the salt content of foods to greater than 5% retards the growth of many Gram-negative foodborne bacteria, including a number of pathogens. As consumers have demanded foods that are closer to being fresh, products are being developed that rely on packaging with multiple barriers to affect several of the factors that influence microbial growth. Typically, no single variable is altered to such an extent that it is sufficient by itself to control microbial growth. Instead, manipulating multiple variables to a smaller degree produces a total impact that is sufficient to prevent growth. It is readily apparent that a large number of combinations of various variables could be effective. The availability of good models that integrate the effects of pertinent variables almost becomes a requirement for the cost-effective design and production of such products.

Two major approaches, probability-based models and kinetics-based models, have been used to describe the impact of various cultural and environmental factors on the growth of foodborne bacteria. The choice of approach and the specific application within an approach are largely determined by the type of microorganism and the number of variables. Typically, probability-based models have been employed with spore-forming bacteria, while kinetics-based models have been used with non-spore-forming species.

### Probability-based models

Most probability-based modeling has used the general approach of Hauschild<sup>10</sup>, who estimated the probability that a single spore of *Clostridium botulinum* would

germinate and produce toxin in a food. This approach helps take into account the strong effect that cultural conditions have on the germination of bacterial spores. For example, Montville<sup>11</sup> reported that almost all *C. botulinum* spores germinated in a medium with no added NaCl and at pH 7.0, whereas only 1/100 000 spores germinated when 2% NaCl was presented and at pH 5.5. Various investigators<sup>12-19</sup> have systematically estimated the effects and interactions of multiple variables on the probability of germination and growth of *C. botulinum*. Regression analysis is used to model the individual contributions of the variables. More recently, investigators have incorporated terms that allow estimation of the probability of when a microbiological event will occur. For example, Genigeorgis *et al.*<sup>18</sup> modeled the effects of temperature, inoculum size and percentage brine on the duration of the lag period before toxigenesis for non-proteolytic *C. botulinum* types B and E in cooked turkey:

$$\log_{10}(\text{LP}) = 0.625 + 6.71(1/T) + 0.0005IT - 0.033T + 0.102B - 0.102I$$

where LP is the lag to toxigenesis (days);  $T$  is the temperature ( $^{\circ}\text{C}$ );  $I$  is the inoculum size ( $\log_{10}$  of the number of spores) and  $B$  is the percentage brine. The model provided reasonable agreement with experimentally derived data. Another group, also working with non-proteolytic *C. botulinum*, recently developed a new primary model that incorporates terms for both time and the relative extent of germination:

$$P_t = P_{\max}/(1 + e^{k(\tau-t)})$$

where  $t$  is the time (days);  $P_t$  is the probability of growth at time  $t$ ;  $P_{\max}$  is the maximum probability of growth over the entire storage period;  $k$  is the rate constant ( $\text{days}^{-1}$ ); and  $\tau$  is the time to  $P_{\max}/2$  (Whiting, R.C. and Call, J.E., submitted). This approach was subsequently used to develop a probability-based model of the effects of temperature, pH and NaCl concentration on the time to toxigenesis.

### Kinetics-based models

The second broad approach is the development of models that mathematically describe the effects of cultural and environmental conditions on a microorganism's growth kinetics, particularly lag-phase durations and generation times. These can be modeled either directly or using mathematical functions such as the parameters of the Gompertz equation. The complexity of the models required varies with the number and independence of the variables being considered simultaneously. While the growth of a microorganism in a food system is potentially dependent on the interaction of a range of variables, for many products growth is overwhelmingly dependent on a single variable. In such instances, it is generally storage temperature that is the most important factor controlling microbiological growth. It is not surprising that a substantial amount of modeling research has concentrated on this variable.

Typically, modeling the impact of temperature using a simple Arrhenius equation is only accurate for a portion of an organism's temperature range. However, modifications of this approach, as well as other explicit equations, have been effective. The three most studied have been the 'non-linear Arrhenius-Schoolfield' equation<sup>20-22</sup>, the 'linear Arrhenius-Davey' equation<sup>23,24</sup> and the 'square root' ('Ratkowsky-Belaradek') models<sup>25,26</sup>. The Schoolfield equation and related nonlinear Arrhenius models were developed to enhance the basic Arrhenius model to achieve better fits at the extremes of organisms' temperature ranges. While reasonably effective, this six-parameter equation is complex and its use can be cumbersome.

The 'square root' equation is probably the most studied and widely used of the simple models for the effects of temperature on microbial growth. Below the microorganism's optimum growth temperature, the square root of the growth rate constant ( $R$ )<sup>25</sup> and the reciprocal of the lag phase duration,  $\lambda$  (h)<sup>27</sup> are linearly related to temperature according to the following relationships:

$$(R)^{0.5} = b(T - T_{\min})$$

$$(1/\lambda)^{0.5} = b(T - T_{\min})$$

where  $b$  is the slope of the regression line;  $T$  is the incubation temperature (K); and  $T_{\min}$  is the notional minimal growth temperature (K), which is derived by extrapolating the linear regression line to zero. As incubation temperatures increase above an organism's optimum growth temperature, growth rates begin to decline. Ratkowsky *et al.*<sup>26</sup> subsequently expanded their model to include a term that took into account this depression of growth rate:

$$(R)^{0.5} = b(T - T_{\min})(1 - e^{c(T - T_{\max})})$$

where  $T_{\max}$  is the notional maximal growth temperature (K) and  $c$  is a constant. This empirical secondary model has been shown by a number of investigators to be effective for estimating the effects of different constant storage temperatures on the growth of variety of microorganisms in foods and model systems<sup>28-33</sup>. One of the clear advantages of this model is its simplicity, both in relation to generating models and subsequent applications. If one limits the temperature range to less than the optimum range for an organism's growth, the model can be generated with simple linear regression techniques.

Zwietering *et al.*<sup>34</sup> investigated the effectiveness of several models for describing the effect of temperature on the growth kinetics of *Lactobacillus plantarum*, an organism for which they had a large database of experimental values. They concluded that two reparameterizations of the 'square root' equation were the most effective for modeling growth rates and maximum population densities, both in terms of fit and ease of use. However, they concluded that the effect on the lag phase was better described by a hyperbolic function:

$$\ln(\lambda) = p/(T - q)$$

where  $\lambda$  is the lag phase duration (h);  $T$  is the temperature (K);  $p$  is a parameter that accounts for the decrease

in lag phase duration as temperature is increased; and  $q$  is the temperature (K) at which lag phase is infinite. Zwietering *et al.*<sup>34</sup> used these three equations to develop a model for predicting the growth curve for *L. plantarum* over its entire temperature range.

A third empirical equation used increasingly to model the effect of incubation temperatures is the linear Arrhenius-Davey equation<sup>23</sup>:

$$\ln(k) = C_0 + C_1/T + C_2/T^2$$

where  $k$  is the growth rate constant;  $T$  is the temperature (K); and  $C_0$ ,  $C_1$ , and  $C_2$  are coefficients to be determined. This equation can also be used to model the effect of temperature on the reciprocal of the lag phase duration<sup>24</sup>:

$$\ln(1/\lambda) = C_0 - C_1/T + C_2/T^2$$

The three coefficients associated with the equation can be generated readily using nonlinear regression techniques to produce the best-fit curve. Studying the growth of *Listeria monocytogenes* on refrigerated beef, Grau and Vanderlinde<sup>35</sup> compared the Davey and Ratkowsky models and found that both were effective.

The above models were developed based on experimental data from cultures maintained at constant temperatures. Substantially less modeling has been done on the effects of fluctuating storage temperatures, though this is currently a very active area of research. Blankenship *et al.*<sup>36</sup> used a modification of the 'square root' equation to model the effects of cooling schedules on the growth kinetics of *Clostridium perfringens* in a cooked meat product (chili). More recently, van Impe *et al.*<sup>8</sup> presented a dynamic model based on a differential equation that combines the Gompertz equation, the Ratkowsky equation and a term to account for the transition to inactivation when a microorganism is shifted to an adverse elevated temperature.

When more than one variable has to be considered to predict the growth rate of a foodborne microorganism, the type of model employed is dependent on the number and independence of the variables. If temperature and another variable are independent of each other, modifications of the Ratkowsky and Davey equations are effective. For example, McMeekin *et al.*<sup>37</sup> studied the combined effects of water activity ( $a_w$ ; parameterized as NaCl concentration) and temperature on the growth of *Staphylococcus xylosus*. They found that at each  $a_w$  tested, the relationship between growth rate and temperature (below the optimum growth temperature) could be described by the 'square root' model, with  $T_{\min}$  remaining constant. The combined effects of the two variables could be described by a simple multiplicative expression:

$$R^{0.5} = b(T - T_{\min})(a_w - a_{w \min})^{0.5}$$

Chandler and McMeekin<sup>38</sup> obtained similar results when the  $a_w$  of *S. xylosus* cultures was adjusted using glycerol, and concluded that the slope and  $a_{w \min}$ , but not  $T_{\min}$  varied with the identity of the humectant. Adams *et al.*<sup>39</sup> examined the combined effects of pH and suboptimal temperatures on the growth kinetics of *Yersinia*

*enterocolitica*. They found that the two variables were independent and could be expressed as:

$$R^{0.5} = b(T - T_{\min})(\text{pH} - \text{pH}_{\min})^{0.5}$$

The notional  $\text{pH}_{\min}$  was dependent on the identity and concentration of the acidulant, and would likely be influenced by the buffering capacity of a food system. Davey<sup>23,24</sup> provided an expanded version of his equation that examined the combined effects of temperature and water activity:

$$\ln(k) = C_0 + C_1/T + C_2/T^2 + C_3a_w + C_4(a_w)^2$$

Grau and Vanderlinde<sup>35</sup> used multiplicative expansion of both the Ratkowsky and Davey equations to model the effects of temperature and pH on the growth of *L. monocytogenes* on beef tissue.

While it is assumed that additional variables could be included using the above approach, response-surface techniques have been the primary method for developing models for more complex foods that are dependent on four, five or more primary variables, particularly if the variables are interactive. This approach employs regression analysis techniques to generate the best-fit, multidimensional response-surface equations that describe the effects and interactions of the experimental variables. Investigators have successfully employed this empirical approach to develop four- or five-variable models for a number of foodborne pathogens, including *C. botulinum*<sup>2</sup>, *Salmonella* spp.<sup>40</sup>, *Listeria monocytogenes*<sup>41</sup>, *Aeromonas hydrophila*<sup>42,43</sup>, *Shigella flexneri*<sup>44</sup>, *Y. enterocolitica*<sup>45</sup>, *Staphylococcus aureus* and *Escherichia coli* 0157:H7 (Buchanan, R.L. *et al.*, submitted).

### Modeling microbial inactivation

There is an extensive knowledge base on the thermal inactivation of microorganisms in foods, including a number of effective empirical and mechanistic models. However, there is surprisingly little systematic data on the non-thermal inactivation of bacteria resulting from the manipulation of other food formulation parameters, such as pH, water activity or the presence of antimicrobials. Parish and Higgins<sup>46</sup> reported that when *L. monocytogenes* was placed in orange serum adjusted to pH 3.6–4.8, the lag period before the initiation of inactivation was linearly related to pH, and could be modeled accordingly. Also using *L. monocytogenes*, our laboratory (Buchanan *et al.*, submitted) found that in a microbiological medium adjusted to a pH  $\leq$  5.5 with HCl, the time to achieve a '4-D' ( $10^4$ -fold) inactivation ( $t_{4-D}$ ) was linearly related to pH and could be described by:

$$t_{4-D} = m(\text{pH} - \text{pH}_0)$$

where  $m$  is the slope of the regression line and  $\text{pH}_0$  is the notional pH for instantaneous inactivation based on extrapolating the regression line to  $t = 0$ . The values obtained for  $m$  and  $\text{pH}_0$  were 197.3 and 2.67, respectively.

The effect of two monocarboxylic acids (lactic acid and acetic acid) on the inactivation of *L. monocytogenes* was also determined (Buchanan *et al.*, submitted). We found that the rate of inactivation was dependent on the

identity of the organic acid, its concentration, and the pH of the system. The logarithm of  $t_{4-D}$  was found to be linearly related to the square root of the concentration of undissociated acid. This allowed the development of the following equation:

$$t_{4-D} = \exp \left\{ 2.303 \left[ m \left( \frac{T/\exp[(\text{pH}-\text{pK})/2.303]}{1 + \exp[(\text{pH}-\text{pK})/2.303]} \right)^{0.5} + b \right] \right\}$$

where, for each acid,  $T$  is the total concentration (mM) of organic acid;  $m$  is the slope of the regression line; and  $b$  is the y-intercept of the regression line. Additional work on the development of response-surface models for the effects of multiple variables on the non-thermal inactivation of *L. monocytogenes* and *Salmonella typhimurium* are currently being completed<sup>47</sup>.

### Applications

Once models have been developed and validated, a key to their successful use is reducing their operation to a 'user-friendly' form. The widespread availability of microcomputers that has helped stimulate interest in microbial modeling is also an important tool in developing such 'user-friendly' applications. For example, our laboratory has developed application software to demonstrate the potential usefulness of predictive microbiological approaches<sup>48</sup>. The program, which is currently in its fourth version, automates the use of available response-surface models for the effects of storage temperature, initial pH, NaCl content ( $a_w$ ), sodium nitrite concentration and oxygen availability on the growth of foodborne pathogens, including *Salmonella* spp.<sup>40</sup>, *L. monocytogenes*<sup>41</sup>, *A. hydrophila*<sup>42,43</sup>, *S. aureus* (Buchanan *et al.*, submitted), *E. coli* 0157:H7 (Buchanan *et al.*, submitted) and *S. flexneri*<sup>44</sup>. The software has been distributed extensively to industry, government and academia, and is being used to provide 'first estimates' of the behavior of pathogens in food, for applications in both designing and evaluating products. Likewise, the software has proved to be a useful tool for teaching food microbiology.

As available knowledge of microbiological modeling and applications approaches becomes more extensive, the simplicity, flexibility and usefulness of application software will be enhanced significantly. One of the approaches that will be particularly important is the use of 'expert systems'. This computer modeling technique formalizes the thinking processes of experts in a field such as food microbiology, coupling this with objective tools such as mathematical modeling. This permits the user of the system to have available for immediate use both objective predictors and the experience of experts. Several corporations are currently developing impressive expert systems for assessing microbiological relations within food products. Likewise, the UK Ministry of Agriculture, Fisheries and Food has developed and recently gone online with 'Food Micromodel', an extensive expert system/database for food microbiology. While the database is physically located in the UK, subscribers can interface with the computer via the telephone to query the system from anywhere in the world.

## Concluding remarks

There is a great deal of excitement among researchers in predictive microbiology in relation to the future. New techniques and findings are being reported almost weekly, and this is likely to accelerate as a developing international network of scientists begin to collaborate and share databases. It seems reasonable to predict that the next five years will see the introduction of increasingly more comprehensive computer-based models and expert systems. This should be enhanced by the introduction of dynamic modeling techniques similar to those employed by engineers to study processing operations. Ultimately, it should be possible to produce an integrated dynamic model that could follow the microbiological impact of each of the different steps associated with the production, distribution and retailing of a food. Such a tool would be an obvious benefit to efforts to introduce and operate HACCP-based food safety systems.

One of the factors that has enhanced the rapid development of predictive microbiology has been the high degree of international cooperation among researchers. This is going to become even more critical as models are expanded to include additional variables. Data collection becomes a limiting factor when interactions among a large number of variables must be considered, and models are only as good as the quantitative data available. Likewise, there is increased need to enlist various laboratories evaluating different types of foods, to help validate the effectiveness of different models. Hopefully, there will be further efforts to enhance the international exchange of modeling concepts and databases.

Predictive microbiology techniques should be a boon to food microbiologists, allowing them to rapidly explore the microbiological impact of varying conditions within a food. Likewise, the development of expert systems will provide a means of making their knowledge readily available to other segments of the food industry, freeing their time for handling the more complex questions. However, care has to be exercised in relation to emphasizing to users that the models are only a means of providing rapid 'first estimates' of microbiological behavior. Models are not a substitute for good laboratory support. Instead, they are a means of allowing a laboratory to function more effectively. This new area of food microbiology research will undoubtedly provide a powerful set of new tools that will allow us to get one step closer to the long-term goal of being able to design microbiological quality and safety into food products, instead of attempting to introduce these attributes after the fact through end-product testing and inspection.

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