

Minireview

Predictive Microbiology : A Review

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Received 23 March 2002/Accepted 24 July 2002

Key words : Predictive microbiology/Types of models/Limitations/Prospects/HACCP.

INTRODUCTION

Predictive Microbiology may be considered as the application of research concerned with the quantitative microbial ecology of foods. The subject is based on the premise that the responses of populations of microorganisms to environmental factors are reproducible and that, by characterizing environments in terms of these factors which affect microbial growth and survival, it is possible from past observations to predict the responses of microorganisms in new, similar environments.

This concept of predictive microbiology is new in its application but has a long history. Early works like those of Esty and Meyer (1922) had used mathematics to determine the survival of microorganisms. Baird Parker and Kilsby (1987) point out that models for the thermal destruction of microorganisms were well established in the literature and industry. Modeling of microbial growth was also being done in the field of industrial microbiology (Monod, 1949). However, it has been recognized that food microbiology should build its own repository of models without copying those used in industrial microbiology, as their objectives are different (Baranyi and Roberts, 1994).

This realization and two related factors contributed to the newly found application of this concept as an organized field of study (McMeekin et al., 1993). The first factor was the increased incidence of major foodborne disease outbreaks during the 1980's, which resulted in an acute awareness and demand for food safety. The second was the realization as noted by Roberts and Jarvis (1983) that traditional microbial end product challenge testing was an expensive and largely negative science and a more systematic and co-operative approach to assure the safety of

foods was needed.

The conflicting demands of consumers for 'fresher' and more 'natural' or less processed foods on the one hand and for safe foods free from potential risks on the other also provided the impetus for the development of this field. At the same time the ready access to computing power hastened the process, as well (Buchanan, 1991a).

The development of predictive microbiology received a huge boost when the Ministry of Agriculture, Fisheries and Food (MAFF) in the United Kingdom reviewed possible topics for new research programs and decided in 1988, to fund a nationally coordinated program of research on the growth and survival of bacterial pathogens in food systems. It was envisaged that the research would develop new modeling expertise and generate a computerized Predictive Microbiological DataBase (Gould, 1989).

Interest in Europe was also being developed through the FLAIR (Food Linked Agricultural and Industrial Research) program with about 30 laboratories in 10 EU countries collaborating to examine the growth responses of spoilage and pathogenic organisms in a wide range of natural products.

In the United States, predictive microbiology research was centered at the Microbial Food Safety Research Unit of the USDA, which described the effect of five variables (temperature, NaCl concentration, pH, nitrite concentration and gaseous atmosphere) on the growth of various pathogens.

These laboratories were involved in describing growth, death and survival responses of foodborne pathogens as affected by temperature, pH, levels of organic acid, salts, nitrite and concentrations of preservatives and gaseous atmosphere. The target organisms included were *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp, *Shigella*, spp, *Staphylococcus aureus*

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and *Yersinia enterocolitica*. The cost involved in funding such huge collaborative studies was justified when compared with the enormous cost of food poisoning outbreaks.

GENERAL FORM

The various needs and strategies for developing predictive models for food microbiology have been summarized by Ross and McMeekin (2000). The primary variable of interest may be the growth rate, death rate or time for some event to happen or some condition to be reached, or the probability that the event will happen within some pre-determined time.

The general form of the linear mathematical model is (McMeekin et al., 1993):

$$Y = \alpha + \beta X_1 + \gamma X_2 + \varepsilon$$

Where Y is the observed response, X_1 and X_2 are the known or set independent variables. α , β , γ are the parameters whose values are determined by the data and are fitted by least square principles so as to minimize the differences between the observed values of the response variable and those predicted by the fitted equation. The stochastic term (ε) indicates the extent to which the predicted response deviates from the observed response.

CLASSIFICATION OF MODELS

Models can be classified based on the microbiological event studied into kinetic and probability models (Roberts, 1989), on the modeling approach used into empirical and mechanistic models (Roels and Kossen, 1978) and by the independent variables (considered for modeling) into primary, secondary and tertiary models (Whiting and Buchanan, 1993).

Kinetic and probability models

Kinetic models are concerned with the rates of response (growth or death). Examples include the Gompertz and square root models which describe the rates of response, like lag time, specific growth rate and maximum population density (McMeekin, et al., 1993; Whiting and Buchanan, 1994) or inactivation/survival models that describe destruction or survival over time (Huang and Juneja, 2001; Xiong et al., 1999).

Probability models, originally used for predicting the likelihood that organisms grow and produce toxins within a given period of time (Hauschild, 1982; Stumbo et al., 1983), have been more recently extended to define the absolute limits for growth of

microorganisms in specified environments, for example, in the presence of a number of stresses which individually would not be growth limiting, but collectively prevent growth. Investigators like Baker et al. (1990) and Meng and Genigeorgis (1993) systematically estimated the effect of interactions of a range of factors on the probability of the germination and outgrowth of *Clostridium botulinum*. Lund et al. (1985) used another probabilistic approach while studying the survival, growth and toxin formation of a mixed inoculum of approximately equal number of spores of two strains each of *C. botulinum* type A, proteolytic type B and type E. Probability models indicate only the probability of growth or toxin production and do not indicate the speed at which they occur (Roberts, 1989). Nevertheless they were the first attempts in predicting the risk associated with foods. (Skinner and Larkin, 1994).

Empirical and mechanistic models

Empirical models usually take the form of first- or second- degree polynomials and are essentially pragmatic, describing the data in a convenient mathematical relationship (curve fitting). An example is the quadratic response surface used by Gibson et al. (1988). Mechanistic or deterministic models are built up from theoretical bases and allow interpretation of the response in terms of known phenomena and processes. Attempts, like those of McMeekin et al. (1993), to find a fundamental basis for the square root model are important steps towards more mechanistic approaches. Draper (1988) considers the mechanistic models to be more preferable than the empirical ones, as they usually contain fewer parameters, fit the data better and extrapolate more sensibly.

Levels of Models

(i) Primary models

These models measure the response of the microorganism with time to a single set of conditions. The response can either be direct/indirect measures of microbial population density or products of microbial metabolism. These primary models include growth models (Buchanan et al., 1989; Gibson et al., 1987), the growth decline model (Whiting and Cygnarowicz 1992), D-values of thermal inactivation (Rodriguez et al., 1988, Abraham et al., 1990), inactivation/survival models (Kamau et al., 1990; Whiting, 1992), growth rate values (McMeekin et al., 1987) and even subjective estimations of lag time or times to turbidity/ toxin formation (Baker et al., 1990; Dodds, 1989).

(ii) Secondary models

These models indicate how parameters of primary

models change with respect to one or more environmental factors (e.g., atmosphere, pH, temperature and salt level). Response surface (Buchanan and Phillips, 1990; Juneja and Eblen, 1999), Arrhenius (Broughall et al., 1983), and Belehraddek (Ratkowsky et al., 1991) are some examples of this type of models. Secondary models may be further categorized as direct or indirect. A model that describes the effect of an environmental variable on a primary model parameter would be a direct secondary model. For example, a response surface equation for the parameters of the Gompertz function would be a direct model. Alternately, a secondary model that relates an environmental variable to a value derived from one or more parameters of a primary model would be an indirect model. Thus a response surface equation that relates environmental variables to values for lag phase duration or generation times that were derived from a Gompertz or logistic would be an indirect secondary model.

(iii) Tertiary models

These are applications of one or more secondary models to generate systems for providing predictions to people not familiar with the modeling technique. These are in the form of user-friendly applications software (Buchanan 1991b and 1993) and expert systems (Adair et al., 1992; Jones, 1992). This level would include algorithms to calculate changing conditions (e.g., transient temperature after 5 days of storage), compare microbial behavior under different conditions (two salt levels), or graph the growth of several microorganisms simultaneously.

Models developed from the combined use of Gompertz function and response surface analysis are well suited for the development of user-friendly applications programs and these have been used with commercially available software (Lotus 1-2-3, Trademark of the Lotus Development Corp.) to develop the Pathogen Modeling Program by the USDA (Buchanan, 1991b). The latest version is available freely on the internet (<http://www.arserrc.gov/mfs/pathogen.htm>).

Another example has been the Food MicroModel, the result of a research project financed by MAFF. In 1994, MAFF granted a licence to Food Micromodel Ltd. jointly owned by the Leatherhead Food Research Association and the software house STD Ltd. to develop and market Food MicroModel software for the personal computer.

VALIDATION OF MODELS

There might be significant differences between

predictions derived in the broth system and actual observations in food because of various reasons. There might be growth-inhibitory or heat-protective factors like organic acids, humectants, etc., which are not accounted for by the model, nevertheless influence the microbial behaviour. The growth condition history of the inoculum or the natural food microflora can affect the subsequent lag phase of the population or the intrinsic heat resistance of cells. Moreover, every step in the model construction process introduces some error and hence model predictions never perfectly match observations. To assess the reliability of models before they are used to aid decisions, a process termed 'validation' is undertaken.

Validation can be carried out on the basis of the same data as the model was set up with, to determine if the model can describe the experimental data sufficiently. This is internal validation, also termed 'curve fitting'. External validation typically involves the comparison of model predictions with analogous observations of inoculated-pack experiments (experiments done with actual food products) not used to develop the model or with values reported in the literature. Internal validation provides an estimate of the "goodness of fit" and shows where additional data are needed. Methods for comparing how well competing models describe the data used to generate them, or for determining whether a fitted model is statistically acceptable relative to the measuring error inherent in the data, have been used in the predictive microbiology literature (Adair et al., 1989; Zwietering et al., 1990 and 1994).

It is also required to test the predictions against microbial behavior in various foods. Models cannot be used with confidence until this validation is done. Growth rates and generation times predicted by the model have been compared to those observed for the same organism in foods (Buchanan et al., 1993; Gibson et al., 1988; McClure et al. 1993; Sutherland et al., 1994; Wijtzes et al., 1993). Graphical methods involve plotting the logarithm of the observed values against those of the predicted values and visually comparing whether these fall above or below the line of identity/ equivalence have been used (Bhaduri et al., 1994; Wijtzes et al., 1993). The distance between a point and the line of equivalence is a measure of the inaccuracy of that particular prediction. Statistical measures like Root Mean-Square Error (RMSE) and regression coefficient, or coefficient of determination (r^2) values were used by Duh and Schaffner (1993) to assess the reliability of predictive equations developed based on measurements in brain heart infusion broth to give values comparable to those in the literature based on measurements in food. These terms

have been described and used to mathematically compare data derived from literature (Giffel and Zwietering, 1999). McClure et al. (1993) compared their models on the basis of the sum of the squares of the differences of the natural logarithm of observed and predicted values.

APPLICATIONS OF PREDICTIVE MICROBIOLOGY AND MICROBIAL MODELS

Predictive microbiology would encourage a more integrated approach to food hygiene and safety and will have an impact on all stages of food production, from raw material acquisition to retailing and handling in homes (Gould, 1989). The field also provides a basis for comparison of data from diverse sources on the growth of microorganisms in foods. It will provide a rational basis for the drafting of guidelines, criteria and standards pertaining to the microbiological status of food (Genigeorgis, 1981).

Whiting and Buchanan (1994) have listed the various applications of microbial models. Microbial models are valuable tools for predicting the growth or survival of microorganisms in foods held under normal or abusive storage conditions. They also aid in the development of hazard analysis critical control point (HACCP) programs by showing what conditions permit growth or survival and thereby identify critical control points. Changes in a food's composition or a new formulation can quickly be evaluated for the pathogen growth or survival potential (Farber, 1986). Tertiary models are valuable educational tools to explain food microbiology principles to non-technical people like food handlers and workers (McMeekin and Olley, 1986; Walker and Jones, 1992). Models can be used to promote efficiency, by designing testing programs and targeting critical areas for research (Gould, 1989). A good review covering the applications of the subject to the dairy industry is presented by Griffiths (1994).

Models with their multiple uses are fast evolving from a subject of research and development into techniques used by the food industry and regulatory agencies in providing food processors, food inspectors and consumers greater confidence in food safety aspects.

LIMITATIONS IN THE MODELING TECHNIQUE

Great caution is required in the use of microbial models as it is questionable that models derived in an experimental system can reliably predict the growth of

the modeled organism in foods. Although there is ample evidence that supports the underlying assumption that a model broth very well mimics a food system (Gibson et al., 1988; Ross and McMeekin, 1991; Wiltzes et al., 1993), exceptions to this assumption are also not unknown (Genigeorgis et al., 1971; Gibson et al., 1987; Raevuori and Genigeorgis, 1975).

Another challenge to the mathematical description of microbial behavior in food is that of microbial interactions which are known to occur in foods but which are seldom taken into account. This is particularly true of fermented foods, which involve lactic acid bacteria capable of producing potent bacteriocins thereby affecting the growth of other bacteria. It has been suggested that these be included in model development (Ross and McMeekin, 1994) and although recent work has addressed these aspects (Jagannath et al., 2001a and 2001b), there is a need to include many more bacteriocins and lactic acid bacteria in the study.

Several workers have also pointed out that models derived in static conditions may not be applicable to fluctuating conditions (i.e., those in which environmental conditions like temperature, pH, gaseous atmosphere and water activity change) during the life of the product (Gibbs and Williams, 1990; Gibson, 1985; Mackey and Kerridge, 1988).

Previous incubation conditions of the test organisms can affect the subsequent rate of growth of organisms (Walker et al., 1990; Fu et al., 1991; Buchanan and Klawitter, 1991). Katsui et al. (1981) have also reported such a history effect on the heat resistance of *Escherichia coli* cells. Fu et al. (1991) termed this, a "temperature history effect" and subsequently other environmental conditions like pH have also been investigated under such history effect.

Hedges (1991) opined that many of the papers in the predictive microbiology literature do not represent real contribution to science because of the empirical nature of many of the models published and that such contributions do not help to elucidate the underlying processes, but merely describe a set of observations. Cole (1991) in reply highlighted the salient uses of predictive models and the power that these models provide for the food microbiologist in decision-making thereby justifying their use.

Most modeling uses a mixture of strains of microorganisms and the growth or survival predicted by the model reflects the fastest growing or hardiest strain in the mixture, respectively. Studies have shown that the growth conditions related to the inocula, like phase of growth (Baranyi et al., 1993), temperature (Gay et al., 1996) and medium used have a significant impact on the subsequent response of the organism.

Significant strides have been made in developing effective models for assessing the effect and interactions of several important variables on the behavior of microorganisms, but few studies have been conducted to study the effect of type and concentration of organic acid on microbial growth (Hsia and Siebert, 1999), the activities of commonly used antimicrobials such as phosphates, sorbates and bacteriocins or the effect of humectants other than sodium chloride. Models employing changing conditions of growth phase and storage are also limited.

PROSPECTS FOR PREDICTIVE MICROBIOLOGY

It has been suggested that models be developed which take into consideration possible interactions among microbial flora present in the product (Griffiths 1994; Ross and McMeekin, 1994). This is especially true of dairy products where lactic starters are used. Antagonistic effects like bacteriocins produced by lactic acid bacteria, preservatives and synergistic effects among organisms have a profound influence on microbial growth and these require consideration in future model development.

Mathematical modeling of fungal growth has not received the degree of interest similar to that which the modeling of bacterial growth has and there is also a need for concerted effort from scientists, food manufacturers and processors to overcome the hurdles faced in modeling fungal growth in foods (Gibson and Hocking, 1997). Spoilage organisms have also not received much attention for development of comprehensive models (Whiting, 1997). Other microbial situations that need microbial modeling are growth in heterogeneous foods, on surfaces or boundaries, in microenvironments and biofilms (Whiting, 1997). Models predicting spore inactivation will also be of immense use to food industries where thermal processing is a crucial step in ensuring the safety and shelf life of processed foods. Construction of such a database from published papers and from experiments performed under defined conditions have been recently attempted (Jagannath, A., Nakamura, I., and Tsuchido, T. A-25 Page 85, Abstract presented at the Annual meeting of the Society of Antibacterial and Antifungal Agents, Tokyo 30-31 May 2002; Nakamura et al., 2000).

The recent foodborne disease outbreaks have highlighted the need for collaborative efforts between the government and the food industry to fund, develop and validate various predictive models for Japanese foods. These data can be included in a large database and made available for public health

assessments and also as research tools.

ACKNOWLEDGMENTS

The first author is thankful to the Japanese Society for the Promotion of Science, Tokyo, Japan for the award of postdoctoral fellowship. This study is also supported by the Kansai University Special Research Fund, 2002.

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