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# Food Safety Assessment

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## Chapter 24

# Predictive Microbiology

### Mathematical Modeling of Microbial Growth in Foods

One of the basic precepts of modern food microbiology is that the growth of microorganisms is a function of the food as an environment. The species most capable of dealing with the environment that a particular food represents will thrive and predominate. Each environment can be considered the integration of a finite number of factors that influence a microorganism's physiological responses. Theoretically, the large number of factors that influence the growth of bacteria in foods could be quantified so that specific information on the growth characteristics of individual foodborne microorganisms would be available for each food. However, consideration of the thousands of different foods eaten worldwide and the high level of biological variation within single foods quickly leads to the realization that such a goal is virtually impossible. Luckily, in most foods the number of factors that are the primary determinants of growth for foodborne microorganisms is limited. If microorganisms' responses to these variables could be derived, their behavior in foods could be estimated. This is the underlying goal of predictive microbiology, a rapidly growing subdiscipline of food microbiology. This includes a primary objective of overcoming the need for an infinite amount of data by determining quantitative relationships between microbial growth or survival and identified primary determinants. The general approach involves the acquisition of data derived under controlled conditions and the use of that information to establish mathematical relationships that can depict the effects and interactions of the variables. The mathematical models derived can then be used to predict how microorganisms are likely to behave in a range of foods based on physical measurements of the primary determinants.

#### Historical

Most successful research on modeling the effects of multiple variables on the growth or survival of foodborne microorganisms has been achieved during the past decade, particularly the development of models related to the growth of pathogenic bacteria. There are a number of reasons for this recent burst of activity, not the least of which is the ready availability of increasingly sophisticated personal computers. However,

attempts to understand and mathematically describe the interactions of various factors have been made throughout the history of microbiology. Much of the early work with microbiological modeling had an emphasis that was not pertinent to conditions associated with foods. There has been extensive modeling of conditions that occur during various industrial fermentations, including the development of a body of equations, such as those introduced by Monod (1), that describe the impact of variables on yield. Fermentation models seldom considered many of the variables of concern with foodborne microorganisms. Further, these models assume a nutrient-limited system that has already reached stationary phase or a steady state, a condition not generally pertinent to the growth of bacteria in food matrices.

The acquisition of data for elucidating the interactions of multiple variables associated with food systems has been underway for several decades, particularly in relation to the determining how the activity of antimicrobials is affected by other parameters. Research characterizing the effectiveness of nitrite in model and cured meat systems was an area of early emphasis due to interest in controlling nitrosamine formation without loss of antibotulinal activity. Nitrite's antimicrobial activity is dependent on its interaction with temperature, pH, water activity, oxygen availability, iron content, etc., and accordingly required the consideration of multiple variables (2-7). Studies of this type provided an understanding of the relative importance of multiple variables, and demonstrated the desirability of modeling techniques. For example, in one of the early applications of response surface analysis techniques to food microbiology, Schroder and Busta (8) demonstrated that only four of sixteen ingredients in a soy-based ground meat analog had a significant impact on the growth of *Clostridium perfringens*. However, little of this earlier research extended beyond limited research applications due to a lack of sufficient databases or effective modeling techniques. Farber (9) has provided an excellent review of the various modeling approaches that were investigated during that period.

The various models that have been developed to describe the growth of foodborne bacteria can be subdivided into two major approaches: probability-based models and kinetics-based models. The choice of approach is largely dependent on the type of bacterium being considered and the impact of growth on the safety of the product. Probability-based models have been usually employed with endospore forming bacteria, particularly *Clostridium botulinum*, where any growth is considered hazardous. Kinetics-based models have been employed more often with non-endospore forming pathogens, particularly those where the microorganism is not considered hazardous until there has been some degree of growth.

### **Probability-Based Models**

Much of the work on probability-based models is similar to that pioneered by Hauschild (10) who attempted to estimate the probability that a single spore of *C. botulinum* would germinate and produce toxin in a food. This approach takes into account the strong effect that cultural conditions have on the germination of bacterial spores. For example, Montville (11) reported that almost all *C. botulinum* spores germinated in a medium with 0% added NaCl and a pH of 7.0, whereas only 1 in

100,000 spores germinated when the salt level was 2% and the pH was 5.5. If the number of spores in a product is low and conditions for germination are non-optimal, the probability that a population of spores includes one that is capable of initiating growth has a large impact on any model for predicting product safety. Other investigators (12-18) have systematically estimated the effects and interactions of various variables on the probability of germination, outgrowth, and toxigenesis of *C. botulinum*. Various forms of regression analysis have been used to model the individual contributions of the variables, providing a series of mathematical expressions that could be used to predict the bacterium behavior in foods. For example, Genigeorgis et al. (17) modeled the effects of temperature, inoculum size, and % brine on the lag time to toxigenesis (which includes time for sufficient growth to yield toxin formation) for non-proteolytic *C. botulinum* types B and E in cooked turkey, deriving the relationship

$$\text{Log}_{10}\text{LP} = 0.625 + 6.710(1/T) + 0.0005(I*T) - 0.033(T) + 0.102(B) - 0.102(I)$$

where, LP = Lag to toxigenesis; T = temperature; I = inoculum size; and B = % Brine.

This model achieved an acceptable degree of agreement between predicted and observed values (Table 1), though the authors concluded that a larger database was necessary for enhanced confidence levels.

The limiting factor for probability based models has been their adaptation for use by non-research personnel. One of the key questions is what is a realistic probability of failure that one should be willing to accept, particularly in relation to potentially fatal intoxications such as those that could occur with *C. botulinum*. Other issues include the level of spores that one could anticipate in products, and translation of the probabilities into values that can be used to set the safe shelflife of a product. This latter question is increasingly being addressed using an integration of probability- and kinetics-based models similar to that employed by Genigeorgis et al. (17) which addressed both the probability of germination and the time to achieve sufficient growth to yield toxin formation.

### **Kinetics-Based Models**

The second major class of models depict the effects of cultural parameters on the growth kinetics of a microorganism, particularly its lag and exponential growth phases. The complexity of the models have varied with the complexity of target food system. Although a variety of factors can influence the growth kinetics of foodborne pathogens, in many instances growth is overwhelmingly dependent on a single prime determinant. For example, the primary determinant of microbial growth in a highly homogenous food such as fluid milk is temperature.

Various models have been developed to depict the effect of incubation temperature on exponential growth rates and/or lag phase durations including the "square root" (19, 20), "linear Arrhenius" (21, 22), and "non-linear Arrhenius" (23-25) models. The "square root" model has been studied extensively, particularly for refrigerated foods.

**Table 1. Comparison of representative predicted versus observed "lag to toxigenesis" for cooked turkey inoculated with spores of Clostridium botulinum**

<i>Temp (°C)</i>	<i>Inoculum (Log cfu/g)</i>	<i>% Brine</i>	<i>Lag to Toxigenesis (days)</i>	
			<i>Observed</i>	<i>Predicted</i>
30	3	0	0.5	0.3
	1	0	0.5	0.5
	2	1.5	0.5	0.6
20	0	0	2.5	2
	3	1.5	1.8	1.3
	1	2.2	2.5	2.5
16	4	0	1	1.2
	2	1.5	2	2.8
	0	2.2	7	5.4
12	2	0	5	4
	0	1.5	9	9
	4	2.2	7	4
8	3	2.2	16	12
	2	0	8	10
	1	1.5	14	17
4	3	1.5	110	101
	0	0	>180	149
	4	2.2	120	95

SOURCE: Based on the probability models of Genigeorgis et al., (16).

For the temperature range below a microorganism's optimum, the relationship is

$$(r)^{0.5} = b(T - T_0)$$

where  $r$  = growth rate constant,  $b$  = slope of the regression line,  $T$  = incubation temperature in °K, and  $T_0$  = notational minimal growth temperature in °K. The latter term is derived by extrapolating the regression line to zero (19). The function is very easy to use once the linear relationship between growth rate and the square root temperature function has been established. Above an organism's optimum growth temperature, its rate of growth declines, and a more complex equation is required (20). This technique has been used successfully to describe the relationship between storage temperature and the microbiological safety or quality of various refrigerated foods (26-30), particularly dairy products. Using a large database depicting *Lactobacillus plantarum* growth in a microbiological medium over a wide range of temperatures, Zwietering et al. (31) assessed various models for describing the effect of temperature on microbial growth. They concluded that two modifications of the Ratkowsky equations were most effective for modeling growth rates and maximum population densities, whereas a hyperbolic function was more effective for lag phase duration. An integrated combination of the three equations was used in conjunction with the Gompertz function to develop a model for predicting the organism's growth curve over its entire temperature range.

Several investigations have extended this approach to develop models describing the combined effects of temperature and water activity (32). A modification of the square root function was used to model the effect of cooling schedules on the potential growth of *C. perfringens* in a meat product (33).

While the above models have been effective in relatively simple food systems, attempts to model more complex systems that are dependent on the interaction of multiple variables have generally used a polynomial or response surface analysis approach (34-38). These approaches employed non-linear regression techniques to generate "best-fit," multidimensional response surface equations that describe the effects and interactions of the variables. This approach to kinetics modeling has been greatly enhanced by coupling it to model equations, such as the logistics and Gompertz functions (35, 39), that can be used to depict growth curves mathematically. Used in conjunction with curve fitting computer routines, these sigmoidal functions allow the growth curve to be described mathematically as a series of coefficients. For example, the Gompertz function describes a growth curve as four values

$$L(t) = A + Ce^{-B(t-M)}$$

where,  $L(t)$  = Log count of bacteria at time (in hours)  $t$ ;  $A$  = Asymptotic log count of bacteria as time decreases indefinitely (i.e., initial level of bacteria);  $C$  = Asymptotic amount of growth that occurs as  $t$  increases indefinitely (i.e., number of log cycles of growth);  $M$  = Time at which the absolute growth rate is maximal; and  $B$  = Relative growth rate at  $M$ .

The Gompertz function has been the one most extensively used due to the combination of its relative simplicity and overall effectiveness (39). Once sufficient databases have been generated, the coefficients (or suitable transformations of the coefficients) of the sigmoidal functions are fitted against the independent variables using either quadratic (36, 38) or cubic polynomial models (37) (Table 2). When effective models are developed, the predicted values of the sigmoidal functions can be used to calculate parameters such as predicted generation times, lag phase durations, or time to reach a designated population density. Fits between predicted and observed values have been satisfactory, providing reasonable estimates of an organism's growth kinetics. For example, a comparison of representative data from Gibson et al. (36), who modeled the effects of temperature, pH and NaCl content on the growth of *Salmonella* (Table 3), indicates that the model provides reasonable predictions of the microorganism's growth rate over a wide range of variable combinations. A similar effectiveness was reported by Buchanan and Phillips (37) who modeled the effect of temperature, pH, sodium chloride content, sodium nitrite concentration, and oxygen availability on the growth kinetics of *Listeria monocytogenes*.

Most response surface models that have been released have been based on experimental data generated in microbiological media (36-38), wherein variables could be controlled rigorously. Although specific databases could be generated for individual commodities, the media-derived, Gompertz-based response surface models provide reasonable "first estimates" of the behavior of foodborne pathogens in a variety of food systems. This is demonstrated in Table 4 which compares predicted values for *L. monocytogenes* (37) against reported values for different commodities. The ability to use media-derived models to predict behavior in foods is an important advantage considering the experimental effort required to acquire sufficient data to generate accurate models when dealing with three or more variables.

Once developed, a key to the successful use of multi-variable models is reducing the calculations to a "user-friendly" form. The USDA/ARS Microbial Food Safety Research Unit (40) recently developed application computer software to demonstrate the potential usefulness of predictive microbiological approaches. The program, which automates the use of response surface models for *Salmonella* spp. (36), *L. monocytogenes* (37), *Staphylococcus aureus* (Smith et al., in preparation), *Shigella flexneri* (Zaika et al., in preparation), *Aeromonas hydrophila* (38), and *Escherichia coli* O157:H7 (Buchanan et al., in preparation), has been distributed widely to food microbiology laboratories in industry, government, and academia. Similar applications software are being developed by researchers in Europe. The development of computer programs of this type must be an integral part of predictive microbiology.

### Concluding Remarks

There is a great deal of excitement among researchers in predictive microbiology as new techniques and findings appear almost weekly and as international teams of scientists begin to share their knowledge and databases. It seems reasonable to predict that the next five years will see the introduction of increasingly more comprehensive

**Table 2. Cubic models for the effects and interactions of temperature (T)(5 - 37 °C), initial pH (P)(4.5 - 7.5), sodium chloride content (S)(5 - 50 g/l), and sodium nitrite concentration (N)(0 - 1000 mg/l) on the aerobic and anaerobic growth of *Listeria monocytogenes* Scott A, using Ln(M) and Ln(B) transformations (37)**

	Aerobic
Ln(M) =	$37.657 + 0.0135T - 13.7331P + 0.4013S + 0.0713N + 0.00372T^2 + 1.9759P^2 - 0.000667S^2 - 0.000007051N^2 - 0.083TP + 0.000842TS - 0.000214TN - 0.1155PS - 0.0167PN - 0.000125SN + 0.0000292T^3 - 0.0935P^3 - 0.00000328S^3 + 0.000286TPS + 0.0000315TPN + 0.0000014TSN + 0.0000175PSN - 0.000384T^2P - 0.00000855T^2S - 0.00000043T^2N + 0.00731TP^2 - 0.0000441TS^2 + 0.00672P^2S + 0.000968P^2N + 0.000294PS^2 + 0.00000062PN^2 - 0.00000016S^2N$
	Degrees of freedom = 308 R2 = 0.967
Ln(B) =	$-47.709 + 0.1631T + 18.6861P - 0.3609S + 0.01N - 0.00161T^2 - 2.7074P^2 + 0.00623S^2 - 0.0000863N^2 + 0.0242TPS - 0.000906TS + 0.000594TN + 0.0671PS - 0.00715PN + 0.000337SN - 0.0000648T^3 + 0.1276P^3 - 0.000029S^3 - 0.000551TPS - 0.0000733TPN - 0.00000033TSN - 0.0000431PSN + 0.000189T^2P + 0.0000549T^2S - 0.00000047T^2N - 0.00222TP^2 + 0.0000459TS^2 - 0.00000002TN^2 - 0.0007781P^2S + 0.000777P^2N - 0.000872PS^2 + 0.0000112PN^2 - 0.00000038S^2N$
	Degrees of freedom = 308 R2 = 0.942
	Anaerobic
Ln(M) =	$89.9195 - 0.5378T - 38.8065P + 1.735S + 0.2175N + 0.00284T^2 + 5.9583P^2 + 0.00962S^2 - 0.000186N^2 + 0.1063TP - 0.00159TS + 0.000397TN - 0.567PS - 0.0574PN + 0.0000813SN - 0.0000321T^3 - 0.3024P^3 - 0.000107S^3 - 0.0000148TPS - 0.0000468TPN - 0.00000118TSN - 0.0000143PSN + 0.000397T^2P - 0.0000126T^2S - 0.00000184T^2N - 0.00964TP^2 + 0.0000487TS^2 + 0.0000001TN^2 + 0.0436P^2S + 0.0038P^2N - 0.000461PS^2 + 0.0000247PN^2 + 0.00000123S^2N - 0.00000001SN^2$
	Degrees of freedom = 211 R2 = 0.974
Ln(B) =	$78.2567 + 0.7928T + 34.3598P - 0.913S - 0.4437N + 0.00218T^2 - 5.3119P^2 - 0.00394S^2 + 0.000233N^2 - 0.2134TP - 0.00174TS - 0.00094TN + 0.3002PS + 0.1272PN - 0.00015SN + 0.0000274T^3 + 0.2693P^3 + 0.0000493S^3 + 0.000442TPS + 0.0000985TPN - 0.00000047TSN + 0.0000304PSN - 0.00104T^2P + 0.00000175T^2S + 0.00000584T^2N + 0.0194TP^2 - 0.0000318TS^2 - 0.00000011TN^2 + 0.0238P^2S - 0.00911P^2N + 0.000215PS^2 - 0.0000298PN^2 - 0.00000068S^2N - 0.00000003SN^2$
	Degrees of freedom = 211 R2 = 0.944

**Table 3. Comparison of representative predicted versus observed times to achieve a 1000-fold increase in the numbers of salmonellae in tryptone soya broth**

<i>Temp (°C)</i>	<i>% NaCl</i>	<i>Initial pH</i>	<i>Time (hr)</i>	
			<i>Observed</i>	<i>Predicted</i>
10	0.82	6.22	176	180
	4.56	6.02	394	372
15	1.33	6.13	41	45
	3.75	5.95	85	70
20	0.77	6.50	14	17
	4.50	5.90	36	38
25	1.32	6.20	11	9
	4.06	6.02	16	17
30	1.32	6.20	10	7
	4.5	5.99	14	17

SOURCE: Based on the response surface models of Gibson et al. (36).

Table 4. Comparison of selected reported growth kinetics values for *Listeria monocytogenes* versus those predicted by the models of Buchanan and Phillips (37)

Food <sup>a</sup>	(°C)	pH	% NaCl	Generation Time (h)		Lag Phase (h)		Reference
				Reported	Predicted	Reported	Predicted	
1. Clarified cabbage juice	30	6.1	0.5	1.6-1.8	0.4	10	3	41
2. Clarified cabbage	30	6.1	2.0	2.2-2.3	0.5	— <sup>d</sup>	3	41
3. Whole milk	10	(6.7) <sup>b</sup>	(0.5)	10	4.4	24	25	42
4. Chocolate milk	13	(6.7)	(2.5) <sup>c</sup>	3.9-4.7	2.1	—	12	43
5. 2% Milk	13	(6.7)	(0.5)	4.4-4.5	2.6	12	16	43
6. Uncultured whey	6	6.2	(0.5)	14.8-21.1	8.9	72	50	44
7. Cultured whey	6	6.8	(0.5)	16.3-17.4	9.4	72	48	44
8. Ice cream mix	9.5	6.4	(4.5) <sup>c</sup>	8.7-13.3	7.5	22-95	33	Smith & Holsinger <sup>f</sup>
9. Whole milk	9.5	6.7	(0.5)	5.2-9.0	4.8	7-12	27	Smith & Holsinger <sup>f</sup>
10. Tryptose broth	4	5.6	0.5	27.1	16.3	144	91	45
11. Tryptose broth	21	5.0	0.5	8.0	1.8	12	16	45
12. Tryptose broth	35	5.0	0.5	1.1	1.1	6	9	45
13. Tryptose broth	35	5.6	0.5	0.8	0.5	3	3	45
14. Tryptic soy broth	4	7.0	0.5	33.5	14.7	—	68	46
15. Tryptic soy broth	13	7.0	0.5	4.8	2.8	—	17	46
16. Tryptic soy broth	35	7.0	0.5	0.7	0.5	—	1	46
17. Tryptic soy broth	30	4.7	0.5	6.2	1.7	—	19	46
18. Tryptose phosphate broth	4	5.6	0.5	26.4	16.2	144	91	47
19. Tryptose phosphate broth	4	5.0	0.5	NG <sup>e</sup>	29.9	NG	144	47
20. Tryptose phosphate broth	13	5.0	0.5	8	5.4	12	38	47
21. Tryptose phosphate broth	21	5.6	0.5	1.8	0.9	6	8	47
22. Tryptose phosphate broth	35	5.6	0.5	0.8	0.5	3	3	47

<sup>a</sup>All food samples were assumed to be aerobic.

<sup>b</sup>Parentheses indicate that value not given and had to be assumed.

<sup>c</sup>An elevated value was assumed to estimate effect of ingredients other than NaCl that would affect the water activity of the food system. The ice cream mix samples had measured  $a_w$  (10C) values of 0.930-0.956.

<sup>d</sup>Value not reported.

<sup>e</sup>No growth.

<sup>f</sup>Unpublished data

computer-based models and expert systems applicable for a range of food products. These techniques should be a boon to food microbiologists, allowing them to quickly explore the microbiological impact of varying conditions within a food. This new area of 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 a product, instead of attempting to infer these attributes after the fact using end product testing.

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