

Predictive Microbiology - Quantitative Microbial Ecology
Culture – March 2004

Dr J. Baranyi¹ and Dr T.A. Roberts²

¹ Biomathematician, Institute of Food Research, Norwich, UK

² Food Safety Consultant, 59 Edenham Crescent, Reading, RG1 6HU, UK
(formerly Head of Microbiology, Institute of Food Research)

Introduction

An area of food microbiology has come to be known as "predictive microbiology" in the last two decades. Checking the search program of the Web of Science (<http://wok.mimas.ac.uk/>), hundreds of papers were published with the keyword "predictive microbiology" in the last 10 years, and the number is steadily increasing.

So what science does "predictive microbiology" cover exactly? In the first book on the subject, published just over 10 years ago, McMeekin et al. (1993) defined it as a quantitative science that enables users to evaluate objectively the effect of processing, distribution and storage operations on the microbiological safety and quality of foods. Later, the same authors used the expression "Quantitative Microbial Ecology of Food", which is a somewhat more generic description. The most recent book on the field (McKellar and Xu, 2003) puts more emphasis on the need to describe the microbial responses to the food environments by mathematical models. The evolution of predictive microbiology into a more and more exact science is well illustrated by this shift of emphasis in its definition.

Food microbiology has adopted modern methods and novel concepts with some reluctance. Many food microbiologists follow the "old fashioned" approach of enumerating microbes at different stages of food storage, identifying the major fractions of the microflora by their phenotypic characters, and gradually building up an understanding of the shelf-life and safety of foods. However fascinating this is to the dedicated food microbiologist, it is slow and expensive, and has not led to a cumulative, structured database of information that can be interrogated quickly.

Study of the effects on microbial growth of single controlling factors such as temperature, pH or water activity, resulted in acceptance that particular microbes of concern would not grow below certain temperatures, or below a certain pH value or water activity. Some scientists recognised that other factors were important e.g. the composition of the atmosphere above the food, preservatives, food structure, but the experiments needed to cover the effects of all those factors appeared enormous and beyond the scope of individual food microbiologists.

Not until the problem was viewed from another perspective was progress made. All foods contain water, have a pH value and a temperature of storage. If the growth response determined by those "controlling factors" could be measured, then modelled, the result would indicate how much growth could be attributed to those three factors. If the differences between the calculated and observed responses were significant, other factors would have to be taken into account.

Comparisons of growth rates published in the scientific literature with “predictions” from such relatively simple models for the same conditions of pH, temperature and water activity were often surprisingly close and encouraged further efforts.

Gradually, using models that had been validated by comparing outputs with independent data became recognised as just as reliable as accumulating results from the scientific literature or spending weeks generating more microbiological data. Occasionally it is important to have an “accurate” estimate of the growth/survival, but more often it is sufficient to have a “reasonable” estimate, but quickly. It is necessary to obtain quick and “good enough” estimations of the shelf-life of foods, in which pathogenic bacteria might grow, in new product development and in risk assessment..

History

Predictive microbiology started as a purely empirical (though quantitative) science. Its earliest appearance is probably Esty and Meyer (1922), who described the thermal death of *Clostridium botulinum* type A spores by a log-linear model, which is still used to estimate the necessary heat processing of low-acid canned foods. This model simply says that, at a given temperature, the *relative* (or: *specific*) death rate of the bacteria is constant with time. In other words, the percentage of the cell population inactivated in a unit time is constant. This is a simple, logical and understandable model, similar to those commonly used in physical and chemical sciences for processes such as dissipation, diffusion, etc, when the force that causes the decrease of a certain quantity is constant with time.

A step forward was taken by Scott (1936), who investigated how the specific death rate depended on the available water, quantified today by the so-called water activity, a dimensionless number between 0 (dry) and 1 (wet). He subsequently studied the effect of the temperature on the specific microbial death rate. Today the most frequently assumed relation in thermal inactivation theory is that the logarithm of the specific death rate decreases linearly as the temperature increases (this is equivalent to the so-called constant z-value theory).

“Classical” predictive microbiology

The above two-step approach to develop predictive models is still in use, and not only for death but also for growth curves. Commonly, the first step in the developmental procedure is to establish the growth/death model in constant environment (primary model); the next step is to determine, how the parameters of the primary model are affected by environmental factors (secondary model – see Fig.1).

While it is accepted that in “smooth” cases, the bacterial population should die/grow at a constant specific rate, several complicating factors arise, even in a constant environment. In both situations, the prior history of the cells affects the transition period, during which bacteria arrive at the exponential phase. In the case of death curves, this is frequently referred a “shoulder”; with growth curves it is called the “lag” (see Fig 2a). In most circumstances limited information is available about the pre-inoculation period, with no satisfactory solution to modelling these transition periods.

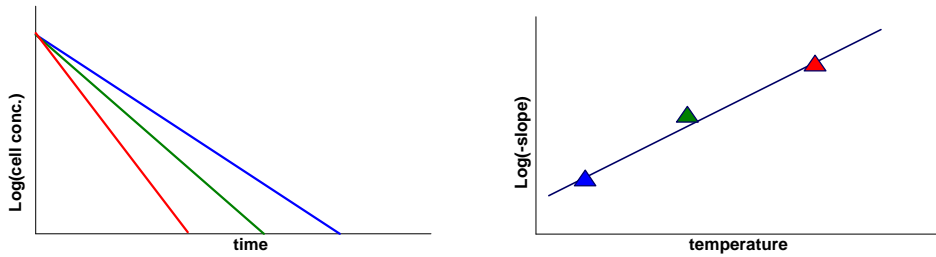


Fig. 1. Primary and secondary thermal inactivation models.

Fig1a. Constant specific death rate: the logarithm of the population size linearly decreases with time; the slope is a function of the temperature.

Fig 1b. Constant z-value theory: the logarithm of the specific rate changes linearly with temperature. The slope of this linear function is characteristic of the microorganism.

Another problem is the post-exponential phase, which is the stationary phase for growth curves (the bacterial population reaches the maximum carrying capacity of the environment) and the so-called “tailing-off” or “tails” with death curves. For growth curves, the problem is not significant from a practical point of view, since the food is inedible by the time the microbial load reaches the maximum population level. However, researchers still do not agree whether “tailing-off” phenomena sometimes observed in thermal inactivation is real, or just experimental artefacts. The problem is that the tails occur at low cell concentrations, where the measurements are unreliable and inaccurate - often around the detection level threshold.

The transitional phases were first described by commonly used sigmoid functions, (see Fig.2a), as an empirical approach to primary modelling. The effects of the environment on the parameters of the primary models were then described by secondary models, usually simple, empirical, multivariate polynomials. The most frequently quoted paper in this respect is that of Gibson et al. (1988), which has since been cited more than 200-times, according to the Web of Science (WoS). That paper used the sigmoid function of Gompertz for the primary model and a quadratic polynomial for the secondary model. The fitting performance of the Gompertz function was also found to be the best by another frequently cited paper, Zwietering et al. (1990) (at January, 2004, its WoS citation index number was 360). This contributed to the fact that, until the mid-90s, the Gompertz function was the most popular to fit sigmoid bacterial growth curves.

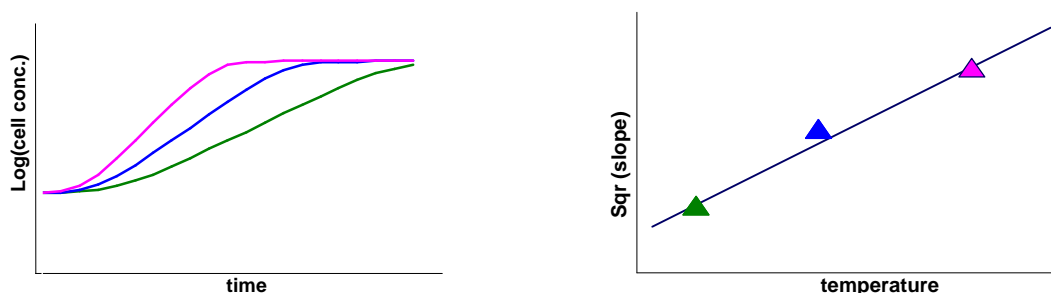


Fig. 2. Primary and secondary growth models.

Fig 2a. Constant maximum specific growth rate: After the lag, in the exponential phase, the logarithm of the population size increases linearly with time, until the stationary phase.

Fig 2b. Square-root model: the Square root (spec.rate) increases linearly with temperature. Notice the analogy with Fig.1

It is generally agreed that the most important environmental factor determining growth is the temperature, followed by the pH and water activity; followed by preservatives, antimicrobials and the composition of the atmosphere. However, while the temperature is controllable during the storage of the food, the other environmental conditions are not. Furthermore, they can be changed by the growing bacteria, and they can affect each other (interactions). This increased the need for dynamic models, when the constant environment would just be a special case (zero-variation) of the general scenario, when the environment can change with time. Another aim was to use more mechanistic models, i.e. to describe the mechanism behind the observed process by models based on laws of fundamental sciences; as opposed to the empirical models driven primarily by data-fitting. The drives for dynamic and mechanistic features in fact strengthened each other, since most mathematical models of various physical, chemical and biological kinetic systems are dynamic (differential) equations: giving the *direction* or the *rate* of the system as a function of the *state* of the system.

To be fair, purely mechanistic models are very rare in practical applications. Models in daily use are, in fact, between the two, using mechanistic elements when possible and completing them with empirical approaches when only observations are available.

A quest for mechanistic foundations

Baranyi and Roberts published three papers (1993, 1994, and 1995) that gave a good mathematical basis for mechanistic modelling of the lag phase. According to its WoS citation index, the Baranyi-model has subsequently been cited in more than 300 papers, and has become the most widely used primary growth model. Though it is useful to fit various (semi-) sigmoid curves (linear phase preceded and/or followed by stationary phases) the main step in the model was its dynamic origin. Namely it describes the transition phases, for either the growth or death situation (see Baranyi and Pin, 2001), in a way that can be also used for a fluctuating environment.

By the 1990s, the square-root (secondary) model of Ratkowsky (1983) had become the most popular to describe the effect of temperature on the specific growth rate (Fig. 2b). Others included the Arrhenius model and its variations, and the Cardinal Temperature model of Rosso (1995). The problem with this approach was that there was no straightforward extension from temperature alone as a single controlling factor to multivariate situations that would retain the good qualitative properties and performance of the original “growth rate vs. temperature” models. Consequently many authors stayed with the simple multivariate polynomials, even when the question was the combined effect of several factors on growth. In fact, all the secondary models were dominantly empirical.

Another direction for the mechanistic basis was the wish to relate the kinetics of the whole population to the physiology and kinetics of individual cells. Baranyi and Pin (2001) gave a mathematical theory how to connect stochastic process models for individual bacteria at the single cell level and a deterministic model for the population level; i.e. how to conclude the behaviour of the population as a whole from observing many individual cells. That theory was recently validated experimentally by Elfving et al. (2004) using a flow chamber and an automated image analyser to enable

observation of divisions of thousands of single cells, and to derive statistical distributions for them. The significance of this technique is that not only kinetic parameters could be characterised by a secondary model, but also their variability, which is vital for quantitative microbial risk assessment.

Unifying efforts for a single database of microbial responses to food environment

Predictive microbiology received a big impetus when the UK Ministry of Agriculture Fisheries and Food initiated, in 1988, a coordinated programme on growth and death of bacterial pathogens, collecting and computerising data in a standardised way. Those collected data served as the base on which the first validated, commercialised programme package, Food MicroModel™ was built. The task of supporting these developments was taken over, when established, by the UK Food Standards Agency (FSA). The FSA, in 2003, released all the data behind the Food MicroModel and funded the development of a program called Growth Predictor, by the Institute of Food Research. The program is freely available today at (www.ifr.ac.uk/Safety/GrowthPredictor). It is the result of a re-modelling effort on all the available growth data (mainly on bacterial pathogens), utilising the scientific developments of the 1990s.

Parallel to these events in the UK, the US counterpart of Food MicroModel™, called PMP (Pathogen Modelling Programme: www.arserrc.gov/mfs/pahogen.htm) was developed at the Eastern Regional Research Center of the USDA Agricultural Research Service. Soon, the coordinators of these research centres and funding agencies on the two sides of the Atlantic recognised that a common, joint, database and unified models would be beneficial for everybody. This is how ComBase, the Combined Database of Microbial Responses to Food Environments (see www.combase.cc) started its life. It is now an internet-based, publicly and freely available database, for research and training/education purposes, for food microbiologists, manufacturers, risk assessors and legislative officers. The original Food MicroModel™ and PMP datasets have been supplemented with additional data submitted by supporting institutes, universities and companies; as well as by data compiled from the scientific literature. Under the funding of the European Union, many EU institutions are also adding their data to ComBase. As written by McMeekin (2003), “*Properly supported, ComBase will be a watershed in the evolution of predictive modelling and its widespread applications*”. Fig.3 shows a query and output screen of the stand-alone version of the ComBase-browser program.

Although collaboration began as an academic exercise, having a single database of information and joint models offers huge benefits to assuring the safety of foods in international trade.

Future

In the state-of-the art summary of our current predictive microbiology knowledge (McKellar and Lu, 2003), readers can find a comprehensive picture of the direction the subject is expected to continue and what is likely to change. The classical primary-secondary model approach will almost certainly be restricted to “smooth” cases, when the microbial population is more or less homogeneous, the population density is high enough to use deterministic models and there are no significant interactions between the environmental factors. Progress is expected in the area of

- *dynamic modelling*: interaction between bacteria and environmental factors;
- *lag modelling*: by means of quantifying and modelling the effect of history via the actual physiological state of the bacteria;
- *growth / no growth boundaries* for bacteria and environment, *probability of growth*: for answering the question “what is the probability that the microbial load is over a specified value, at a specified time ?” (for Quantitative Microbial Risk Assessment purposes);
- *more advanced quantification of the structure of the food environment*;
- *modelling individual cell kinetics by stochastic birth/death processes*: Connecting deterministic modelling at population level to statistical assessment and variability characterisation at single cell level;
- *relating predictive microbiology and molecular microbiology*: using data on how genes are switched on as function of the (dynamically changing) environment; characterisation of variability and stress-tolerance;
- *computational microbiology and bioinformatics development*: data storage and retrieval in a more advanced way.

These tasks require the interdisciplinary collaboration of food microbiologists and mathematicians; food technologists and computing scientists; molecular microbiologists and statisticians.

Just 20 years ago very few food microbiologists believed that models of microbial growth and death would ever be sufficiently reliable to be used in the food industry, or by food regulators. From the early empirical models, a new generation of modelling approaches, together with international collaboration, have opened the door to the possibility of predicting growth and death properties for the key microorganisms in food.

Acknowledgement

J.B. thankfully acknowledges the support of the UK Food Standards Agency (project no: FS 3113) and the European Commission, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 on Food, Nutrition and Health; Contract n°QLK1-CT-2002-30513 (e-ComBase).

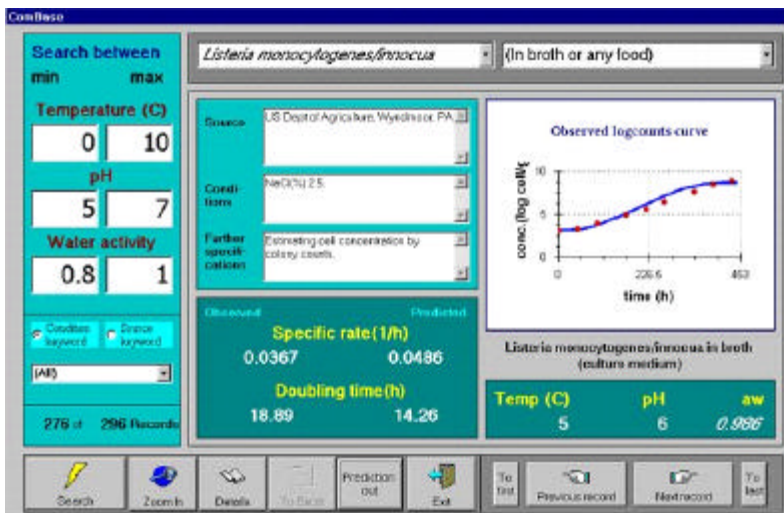


Fig.3. Query and answer screen produced by the stand-alone (not internet-based) version of the ComBase browser. It shows that, at storage temperatures between 0 and 10°C, altogether 296 records were found on the microbial responses of listeria, with pH between 5 and 7, and water activity between 0.8 and 1. This particular “record 276” shows a growth curve measured at 5°C, pH 6, and $a_w = 0.986$. The raw data (red dots) can be compared with prediction (blue curve) generated by Growth Predictor.

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