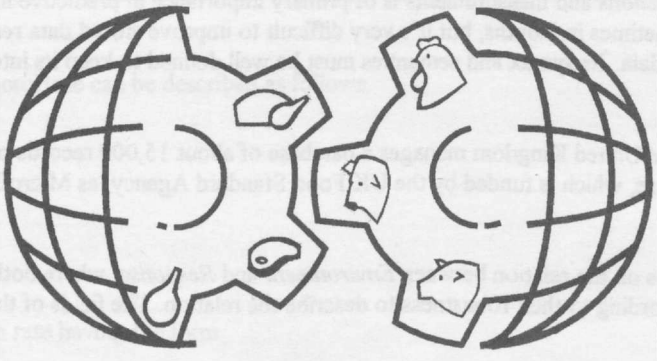
Session 6.II

Meat safety

46th ICoMST



Argentina, 27 August - 1 September, 2000

PREDICTIVE MICROBIOLOGY FOR THE MEAT INDUSTRY

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Background

Food microbiology has been applying mathematical concepts and techniques at an increasing rate. This is partly due to the demand to analyse microbial data, partly due to the dramatic development of the mathematical, computational means to process those data, improving in both quality and quantity. With the advent of desktop computers having greater power than top-range mainframe computers one or two decades ago, it has become possible to solve large and complex numerical problems with sufficient accuracy. This is especially important in food microbiology where, because of the inherent bio-diversity, observations and measurements are generally less accurate than in physical sciences.

According to the definition of Ross at al (1999): *"Predictive microbiology is concerned with the quantitative microbial ecology of foods ... (i.e.) with growth, survival and death of bacteria and fungi in foods. Its basic hypothesis is that the responses of populations of micro-organisms to environmental factors are reproducible and that, by characterising environments in terms of those factors that most affect microbial growth and survival, it is possible, from past observations, to predict the responses of those micro-organisms in other, similar, environments. This knowledge can be described and summarised in mathematical models which can be used to predict quantitatively the behaviour of microbial populations in foods, e.g. growth, death, toxin production, from a knowledge of the environmental properties of the food over time. "*

These indicate that mathematical modelling and computational techniques are going to play a key role in the future improvements of the microbiological safety of meat. In what follows, some aspects of this development will be discussed.

Objective

A ready-to-use predictive model is based on a database of observations and a set of suitable mathematical models:

DATABASE ----------
→ PREDICTIVE SOFTWARE
MATHEMATICAL MODELS ------

In what follows, we show the typical features of the process of creating and validating predictive models of microbial growth in food and demonstrate their overall performance in meat products.

Methods

1. DATABASE
A well-organised database of observations and measurements is of primary importance in predictive microbiology. Mathematical models can (and does) improve, sometimes in months, but it's very difficult to improve stored data retrospectively.
A database is not merely a dump of data. Its syntax and semantics must be well defined to keep its integrity. Below an example is given for such a database.

The Institute of Food Research in the United Kingdom manages a database of about 15,000 records on microbial responses to food environment. We refer to this database, which is funded by the UK Food Standard Agency, as MicroBase. Its structure can be summarised in a scheme as follows:

The database consists of observations on the relation between *Environment* and *Response*, where both sides are characterised by primary and secondary variables according to their robustness to describe the relation. The fields of the database can be divided into four major groups:

a) Administrative fields (source of data; organism observed; measurement method; observation time; etc)

b) Environment

- Primary environmental variables (temperature; pH; a_w);
- Secondary environmental variables (CO_2; additives; etc);
- Non-quantified environmental variables (food/culture media; its composition; initial physiological state of the cells; etc).

c) Response

- Primary response variables (cell concentration profile during the observation time; maximum specific growth/death rate or doubling time/D-value; variation of cell concentration during the observation time or time to reach a certain change in cell number; probability of growth; etc)
- Secondary response variables (toxin production; competition parameters; etc)
- Non-quantified response variables (growth/no growth; survival/no survival; etc)

d) Quality comments (error estimation; expert comments; etc)

Temperature, pH and water activity are the main environmental factors to determine the response of an organism (McMeekin et al, 1993), this is why these variables are among the primary variables of the *Environment*. Note that a dynamic environment (when one or more of the quantitative variables change with time during the observation) can also be recorded in MicroBase. In that case, it is not a single quantity that is recorded for the respective environmental variable but the name of the table storing the environmental profile in time.

The secondary variables are, most commonly, inhibitory factors. Together with the non-quantified variables, their possible number can be quite significant (in MicroBase it is 40) but a given study commonly aims to observe the effects of only a few.

The viewpoint according to which a response variable is primary or secondary is similar to that seen at the environmental variables. Commonly, the maximum specific growth/death rates or their equivalents, doubling times/D-values, are the most characteristic of the studied environment-response relation, and generally it is also easier to model these quantities.

Just as a dynamically changing (time-dependent) environmental profile, also a response profile, such as a whole growth or death curve, can be recorded in MicroBase. To be exact, growth and death parameters are estimated values extracted from those profiles, anyway.

Frequently, it is difficult to decide (and even non-scientific considerations, such as economical or equipment-related issues can influence) what environmental factors and what response observations should be recorded at all. By computerising and modelling, therefore categorising the *Environment* and *Response* variables, some loss of information is inevitable. However, even with this loss, a well-organised database with sufficient amount of records is invaluable because of the fast access to the data and because of the possibility to build mathematical models. Those models can be used to predict situations on which there are no records available.

2. MATHEMATICAL MODELS

A mathematical model is a set of simplifying assumptions that disregards negligible details of the phenomenon to be described. Frequently, models differ only in what extent they omit certain details. There is no recipe for deciding what can be neglected.

Mathematical models are frequently classified as mechanistic and empirical models. The solution to practical problems, however, is always between the two. The simplest models used in predictive microbiology, such as multivariate polynomials, are empirical models and aim at the smooth representation of computerised microbiology data.

When both the environment and the response variables are *dynamic* (the measured quantity changes with time), the correct mathematical model, too, must be dynamic, described by differential equations. This point is frequently missed in current research on predictive modelling.

The growth model built around MicroBase can be described as follows

$$\frac{dx(t)}{dt} = \mu(t)\,x(t)$$

where

$x(t)$: cell concentration;
$\mu(t)$: instantaneous specific growth rate having the form

$$\mu(t) = \alpha(t)\,\mu_{max}(D(t))\,u(t)$$

where

$\mu_{max}(D)$: potential maximum specific growth rate characteristic of the D environment;
$D(t)$: time-dependent *vector* of extracellular conditions (temperature, pH, etc.);
$\alpha(t)$: monotone increasing adjustment function with values between 0 and 1. Its initial level ($\alpha_0 \leq 1$) is determined by the history of the cells until the inoculation. It characterises the transition from the lag to the exponential phase;

u(t) : monotone decreasing inhibition function with values between 0 and 1. It characterises the transition from the exponential to the stationary phase.

This growth model, because of the dynamic background, is suitable to predict bacterial kinetics under environments changing with time (Baranyi and Roberts, 2000). This is an important advantage. For example, a newly developing technique, called TTI (Time Temperature Integration) applies dynamic models (Taoukis et al, 1999).
In MicroBase, the internal function $\mu_{max}(D)$ is approximated by empirical multivariate polynomials. A basic, three-variable quadratic polynomial describes how the primary environmental variables, temperature, pH and water activity, affect the maximum specific growth rate. Other, secondary environmental variables only modify the basic model, *i.e.* if an additive is 0, we get the basic model back (Masana and Baranyi, 2000). This approach is summarised in Fig1.

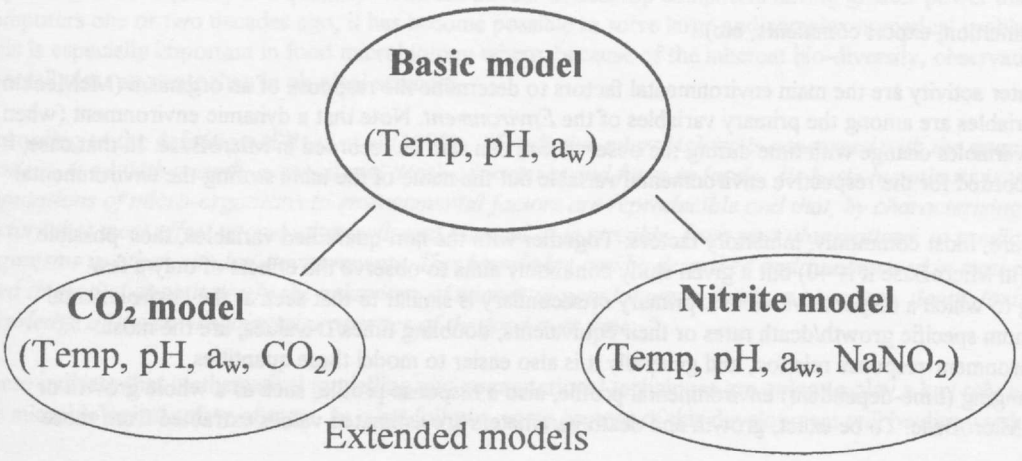

Fig1. Modelling the effect of secondary environmental variables: extending the basic model.

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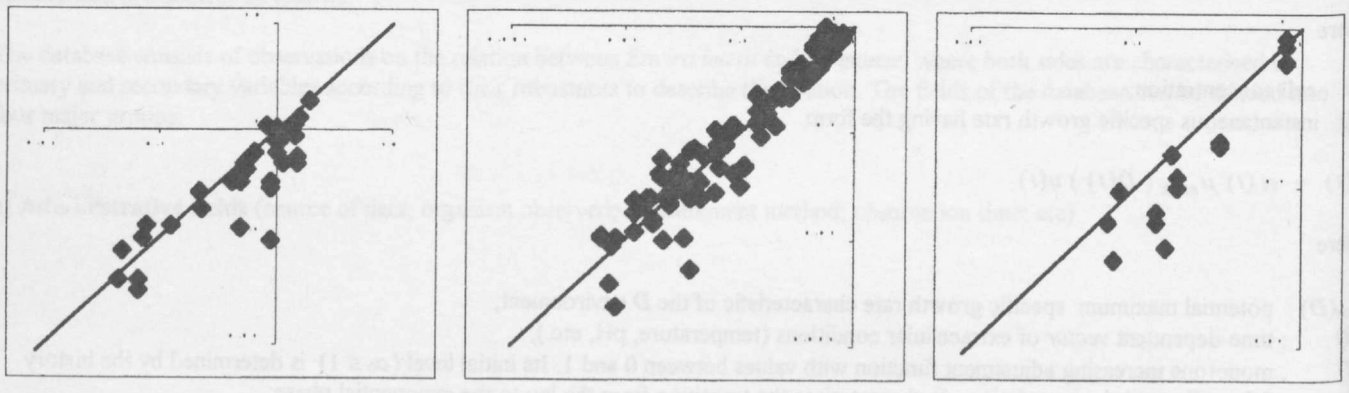
The model parameters are estimated from growth data obtained in culture media. (Regarding other computational aspects of fitting and model creation, see Baranyi and Roberts, 2000). Therefore, this is a worst-case-scenario approach because, apart from the pre-set environmental variables, the conditions are set to be optimum for growth. This way, when validating a growth model in food products, the predicted growth is expected to be higher than observed in food. This is demonstrated specifically for meat in what follows, meat being one of the main targets of predictive models.

Results

In MicroBase, about 6,000 records are derived from publications on the responses of various pathogens to different combinations of environmental conditions observed in food. When a published specific growth rate is plotted against its predicted values, the closer the resultant point is to the line of equality, the more accurate is the prediction. The set of points obtained this way is called the validation plot. For demonstration, three pathogens are selected and their validation plots in meat is shown in Fig2. Observe that whenever the error of a prediction is relatively big, it is always on the safe side. This is, as mentioned above, a consequence of the worst-case-scenario approach.



Altogether, the following rough picture can be drawn as an assessment of the predictive models currently in use. In growth-supporting environment, the maximum specific growth rate can be determined with 5-10% accuracy. That means that the error margin of a measured rate is about 5-10% of the measured value. This is usually confirmed by the standard error of fitting when we regress the logarithm of the maximum specific growth rates against temperature, the most dominant environmental factor.

When modelling the combined effect of other environmental factors, each further factor increases this error by another 10% . This means that the basic three-factor models, describing the effect of temperature, pH and water activity on the specific rate, perform at around 30% accuracy. Because of the worst-case-scenario design, it is almost excludable that, for example, the observed doubling time of an organism would be 2-3 times less than predicted by a three-factor model based on sufficient amount of data. (However, it can be 2-3 times bigger, depending on the specific food). Note, however, that these estimations are very rough, averaged out indicators; the exact figures can vary significantly, depending on the studied organism and the region of environmental factors.

Future

The models discussed in this talk belong to the family of so-called deterministic models. These are useful to describe the behaviour of a culture only if the cell number is relatively big. Crucial questions of food safety studied at low cell concentrations, such as lag time, probability of survival and growth, should be modelled by stochastic processes. The validation of stochastic models describing the variation of individual cells, however, requires different measurement systems.

An simulation study is given below to demonstrate the situation where deterministic models are not suitable and stochastic approach is necessary.

Suppose that during heat inactivation, the resistance of a cell population remains homogeneous, i.e. the survival time for each cell follows the same (exponential) distribution. Therefore, the concentration of cells decrease exponentially. The output of a simulation program, with parameters characterising the inactivation process of *E.coli* at around 65°C, shows the "real" inactivation curve as square symbols in Fig3.
The measurements, in this thought-experiment, are made by sampling where the sample contains 10% of all the cells. In Fig3, triangles represent the measured cell concentrations. The star symbols at logcount=-1 represent measurements when no cell was detected, though there were still cells alive (so the stars are "unvisible" for the observer).
If a deterministic model is fitted to the measured counts (see the curve fitting the triangles), one can easily "cry wolf", i.e. can find tailing in this inactivation curve. The tailing shape, however, is a simple consequence of the fact that the effects of biovariability and measurement error are much more significant at low cell number than at high population level. In fact, it is impossible to decide from a few measurements, if the tailing is real or not. True replicates are necessary at low concentration to validate models of the variability of single cells.

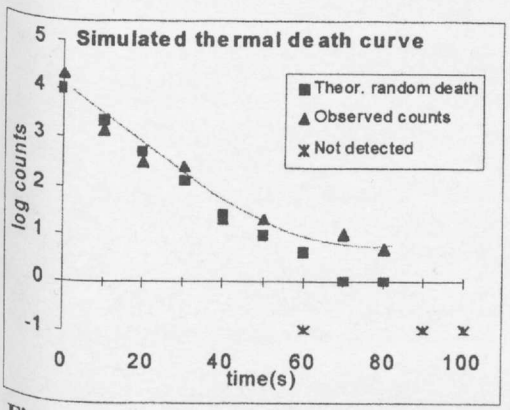


Fig3. Simulated death curve of a cell population with homogeneous resistance and with measurement (sampling) errors. The apparent "tailing" (fitted to the triangles) is not real, only a consequence of randomness having greater significance at low cell numbers.

It can be expected that the future will direct us to seek single cell measurements in order to characterise the distribution of individual kinetic parameters. Then, stochastic mathematical models can be applied to study probability of survival and growth. It is important to see that no conclusion can be drawn from the variance of kinetic parameters determined at population level to the variance of the respective individual kinetic parameter. For example, as Baranyi (1998), and later Baranyi and Pin (1999) pointed out, the individual and population lag are different concepts and the consequences of this difference are far-reaching.

In order to further improve predictive food microbiology, it is expected that automated measuring systems will be developed, able to detect properties of individual cells and to produce large amount of data on repeated observations in order to study the variability of the cell population . Image analysis of colonies generated by single cells, turbidity measurements of populations as successors of single cells, flow cytometry are feasable candidates to perform such measurements.

Conclusion

Further fast developments can be expected in food safety research based on using computational means more extensively and intensively. Microbiological data are to be made suitable to computerised storage (science of Bioinformatics, which is unfortunately meant today mainly for gene banks), and the applied mathematical tools will be more and more sophisticated, helped by the dramatic increase of the computing speed and more and more user-friendly and convenient software packages. This "computational microbiology" is no doubt going to play significant role in helping to ensure the microbiological safety of food.

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