SESSION 6

MICROBE CONTROL IN MEAT

6-L1

COMPUTATIONAL MICROBIOLOGY AS A TOOL FOR MICROBIAL CONTROL DURING MEAT PROCESSING

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Introduction:

It has recently been estimated that approximately 25% of the world's raw agricultural products' volume is lost as a result of microbial activity (Huis in't Veld, 1996). Similarly, the economic impact of food contamination with pathogenic and spoilage organisms on the global food *trade* is regarded as substantial, although less easy to quantify (Buzby and Roberts, 1997). Of the various foods, meat and meat products are of major concern. For instance, the human morbidity rate attributable to meatborne pathogens in the US has been estimated to be 5×10^5 *per annum*, of which approximately 10^3 were fatal (FSIS, 1996).

Various microbial agents may cause muscle foods to deterioriate or become hazardous for public health. Within the framework of this presentation, we will focus on bacteria as records indicate they cause most of foodborne diseases of *known* etiology (e.g. at the beginning of this decade 73%-100% of cases in Europe were attributable to bacteria (Todd, 1997).

Until fairly recently, microbial control strategies were largely based on empiricism and/or preconceptions ('educated guesses'). Although this traditional approach has, in some cases, had a certain degree of success, microbiologists the world over are now increasingly recognizing the potential of exclusively basing safety strategies on sound mathemathical principles ('computational microbiology'). None of the authors of this script are mathematicians. Nonetheless, we hope to illustrate that - even when relying on relatively simple mathematical functions - microbial behaviour can be described with reasonable accuracy. Such does not only save considerable time and effort, but also allows for better prediction of safety and quality aspects of muscle foods under a range of processing conditions prevailing in today's meat industry. For this purpose we will refer to classical examples - based on experience and tradition - as well as to more recent 'computational' approaches.

End-product control:

The traditional way of assessing the safety and stability of meat and meat products has been 'endproduct-oriented' i.e. by inspection at the end of the slaughterline and by testing of further processed muscle foods. This was justifiable at times when only limited knowledge of the history of the product or the process was available. Evidently, such an approach suffers from inherent shortcomings such as the significant additional time- and money investments involved in such testing. More importantly, in most cases, 100% assurance is only achieved provided *all* products are tested and this is only feasible when a non-destructive test, which does not alter the sensory food properties, is available. Yet, such an approach is totally unpracticable. For instance, storing and checking all items of a batch of canned meat for bulging or liquefaction is unrealistic, while testing only one (or few) representative sample(s) cannot possibly guarantee the safety of the whole batch. Although additional testing (e.g. of raw materials and intermediate products at various steps of production) may increase the performance of endproduct control, the shortcomings essentially remain.

The implications of this statistically unsatisfactory argument were extensively discussed in a book issued by the International Committee on the Microbiological Specification for Foods (ICMSF, 1986). Some of the main problems associated with endproduct-based control as recognised by ICMSF were: a) the need for a clear definition of a 'batch' and a 'representative sample', b) the need to significantly increase the number of samples tested before one can - with some confidence - claim to have generated a 'safer' product, and, c) the need to attune the sampling plan [number of samples, limits, accept/reject (so- called two classes plan) or good/marginal/unacceptable decisions (3 classes plan)] to the possible hazard. Even if the number of test samples is increased, the probability of *finding* a defective, when the percentage of defective products is < 1%, is very low (ICMSF, 1988).

In a nutshell, ICMSF's approach is that quality must be built-in in a product during production, rather than inspected by determining non- conformities at a stage when corrective measures are no longer possible. This means, firstly, that *the process* rather than the product must be the primary target of control actions (because control- and documentation of process parameters make product testing largely obsolete) and, secondly, that of the variety of process factors, first and foremost the 'critical' ones need to be

kept 'under control' (*vide infra*).

Intervention strategies

Single-point intervention:

Unfortunately, it is not always obvious which steps are critical for product safety and to what extent. Also, with the possible exception of processes like irradiation or *lege artis* thermal treatment of low - acid canned foods (obviously assuming suitable package integrity is in place), there are practically no options for single-point intervention that allow sufficient microbial safety assurance of a food product.

The implications of switching from a 'black box' approach to intervention measures based on adequate understanding of the process can be illustrated using canned (muscle) foods as an example. Steering of the microbiologial events during and after canning followed the discovering and description of the linear relationship of logarithmic microbial counts and heating time. This major first step, generally known as the D-value concept, is valid for fixed temperatures only. In industry practice a sterilisation process is rarely achieved at a constant temperature. Therefore, to describe the effect of changing temperature, z-values were introduced. Now the total amount of thermal energy applied to a can during the heating-holding-cooling cycle could be calculated. When lower temperature limits for microbial inactivation were set, the total microbicidal thermal effect could be described as a sort of weighed time-temperature integral, known as the F-value. This process of refinement of parameters provided a better description of microbial inactivation in canned meats. It thus allowed defining the effects of complex thermal regimes on microorganisms as one distinct value, which is easily compared to that of other possible regimes. Although purely descriptive, this approach has proven to be a most valuable tool for the prediction of safety of canned foods (McClure and Roberts, 1992).

Multiple-point intervention:

When discussing the various points of intervention in the meat industry, those approaches deserve particular attention that can be easily integrated in meat processing as we know it. It is useful to group the proposed actions into measures aiming at: a) prevention of microbial contamination, commonly called Good Manufacturing Practices (GMP's or 'hygienic practices') and b) those effecting removal or inactivation of microbes, or interrupting their metabolism. In the latter category, the methods may represent either an integrated part of the process - which, for instance, applies to curing, salting, drying and heating - or a distinctly separate (often terminal) 'microbial decontamination' step.

Considering the design of most meat (or meat product) processing lines, single- point intervention approaches are generally not effective. A number of steps are critical and often the various options available to keep them 'under control' are delicate, not always fully effective or widely (scientifically or politically) accepted. Decontamination of muscle foods by treatment with physical or chemical agents belongs to the latter category (Smulders and Greer, 1998).

This realisation has led to the development of modern safety assurance relying on a 'cumulative' approach, addressing various points along the entire production process 'from conception to consumption'. To this end the whole process is made subject of study by a multidisciplinary team of experts, who identify all aspects of the processing of a certain product, potential risk areas, the severity and likelihood of occurrence of these risks and what measures are available to effectively tackle them. Finally, the team establishes methods for monitoring of- and documenting the compliance with proposed intervention strategies. This approach has become known as the 'Hazard Analysis Critical Control Points' (HACCP) concept (e.g. Baird-Parker, 1987). At this point it is essential to realize that almost no CCP laid down in HACCP plans, for instance those issued by the ICMSF (1988), affords 100 percent control (CCP-1). The majority of CCP's are therefore classified as CCP-2. Furthermore, unfortunately, for some risk areas control options do either not exist or are not accepted for a variety of reasons.

Although HACCP plans largely rely upon experience with (occasionally additional testing of) the raw materials and on (mathematical) descriptions of the process under study, they would benefit from including mathematically determined probabilities of hazards occurring and efficacies of intervention measures. To allow proper assessment of the efficacy of theoretically possible and practically achievable intervention measures, it is essential that one gains a deeper understanding of the microbial response to environmental conditions, preferably in a formalised, mathematical way.

The need for mathematically determined data on microbial behaviour:

Long before the more recent concepts of mathematical modelling (*vide infra*) were introduced, the basics for a computational approach to food microbiology were established. Two major achievements need to be mentioned. Firstly, the various factors relevant for microbial growth in foods were identified (Mossel and Ingram, 1955). These can be grouped as 'intrinsic' (food-specific, e.g. pH, a_W, structure..), 'extrinsic' (the food environment, e.g. temperature, partial pressure of oxygen ..), 'implicit' (e.g. properties of the microbes under study, antagonistic effects by other organisms..) and 'process' factors (e.g. food processing parameters such as heat treatment). The second breakthrough was the so-called 'hurdle concept' introduced by Leistner (1978; 1992; 1994; Leistner and Gorris, 1994). This term is used to describe an approach to food safety relying on the cumulative effects of manipulating the various afore- mentioned factors, all of which form a complex cascade of time-dependent and sometimes interdependent hurdles for microbial growth.

As outlined above, when pursuing a 'risk-' rather than a 'hazard-' 'based safety assurance approach, data have to be provided on the nature of the food and the kinetics of the conditions under which it is produced. To be able to assess microbial safety and - stability of food, data on microbial growth and decay are necessary. The available information in this area has recently been compiled by the ICMSF in volume 5 of the 'Microorganisms in Food' series (ICMSF, 1996). The conditions tabulated in this books do not always cover all prevailing conditions in food industry practice. Whenever the production variables (e.g. commodity definition, atmosphere, temperature etc. ..) differ from those described by ICMSF or when they vary, ICMSF's data clearly do not apply *sensu strictu* and the manufacturer runs the risk of producing with a false sense of security. Consequently, performing end-product or challenge tests remain necessary sometimes. Such an approach is usually considered expensive, slow, demanding on facilities and on microbial skills of the staff involved, and, again, the results and conclusions drawn from these validation tests only apply to a certain situation and will not contribute to a better *general* idea on microbial risks: the knowledge obtained is 'non- cumulative' (Baranyi and Roberts, 1995). Admittedly, (minor) changes in product formulation and in time-temperature history may result in a 'similar' product, but, as long as microbiologists lack fundamental understanding of the factors influencing microbial activity and to which extent these factors apply, 'building-in safety' has not been achieved (McClure and Roberts, 1992). Nevertheless, it is occasionally possible to make valuable calculations for microbial safety and stability of food using quite simple mathematics, even when only a few data on food composition and microbial growth are available. A classical example is the thermal processing in the canning industry which relies on mathematics for estimating the elimination of (spores of) the most infamous anaerobic organism, *Cl. botulinum* (Esty and Meyer, 1922). It is a typical empirical (statistical) approach. Meanwhile, this concept (where a 'P' - instead of the 'F' - value is used) has gained importance for pasteurization processes too (Weber, 1996).

Another, rather simple and straight-forward example is a method for shelf life calculation which was recently presented by McMeekin and Ross (1996). Although the subject of study of these authors was pasteurized milk, their concept can easily be applied to for instance heat-treated meat products. The following assumptions were made: a) a mere 5 spoilage bacteria present in one liter of milk (= 0.005 /ml or -2.3 $\log_{10}$ units/ml), b) a standard deviation of microbial numbers of s = 0.4 log cycles (/ml), c) spoilage observed at 7.5 $\log_{10}$ *Pseudomonas* sp. per milliliter milk, d) storage temperature 4 °C, and finally, e) doubling time for *Pseudomonas* sp. at this temperature of about 5.5 hrs. From these data, the difference between initial and final (spoilage) level was calculated to be 9.8 [=7.5-(-2.3)] $\log_{10}$ cycles. To reach a multiplication to the 9.8 $\log_{10}$ level, 32.5 doublings (i.e. $10^{9.8} \sim 2^{32.5}$) of the initial 5 microorganisms are necessary. Consequently, the time to spoilage is 7.5 days (32.5 x 5.5 = 178.75 hrs.~ 7.5 days). Assuming there is a normal distribution, 95% of the initial contaminating flora are distributed in the range from 5 ± 2 times the standard deviation s , i.e. a range from 0 to approx. 31.5 microorganisms/l (= -1,5 $\log_{10}$ units/ml) milk. As shelf life prediction should be set to this worst-case scenario, the difference between starting conditions and spoilage level is 9 [=7.5-(-1,5)] $\log_{10}$ units, which corresponds with approx. 30 doublings. For 30 doublings, 164 hrs, i.e. 6.8 days are needed to reach spoilage levels.

Similar calculations for the spoilage of fish have been presented by Dransfield and Scheffer (1991). These authors showed that the rate of growth (R) of spoilage bacteria at a given temperature t as compared to 0 °C can be expressed as $R = (1 - 0.1\ t)^2$. This simple equation represents a quantitative description of the well- known fact that a decrease in temperature rapidly reduces spoilage rate.

Obviously, not all of the questions and problems in today´s food industry can be dealt with in such a simple way. The afore-mentioned examples focus on one *extrinsic* factor (in the milk spoilage example: storage temperature) or on one *process* factor only (in the can sterilisation example: cooking regime, i.e. again temperature). However, the problems faced by meat industry are usually far more complex. This situation demands a new approach for describing microbial response to environmental conditions.

This modern (and in essence purely analytical) approach implies studying the behaviour of microorganisms, not necessarily under

the entire complex of conditions, but rather by concentrating on the microbiological effects of a set of limited but well- defined conditions. From these simplified response studies, 'models' can be generated. 'Model' in this context is understood to represent a mathematical function denoting a common inherent structure - the 'mechanism' - which describes parameters of microbial growth or decay as a response of environmental factors. To date, several databases (though still subject to continuous fine-tuning) have been made available. Two examples are the USDA's 'Pathogen Modelling Programme' and the British 'Food Micro Model'. The following section aims to introduce the readership to the essentials of obtaining microbiological raw data and how to process them mathematically.

The Basics of Microbial Growth

Growth curve description:

The essential stages of bacterial growth are common knowledge. Based on the mathematical description by Monod (1949) and Hinshelwood (1952) of the substrate- limited microbial growth curve and related cell kinetics, three steps are currently recognized. Multiplication of microbes will only occur after a certain adaptation ('lag') phase has been gone through. This lag is followed by accelerating-, then exponential growth (the 'exponential phase'), which decelerates to a plateau, referred to as 'stationary phase', where multiplication and decay are in equilibrium. Important variables to define such growth curves are the initial population (x_0 at t_0), the population at the end of growth (x_{max}) and the maximum specific increase of the microbial population per time unit (Dransfield and Scheffer, 1991). The latter variable is of particular interest. The specific growth rate $\mu(t)$ is defined as the change of population per time unit divided by the number of microorganisms at a given time and represents the slope of the logarithmic growth curve. For this purpose, microbial numbers are expressed as their natural logarithms (ln) and are plotted against time. (In curves, where microbial numbers are given as decadic logarithms ($\log_{10}$), a correction factor of ca. 2.3 is necessary.)

As microorganisms multiply by doubling, the time needed for doubling microbial counts can be described by the terms 'generation time' (or 'mean generation time') and 'doubling time'. Although these expressions may seem to be equivalent, they are in fact only synonymous provided doubling takes place simultaneously for all microbes in a system. In asynchronously growing cultures - which is most frequently the case - only the doubling time can be measured.

Key-factors affecting the growth curve:

When growth curves for particular microorganisms need to be determined, one usually also wants to get an idea of the impact on microbial behaviour of certain external and internal conditions. Although the key-factors that control microbial growth (intrinsic, extrinsic, implicit, process factors) are well-defined, McClure and Roberts (1992) concluded that the understanding of the *relative contributions* of these factors to the safety and shelf-stability of food products is surprisingly poor. Consequently, they proposed another approach.

Considering that tools for description of microbial growth are available and key-factors have been defined, it is feasible to create sets of data describing the different microbial response when one (or a few well-defined) key-factor(s) is (are) *varied*. Current efforts rely on two approaches to mathematical modelling - often used in combination - i.e. including in a model a 'descriptive' component (a most exact statistical description of observed facts) and a 'mechanistic' component, which results from expressing in mathematical terms a rational structure that generally explains the mechanism leading to the growth curves one observes. This approach is briefly discussed further in the following.

Expressing microbial behaviour in mathematical terms: 'Modelling' microbial growth:

The definition of a 'model':

Dransfield and Scheffer (1991) explained the essential differences between the properties of mathemathical models used in meat research in general (see Table 1). They distinguished the 'black box' (descriptive) approach, which aims at exact reproduction of particular data, using regression analysis, and a 'mechanistic' approach, aiming at identifying the common structures behind various sets of observations. In short: the first provides the answers to 'how?', the second to 'why?'. By consequence, the authors explained the term 'mathematical model' not as a fixed (set of) formula(e), but as an idea of how things might function, in the case of microbial growth as 'a set of basic hypotheses on microbial behaviour'. Baranyi and Roberts (1995) used a similar expression. Dransfield and

Scheffer (1991) concluded that, often, mathematical modelling efforts rely on a mixture of both (as was also stated by Box and Draper in 1987), but they suggest a simple statistical reflection or representation of results lacking the quality to elucidate the underlying mechanisms (and hence predict the outcome under *differing* circumstances) must be distinguished from real models.

Important considerations and caveats *when developing and applying models:*

Essential steps from observations and data towards establishing a model have been described by McClure and Roberts (1992). First step is to fit sigmoidal curves to growth curve data. This will result in a number of mathematical functions. The second step is to describe how the fitted parameters of each curve are affected by the various controlling factors. For this purpose, each curve is represented by a few parameters; most notably the maximum specific growth rate. This key value is plotted against the conditions under study, such as pH, NaCl content and temperature (Baranyi and Roberts, 1995), resulting in a 'response surface'. Often, polynomial functions of a second order (i.e. quadratic) have been applied successfully. From these functions, predictions of microbial behaviour can be made and subsequently (the third step) be compared with independently acquired data, e.g. those available from literature. If this 'validation' step is successful, one may consider applying the model to real situations.

This concept implies that first those variables need to be defined, by which the food and microorganism under study can be described (e.g. temperature, pH, a_W etc.). When indeed these fall within the range of the model, it should be possible to predict situations where a risk of microbial activity may occur. Obviously, a great number of potential combinations of different growth conditions prevail under industrial circumstances. On the other hand, the availability of a model now enables us to focus attention on a significantly reduced number of conditions, i.e. those most likely to occur in practice. Only these have to be investigated via challenge tests. This approach reduces analytical efforts considerably.

The considerations of what to include in a model and what not, have been discussed by Baranyi and Roberts (1995). For instance, experiment-dependent factors (e.g. what number of bacteria is used as inoculum) are usually not part of the model. On the other hand, intra- and extra-cellular conditions - subject as they are to changes in bacterial metabolism or changing independently thereof - usually *are* included, as they affect microbial kinetics.

In studying microbiological data generated in laboratory or industrial trials, one may find that sigmoidal curves do not always suitably fit the data points present. Mathematical functions with additional 'valleys and/or peaks' seem to perform better. From a mathematical viewpoint it is no problem to make provisions for these 'anomalies', e.g. by using functions of higher than a second order. Implications of the latter approach ('higher order function → better fit → better model') have been discussed by various authors. For instance, McClure and Roberts (1992) compared a quadratic model for *L. monocytogenes* (designed at the Institute of Food Research in the UK) with a third-order model suggested by Buchanan and Philips (1990). Various types of food with different pH, a_W and stored at different temperatures were analysed, adding up to a total of 28 different situations. It was shown that predictions from both quadratic and cubic models reflected the situations under study well. Indeed, the cubic model never predicted doubling times to be longer than those reported in literature - which means it was always 'on the safe side' - while the quadratic model did so in 6 cases. On the other hand, the 'better fit' advantage of this third- order model for *L. monocytogenes* was impaired by the fact that it gave unreasonable predictions under some conditions, e.g. faster growth when nitrite concentration increased.

The idea of 'upgrading' the performance of models by relying on third- or even higher-order mathematical functions to describe microbial responses was adopted by Hudson (1993). On the other hand, Baranyi and Roberts (1995) demonstrated that a set of measuring data [maximum specific growth rates μ_{max} plotted against pH] fitted a model based on a second - order polynome rather well; although R^2 and F-test revealed that a third - order function afforded an even better fit for *most* of the data, it generated a 'valley' where this is unlikely to occur, i.e. in the stationary phase of microbial growth. The latter is clearly contradictory to all knowledge of microbial behaviour. Hence, using cubic or higher - order polynomial response surfaces functions is not recommended.

Moreover, one must be very cautious in one's attempts to pursue 'best fitting' under all circumstances for other reasons. For instance, it must be borne in mind that by doing so one may in fact be translating measuring errors rather than real-life situations into the model. The traditional way of assessing 'good fit' by 'mean square error (MSE)' or 'regression coefficient' calculation and application of statistical tests (such as the F- test) may be of limited use in some situations, e.g. the one described by Baranyi and Roberts (1995) above. Ross (1996) further discussed the best-fit issue and introduced two factors, which are basically calculated as ratios of predicted to observed values to describe *systematic* over- or underprediction ('bias factor') and the 'best average fit' ('accuracy factor') (see Table 2). A 'bias factor' of 1 indicates two possible situations: (a) the observed and predicted curves fit very well or (b) the observed data lie above and below the predicted curve resulting in no *average* systematic deviation. However, through

considering the 'accuracy factor', case (a) is easily distinguished from the undesirable case (b). In short, both values are used for validating various models of microbial growth against a practical situation (Baranyi and Roberts, 1995; Te Giffel and Zwietering, 1999).

Table 1: Types of Models for Microbial Growth (simplified after Dransfield and Scheffer, 1991):

Type	Statistic	Deterministic
Synonyms	Stochastic Descriptive Empirical	Analytical Theoretical Interpretative Mechanistic
Building up the model	'Black Box' approach: Fit curve to measured data points.	Understanding of the mechanism is essential: various factors known to have an influence (temp., pH,.....) are included. Based on a mathematical function, correction factors are applied to give better fit, when necessary ('tuning').
Mathematics	Data point y related to x - e.g. y= ax +b - as in linear regression.	relationship of y to x described by a function taking into account internal and external factors (parameters for the factors obtained from literature).
Data needed	No extra data needed.	Additional experimental data for single factors necessary.
Causality	Causality problem: erroneous correlations possible.	Causal approach ('mechanistic'). With 'tuning' (see above) causality problem is introduced to a certain extent.
Accuracy of fit	Best fit applies only to a particular situation! Sets of data points which are simulated are affected by measurement errors and biological variability.	Simplification and abstraction. A perfect fit is illusory, as this would be reality itself.
Conclusion	Tells HOW a *particular* process looks like	Tells WHY, WHEN and HOW *similar* processes look alike

Table 2: Parameters to Describe Validity of Mathematical Models (after Ross, 1996)

Term	Significance	Graphical interpretation	Expression
Bias factor (BF)	Describes systematic over- or underprediction.	Do observed values (on average) lie over or under the predicted curve ?	ratio; BF = 1 means no systematic error ('shift'); BF < 1 means that the observed generation times are larger than those predicted (= 'fail - safe'); consequently BF > 1 indicates an unsafe situation.
Accuracy factor (AF)	Describes average difference between observed and predicted values.	Distance between observed data point and predicted curve (closeness).	ratio; AF = 1 means no *average* difference; AF = 2 *means that the prediction is a* factor 2 different from the *observed data.*

Usually, mathematical functions based on growth experiments in laboratory media will correspond with reality to a fair degree (McClure and Roberts, 1992; Ross, 1996). Major factors contributing to discrepancies have been extensively discussed by Baranyi and Roberts (1995). Their findings may be summarized as follows: each step of model generation contains a certain error. Some major categories of errors may be distinguished: All errors originating from the mathematical procedures applied are termed *'numerical procedure errors'*. Simplifications of and assumptions on microbial behaviour (i.e. assuming a homogenous bacterial population and disregarding the natural biological variance) lead to *'homogeneity errors'*. Finally, restriction to a few environmental 'key' factors means incompleteness and therefore results in *'completeness errors'*. Although decreasing the latter two errors is

desirable when models are to be applied in practice, this often leads to an unnecessarily complicated, not generally applicable model. It thus impairs the main advantages of mathematical modelling.

While the previous text focussed on the logarithmic (exponential) phase of microbial growth, the relationship between lag-time and temperature has been shown by Dransfield and Scheffer (1991) to be also relevant e.g. for *Staphylococcus aureus*. The latter consideration is of particular importance when effects of environmental conditions, such as changes in temperature, are under study, as microbes react to these with an adaptation phase, during which metabolic or structural changes take place and multiplication is suspended. Changes in meat production and processing environment *are* mostly temperature changes.

Modelling the genetic variance of microorganisms:

When discussing descriptions of microbial behaviour, one usually focusses on multiplication, i.e. on the increase of biomass, for which purpose some simplifications are made as regards bacterial activity (Baranyi and Roberts, 1995). However, in real life situations microorganisms are anything but representing a constant factor. This is illustrated by the fact that microorganisms have the ability to adapt to most treatments in fresh meat processing and meat products manufacture, such as heating, refrigeration, acid environments, high osmolarity or pressure (Liu et al., 1969; Miller and Caspar., 1994; Lou and Yousef, 1997). Even irradiation procedures might lead to more irradiation-resistant microorganisms (Farkas, 1998). However, resistant microorganisms develop mainly if the treatment for purposes of food-processing or -preservation fails to fully eliminate the microorganisms. If they survive without irreparable damage, microorganisms may adjust to their new habitat and will subsequently possess a selective advantage over competitive flora. For example, genetic adaptation of pathogens in the host, adaptation to antibiotic treatment or to host-cell immune response (Tollefson et al., 1998) are well-known facts and have been studied during infection (for review see Robertson and Meyer, 1992).

Unfortunately, not much is known about the genetic dynamics of microorganisms during food processing. Similarly as is the case for acquired antibiotic resistance of pathogens, environmental adaptation to certain food processing environments is acquired by genetic adaptation. The resulting mutants become resistant by transfer of genetic elements (transposons) such as plasmids, phages or insertion sequences. Microorganisms are able to transfer these elements to closely related organisms. In the worst case, spoilage bacteria might transfer abilities to pathogens. Such a transfer may even occur following the death of resistant microorganisms during which transposon elements may be set free. Genetic variance may also be acquired by the accumulation of point mutations in specific genes. Both mechanisms might lead to a phenotype being more resistant to one or the other treatment. Many food processing technologies can induce mutagenic changes in bacteria (Thompson et al., 1983; Felton et al., 1984), although those mutations are not necessarily leading to a more resistant character. Yet, the altered phenotype resulting from some of these mutations may complicate their diagnosis and/or assessment of their growth characteristics (Farkas, 1998). For mutants to develop enhanced virulence, host - pathogen interaction is usually necessary, but it has also been observed to occur in the (processing) environment (Farkas, 1998).

A number of mathematical models predicting the development of genetic variance during host infection have been developed for viruses such as influenza (Meyer et al., 1993; Rekik et al., 1994). For bacterial mutation, only few models exist and the ones published have a less impressive predictive performance. The accuracy of these models could be enhanced if one could rely on more experimental data (Wagner et al, 1998). Models predicting microbial behaviour in a food processing environment should preferably be based on experimental data on *all* specific technologies applied, because every single one of them may have caused the emergence of resistant microorganisms.

When modelling genetic adaptation of pathogenic microbes, changes within the host are most relevant. Unfortunately, to date, data are lacking. Hence, future work needs to concentrate on a range of effects of host : pathogen/commensil interaction, such as tolerance for the acid environment of the stomach, for bile salts or for microbicidal compounds produced by the host (for instance nitric oxide, interleukins, neutrophilic granula). All of these might effect phenotypic changes in orally ingested pathogens (Groisman and Heffron, 1995) and need to be taken into consideration.

Concluding remarks:

When establishing integrated quality control concepts and safety assurance systems along the meat production line, the definition of characteristics of raw material, product- and process parameters is usually relatively easy. However, there is an urgent need for assessment of microbial risks. To assess quantitative aspects of microbiological risks, a mathematical approach to describe the

dynamics of the microbial population is necessary. Experience shows that describing microbial growth as a response to external factors in an empirical way is feasible and rather accurate even by using simple mathematics. Several successful applications are known. When mathematical analysis is not simply aiming to reflect microbial growth under particular conditions, but also to describe the general 'mechanism' of growth of a certain microorganism as a response to external conditions, a usually more complicated 'model' needs to be generated. Experience with those available suggests that models are very useful to predict the risk associated with microbial growth and may thus largely replace traditonal challenge tests in a cost- effective manner. This approach is particularly valuable, when novel foods are designed or when several product- or processing factors for a 'safe' food have to be changed. However, when generating models and using these for 'predictions', one must remain cautious.

Current attempts to mathematically model microbial growth have huge potential for generating strategies that might help to 'build in' food safety. Their further application in industry practice holds great promise for facilitating microbial control in the meat industry in the future.

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