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Predictive microbiology: providing a knowledge-based framework for change management

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Abstract

This contribution considers predictive microbiology in the context of the Food Micro 2002 theme, ''Microbial adaptation to changing environments''. To provide a reference point, the state of food microbiology knowledge in the mid-1970s is selected and from that time, the impact of social and demographic changes on microbial food safety is traced. A short chronology of the history of predictive microbiology provides context to discuss its relation to and interactions with hazard analysis critical control point (HACCP) and risk assessment. The need to take account of the implications of microbial adaptability and variable population responses is couched in terms of the dichotomy between classical versus quantal microbiology introduced by Bridson and Gould [Lett. Appl. Microbiol. 30 (2000) 95]. The role of population response patterns and models as guides to underlying physiological processes draws attention to the value of predictive models in development of novel methods of food preservation. It also draws attention to the paradox facing today's food industry that is required to balance the ''clean, green'' aspirations of consumers with the risk, to safety or shelf life, of removing traditional barriers to microbial development. This part of the discussion is dominated by consideration of models and responses that lead to stasis and inactivation of microbial populations. This highlights the consequence of change on predictive modelling where the need is now to develop interface and non-thermal death models to deal with pathogens that have low infective doses for general and/or susceptible populations in the context of minimal preservation treatments. The challenge is to demonstrate the validity of such models and to develop applications of benefit to the food industry and consumers as was achieved with growth models to predict shelf life and the hygienic equivalence of food processing operations.

Keywords: Predictive microbiology; Risk assessment; Hazard analysis critical control point

1. Introduction

At the 3rd International Conference on Predictive Modelling in the Food Industry, held in Leuven, Belgium, in August 2000, the University of Tasmania made a contribution entitled ''Predictive microbiology: towards the interface and beyond'' (McMeekin et al., 2002). In that review, we considered the concept and history of predictive microbiology, tracing the development of kinetic and probability modelling approaches, growth models, growth/no growth interface models and non-thermal death models. Attention was also drawn to the interface of predictive micro-

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biology with microbial physiology and, briefly, with information technology and food safety initiatives such as hazard analysis critical control point (HACCP) and risk assessment. It was concluded that "Predictive microbiology (the quantitative microbial ecology of foods) has, after a considerable gestation period, emerged strongly as an essential element of modern food microbiology". Furthermore, we foreshadowed "that consolidation of existing and development of new interfaces will lead to acceptance of predictive microbiology as a mature subdiscipline of microbiology".

So, is predictive microbiology really an essential element of modern food microbiology and can we claim that it has achieved, or will soon achieve, the status of a mature subdiscipline of microbiology? A measure of the veracity of these claims may be made if it can be demonstrated that predictive microbiology has made, or has the potential to make, an impact on other branches of food microbiology. The aim of this review is, therefore, to demonstrate that predictive microbiology provides a knowledge-based framework to deal with change by considering the topic within the Food Micro 2002 theme "Microbial adaptation to changing environments" and relating it in particular to the other topics (Risk Analysis and Preservation) identified by the conference organisers in the "what can we do" category [www.matforsk.no/web/foodm.nsf].

2. A reference point and general recognition of changes affecting food microbiology

In any treatise dealing with the effects of change, it is useful to start with a reference point and for this purpose we have selected the text "Microbiology of foods—occurrence, prevention and monitoring of hazards and deterioration" (Mossel, 1977). This choice reflects the standing of Professor Mossel as a leading 20th century food microbiologist, the fact that his thoughts (condensed in 89 pages) were supported by 2310 citations (although some references were duplicated), and that the mid-1970s was a period during which the pace of social, demographic and technological changes began to have significant impact on the food industry and consumers.

Many commentators have categorised changes causing the increased incidence of food-borne disease in the last 25 years and most have identified a primary division into those associated with social, demographic and behavioural changes in the human population and technological changes (Maurice, 1994; Alterkruse et al., 1997; Miller et al., 1998). The former category includes changes in eating habits, particularly increased reliance on the food service sector; increased international travel and commerce; globalisation of the food industry; population pressures leading to breakdown of public health infrastructure; and consumer demand for less additives in foods and for minimally processed foods. Changes in technology and industry start at the production level with activities such as intensive agriculture and aquaculture and follow through the food production continuum with novel, less severe technologies for food preservation, longer food distribution chains, and the increased scale of food-processing operations.

An excellent account of the effect of these changes was presented by Tauxe (1997) in which those pathogens that have emerged since the 1970s and are predominantly food-borne were listed: *Campylobacter jejuni*, *Campylobacter fetus* ssp. *fetus*, *Cryptosporidium cayetanensis*, *Escherichia coli* 0157:H7 and related *E. coli*, *Listeria monocytogenes*, Norwalk-like viruses, *Nitzschia pungens*, *Salmonella Enteriditis*, *Salmonella Typhimurium* DT104, *Vibrio cholerae* 01, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica*. A similar chronology of pathogenic microbes and infectious diseases recognised since 1973 compiled by the NSTC-CISET Working Group on Emerging and Re-emerging Infectious Diseases in 1996 was presented by Lederberg (1997). This lists 29 pathogens of which only ~5 would be considered food-borne and illustrates the point that the impact of change is not confined to food-related diseases and is paralleled by the emergence and re-emergence of infectious diseases from other sources.

In addition to new food-borne pathogens, Tauxe (1997) considered new vehicles of transmission: "Traditionally, the food implicated in a food-borne outbreak was undercooked meat, poultry or seafood or unpasteurised milk. Now, additional foods previously thought safe are considered hazardous." Examples given included the contents of eggs and products derived from them as a result of internal contamination with *S. Enteriditis;* apple cider (*E. coli* 0157:H7)

and orange juice; raspberries (*Cyclospora*) and other fresh produce including cantaloupe, tomatoes, strawberries, scallions, sprouts, and lettuce. Many of these products were contaminated by organisms of faecal origin with contamination opportunities arising during production and harvesting, initial processing, distribution and final processing, emphasising the need for 'farm-to-fork' control. When coupled with the widespread distribution of product and large scale of operation, Tauxe (1997) pointed to a significant change in outbreak scenarios with localised, acute events being replaced by diffuse and widespread outbreaks that may even cross national boundaries (Tauxe and Hughes, 1996).

In turn, this has led to changing surveillance strategies with increased reliance on molecular approaches and electronic networking between laboratories and regulatory authorities providing a real-time opportunity to identify the source and limit the spread of an outbreak (Swaminathan et al., 2001). Consistent with proactive rather than retrospective surveillance is the increasing adoption of alternative approaches to the prevention of food-borne disease. This is epitomised by the HACCP concept, the essence of which is to build safety into food processing and preparation rather than placing undue reliance on end-product testing. The chronology of HACCP adoption by the food industry is considered later, but it is noteworthy that, in the reference point text, Mossel (1977) drew attention to the shortcomings of "the retrospective or repressive system" of end-product testing that had "the character of a post mortem" and advocated following a preventative approach citing van Oyen (1919) and Wilson (1955). Joining HACCP in the armoury of changing food safety measures is quantitative microbial risk assessment (QMRA) which has adopted increasing prominence since the mid-1990s. However, risk analysis for microbial food safety was discussed by Mossel (1978) and Mossel and Drion (1979) apparently entering a quiescent phase in the interim. This may possibly be attributed to the need for increased computing power and appropriate software to deal with the complex issues involved in QMRA as indicated by Morgan (1993). In a subsequent section, we will argue that the effectiveness of both HACCP and QMRA depends, to a large degree, on the application of predictive microbiology models.

3. A reference point and general recognition of predictive microbiology in the microbial ecology of foods

In considering general principles of food microbiology, Mossel (1977) recognised the customary division of the field into two main areas of interest: (1) the protection of the consumer against food-borne microbial diseases, and (2) the prevention of food spoilage due to microbial activities. He also noted the availability of a great variety of fermented foods consumed all over the world containing "milliards of viable cells" and that the central theme of his book was the microbial ecology of foods. The approach he used to describe the microbial ecology of foods is based on Mossel and Ingram (1955) and Mossel (1971) in which the phenomena of contamination of foods and microbial proliferation in foods were separated and the latter considered in the now standard categories of intrinsic, extrinsic, processing and implicit factors.

Despite the very extensive reference list, one finds no comment per se on predictive microbiology, although there is a hint of the possibility of predicting the keeping quality of "very perishable commodities like fresh meats, poultry, seafood, and vegetables" by indirect techniques:

"Experience shows that for every particular spoilage association, the time taken for overt deterioration to develop is directly related to the generation time γ of those organisms that play the predominant role in such an association. This immediately follows from the Monod–Hinshelwood approach, if it is assumed that the specific spoilers occurring on perishable foods will be out of the lag phase. Then the relation applies

$$t_s = \frac{\log N_s - \log N_o}{\log 2}\gamma$$

where t_s = days to develop spoilage under a given set of intrinsic and extrinsic conditions; γ = generation time under these circumstances; N_s = cfu/g or cm^2 at the time of spoilage; N_o = cfu/g or cm^2 initially present. Once N_s and γ have been determined for given storage conditions, it is very easy to derive from the logarithmic plot, the time to spoilage, hence keeping quality, under these conditions from N_o. Thus, all that is required to calculate the probable storage life is an estimation of the N_o count." A similar approach was used by Mossel and Ingram

(1955). Thus, while the effect of temperature on the characteristics of the bacterial growth curve was determined with subsequent transformation to a plot predicting temperature dependence, it does not appear to have been recognised as part of an emerging research area in Food Microbiology.

General articles on, and reviews of, Predictive Microbiology, began to appear in the mid-1980s (Roberts and Jarvis, 1983; Farber, 1986; McMeekin and Olley, 1986; Baird-Parker and Kilsby, 1987). This first flush of reviews was followed in the early 1990s by a series of letters to the editors of various journals providing varying opinions on the topic and indicating a phase of intense research activity on microbial modelling (Kilsby, 1989; McMeekin et al., 1989; Cole, 1991; Hedges, 1991; Davey, 1992; Riemann, 1992; Whiting, 1992). This period also included the first international meeting on the subject (Application of Predictive Microbiology and Computer Modelling Techniques to the Food Industry, held in Tampa, FL, in April 1992). The proceedings of this meeting were published as 32 papers in a special issue of the Journal of Industrial Microbiology [12 (3–5) (1993)] and in the same year the first monograph, "Predictive Microbiology: Theory and Application" was published (McMeekin et al., 1993). Since that time, two further international meetings (Hobart, Tasmania, 1996, and Leuven, Belgium, 2000), the latter resulting in 31 papers in a Special Issue of the International Journal of Food Microbiology [73 (2–3) (2002)], and many further reviews attest to the increasing maturity of predictive microbiology as a recognisable sub-discipline of Food Microbiology.

Mossel (1977) is also a useful starting point from which to consider the impact of changes on food microbiology in that three rules in food microbiology were promulgated.

- "It is possible for a food to contain dangerously high numbers of pathogenic organisms or levels of microbial toxins and yet to appear perfectly wholesome to the consumer.
- Measures that are effective in preventing growth of pathogenic organisms will not necessarily control microbial spoilage of foods.
- The converse, that is, inhibition of pathogens when spoilage is prevented, usually does hold, especially when this is achieved by proper refrigeration. However, these measures cannot actually eliminate dangerous pathogens. Viruses, protozoa and worms can be infective in very low doses. This applies to bacteria under certain circumstances. In addition, the risk remains of cross contamination onto initially sound products."

At this point it is pertinent to enquire if the three rules, formulated 25 years ago on the basis of the extent of the knowledge base available at that time, are still applicable?

Rule 1 continues to hold and may well be more important due to the development of new technologies for minimally processed foods with extended shelf life. Rule 2 also continues to hold, but Rule 3 may require reassessment due to the emergence of significant psychrotrophic pathogens such as *L. monocytogenes* and strains of *Clostridium botulinum* not foreseen at that time. Note, however, that Rule 3 includes several caveats indicating the potential to encounter pathogens (including bacteria) with very low infective doses and the risk posed by cross-contamination events. These comments, qualifying the general rule, contain a prophetic element when one considers:

- the emergence of parasites such as *Cyclospora* and *Cryptosporidium* as significant causes of food-borne disease due to changes in international trade in food,
- the emergence of *E. coli* strains with very low infective doses initially attributed to changes in agricultural practices,
- major outbreaks of food-borne disease resulting from post-processing contamination such as the *L. monocytogenes*/frankfurther outbreak (Anon., 1999).

4. The emerging paradigms of microbial food safety: HACCP, risk assessment and predictive microbiology

4.1. HACCP

The hazard analysis critical control point concept was introduced to food microbiology as a proactive, preventative system of quality control to replace the "sample-consumptive" nature of food analysis (Hart-

mann, 1997) and is now widely accepted by regulatory authorities and industry as the tool by which safety is built into food-processing operations.

Hartmann (1997) traces the chronology of HACCP development and application as follows:

1959	Origin of HACCP to produce safe foods for the space program
1971	First public awareness of the HACCP concept
1985	Endorsement of HACCP by the US National Academy of Sciences Subcommittee on Microbiological Criteria for Foods and Food Ingredients
1989	NAS publication "HACCP Principles for Food Production" (revised 1992)
1993	Approved by the Codex Alimentarius Committee of Food Hygiene
1995	USDA, Food Safety Inspection Service regulations proposed requiring all slaughter and processing plants to develop and implement a HACCP program

The period from concept development to widespread utility of HACCP, therefore, spans a period of >35 years starting with research in industry, sponsored by government, to provide a solution to a specific problem. After 25 years, its widespread applicability gained the endorsement of a powerful US scientific committee leading to regulatory use at national and international levels. These endorsements, leading to status as a paradigm of modern food microbiology, were no doubt aided by changes in the urgency of the food safety debate engendered by the emergence of dangerous pathogens with 'unusual' characteristics viz.: *L. monocytogenes* and psychrotrophy and toxigenic *E. coli* strains with very low infective doses.

4.2. Quantitative microbial risk assessment

The time frame for QMRA to reach food safety paradigm status is relatively short compared to that for HACCP. In a Special Issue of the International Journal of Food Microbiology [58 (3) (2000)] based on the ILSI Europe Session on Microbiological Risk Assessment at Food Micro '99, the earliest of the references cited to illustrate microbial risk assessment applied to foods was published in 1994. The short period is probably attributable to the discussion being initiated at the international level through the Codex Alimentarius Commission in response to the World Trade Organisation, General Agreement on Tariffs and Trade, Uruguay Round Agreements on Sanitary and Phytosanitary measures and Technical Barriers to Trade (Hathaway, 1993). Also, as the requirement was to provide a widely applicable, objective measure of the effectiveness of food safety initiatives expressed in terms of public health impact, the need for a period of 'translation' from specific to widespread application was not required. It is pertinent to note that, despite official endorsement at the highest level, QMRA is still developing, there are as yet no universally agreed methods and its efficacy, for example, in judging objectively the hygienic equivalence of foods in international trade, remains to be tested.

4.3. Predictive microbiology

Several authors have suggested the origin of predictive models for foods is that developed by Esty and Meyer (1922) to describe thermal processes sufficient to destroy 10^{12} spores of *C. botulinum* type A (Whiting and Buchanan, 2001; McMeekin et al., 2002). This model described a process with a very large safety margin and, whilst that probably accounts for its continued use, it perhaps also inhibited its widespread recognition as a predictive model.

The origin of "modern" predictive microbiology can be traced to the 1960s and 1970s when kinetic models were used to address food spoilage problems (Spencer and Baines, 1964; Nixon, 1971; Olley and Ratkowsky, 1973a,b), followed by the use of probability models to address food poisoning problems, particularly botulism and other intoxications (Genigeorgis, 1981; Roberts et al., 1981). One gains the impression from these writings that the authors already had in mind the general concept of predictive microbiology and perceived it to have widespread utility. This is confirmed by Olley and Ratkowsky, who in 1973 recognised the fundamental similarity of the response to temperature of many spoilage processes and proposed a "universal spoilage curve".

Despite this, the time frame for acceptance of predictive models is similar to that for HACCP and for 30–40 years, it remained largely a researcher-driven activity. However, its value is now recognised as the scientific basis required to underpin HACCP

and QMRA. Predictive microbiology assists the formulation of HACCP plans by identifying hazards and critical control points and in specifying limits and corrective actions (Ross and McMeekin, 1997; Miles and Ross, 1999). With QMRA, predictive models have a particular role in providing exposure assessment information.

4.4. The food safety triangle

Predictive microbiology offers to provide a sound scientific underpinning to meet the ongoing needs of food safety (see e.g. Ross and McMeekin, 1997; Hathaway and Cook, 1997; Coleman and Marks, 1999) whether through the use of risk assessment tools to determine strategies for improved food safety, or the design of specific HACCP plans to enact those strategies. The triangular interplay between these three paradigms of modern food microbiology can be visualised as scientific knowledge underpinning the mechanism(s) of proactive food safety and assessment of its efficiency.

4.4.1. The role of predictive microbiology

At the core of both HACCP and risk assessment is the desire to produce safe foods using strategies based on understanding sources and magnitudes of hazards. Risk assessment is part of the process of deciding what we mean by "safe" but, to assess risk, we must assess human exposure to pathogens in foods. Clearly, this information is rarely available. Predictive microbiology models are used in the assessment of human exposure to food-borne pathogens as surrogates for enumeration of bacteria in the food.

To translate the agreed level of safety (expressed as Food Safety Objectives) into practical actions, the mechanism of HACCP is used. While the HACCP concept involves a systematic approach to food safety based on hazard identification and control and is based on identifying and evaluating key steps in the food production chain which have the greatest effect on risk associated with hazards, it is often applied subjectively. That is, HACCP is a quantitatively based risk management system that relies on qualitative risk evaluation. The subjectivity involved in the application of HACCP, predominantly due to the lack of quantitative information available, has been alluded to by several authors.

Exposure assessment can also be structured using the Process Risk Model approach (Cassin et al., 1998) or the Modular Process Risk Model (Nauta, 2002) approach so that the steps in the farm-to-fork continuum that most significantly contribute to risk are identified using the techniques of sensitivity or importance analysis. Thus, risk assessment techniques can contribute directly to HACCP plans.

4.4.2. HACCP and predictive microbiology

To establish HACCP limits and responses, requires scientific data. In the context of microbiological hazards, this information is increasingly becoming available through predictive microbiology models. Tolerable limits at each step can be determined in the context of an overall risk assessment. Thus, when the tolerance for a particular step is set, predictive microbiology can specify the various combinations of factors (e.g. temperature, organism, time, pH) which achieve compliance with that tolerance limit. For microbiological hazards, predictive microbiology aids HACCP because it provides a quantitative link between "real-time" measurements used to monitor processes, such as temperature, pH, salt concentration, relative humidity and time itself, and the potential for growth or death of specific microbes of concern. Models can be useful in various steps of HACCP, such as:

- *hazard analysis*: models (particularly growth/no growth) can be used to show which organisms will grow on the product, and if so, how fast they will grow, therefore identifying them as potential hazards;
- *identification of CCPs*: by defining the process in terms of parameters such as temperature, water activity, pH, etc., it is possible to identify steps at which (significant) growth or death is possible and whether critical control can be achieved or lost;
- *specification of limits*: "what-if" scenarios can be performed for different product formulations, to see if alterations will allow new hazards to emerge, or increase the risk of an existing hazard;
- *specification of corrective action*: if a loss of control occurs at a CCP, the change in microbial numbers associated with the process deviation can be quantified and appropriate corrective steps specified.

4.4.3. Risk assessment and predictive microbiology

Dose response relationships for the infection process indicate that risk is related to dose. Thus, to assess food safety risk requires knowledge of the number of organisms in foods at the time of consumption. Clearly, this information is rarely available, but as stated earlier, predictive models can assist to meet the need.

Microbial food safety hazards can self-amplify (microbial growth) or can be eliminated or reduced by food preservation and decontamination processes (e.g. cooking). To estimate the numbers of microorganisms present at the time of eating from levels known at some earlier time in the product's history requires a sound and quantitative knowledge of the responses of microorganisms to the environmental conditions (temperature, nutrients, chemicals, preservatives, other microorganisms) they experience in the foods, and for how long. Without predictive microbiology models, this would not be possible. Several authors (Buchanan, 1997; Walls and Scott, 1997a; van Gerwen and Zwietering, 1998; Coleman and Marks, 1999; Lammerding and Fazil, 2000; Whiting and Buchanan, 2001; Nauta, 2002) have discussed the nexus between predictive microbiology and quantitative microbial food safety risk assessment.

5. Microbial adaptability and variable responses: implications for predictive microbiology

Adaptability and response to change are well known as the stock-in-trade of microorganisms as might be anticipated from biological entities with generation times that are measured in minutes rather than years or decades. Combining this trait with small size, ease of dispersal, physiological diversity, remarkable survival capabilities, tolerance of extreme conditions and ready exchange of genetic material leads to a situation where microorganisms have colonised almost every conceivable habitat on earth (and probably beyond). Their associations with plants and animals range from symbiosis to pathogenesis and when those plants or animals are harvested or killed for food, the high level of nutrition available provides ample opportunity to reach very large populations. As an example, the carrying capacity of proteinaceous foods is equivalent to that of rich laboratory media and yields up to 10^9–10^{10} cells/g, ml or cm^2. Even greater populations may be reached in the gastrointestinal tracts of humans and animals with the potential to complete the food-chain cycle or wider environmental cycle central to the transmission of food-borne disease.

Thus, food and gut environments provide for large populations of microorganisms containing subpopulations with the capacity to react to change in a dichotomy described by Bridson and Gould (2000) as classical versus quantal microbiology. In their scheme, it is argued that "Large populations of microorganisms obey the rules of taxonomy but the individual cells exhibit uncertainties (caused by mutations and fluctuating local environments) which are buried within the macropopulations." Further, "The functional stability of classical microbiology masks minority subpopulations which, nevertheless, contribute to the complex dynamics of microbial populations."

Predictive microbiology is concerned with the complex dynamics of microbial population behaviour and, as observed by Monod (1949), "The growth of bacterial cultures, despite the immense complexity of the phenomena to which it testifies, generally obeys relatively simple laws which make it possible to define certain quantitative characteristics of the growth cycle, essentially the three growth constants: total growth (G), exponential growth rate (R) and growth lag (L). That these definitions are not purely arbitrary and do correspond to physiologically distinct elements of the growth cycle is shown by the fact that, under appropriately chosen conditions, the value of any one of the three constraints may change widely without the other two being significantly altered. The accuracy, the ease, the reproducibility of bacterial growth constant determinations is remarkable and probably unparalleled so far as biological quantitative characteristics are concerned." It should be noted, however, that in the introduction to his paper Monod (1949) added the rider that "The discussion will be limited to populations considered genetically homogeneous."

Bridson and Gould (2000) drew a distinction between quantal and classical microbiology and an analogy with quantum and classical physics. In this they portrayed the phases of the growth curve that exhibited predominantly classical microbiology char-

acteristics (exponential and stationary phases) and those where quantal microbiology traits are predominant (the lag phase and the terminal part of the death phase). Whilst these are normally regarded as opposite ends of the growth curve, they are, in fact, intimately juxtaposed. Indeed, in some circumstances they may be regarded as a single phase, eventually leading to proliferation of those cells best adapted to survive the rigours of their historical environment but continuing to encompass sufficient heterogeneity to deal with insults or exploit opportunities imposed by a new set of environmental conditions. Both classical and quantal microbial behaviour have implications for predictive microbiology: the former in regard to variability in population behaviour and the latter in relation to the adaptability of individual cells within that population. There must, however, be a gradation in the influence of overall population behaviour (classical microbiology) and that of individual cells within a population (quantal microbiology) on the ability of predictive models to describe adequately microbial behaviour.

Monod's statement (1949) on the reproducibility of the bacterial growth rate constant is borne out by many predictive microbiology studies under conditions that permit rapid growth and lead to large populations (the realm of classical microbiology). These studies also demonstrate that estimates of lag phase duration (where quantal microbiology dominates) are more variable than growth rate estimates. However, both response times exhibit progressively greater variability as a population experiences harsher conditions and generation and lag times become longer. When population development is inhibited by decreasing temperatures and/or water activities, equivalent cell yields are obtained over a wide range of temperature and/or water activity values until conditions approach those too extreme to allow growth to continue. Variability in response times may, therefore, also contain a component attributable to "metabolic efficiency" as well as population density per se.

Conditions under which the adaptability of individual cells will influence the performance of a predictive model include those where small population sizes are the norm and inimical conditions lead to a decline in the viable population. The outcome of this situation, commonly encountered in food-processing operations, will depend on the propensity of a few cells to retain reproductive capacity and to seed the next burst of active growth after a period of physiological adjustment or repair. This emphasises the points made by Bridson and Gould (2000) that "quantal microbiology does not merely describe a few microbial cells, it indicates that the microbiological outcome of further cultivation is unpredictable. The normally generous inoculation of cells into culture media that is commonly used is often predictable. . ..However, very low inocula of stressed cells distributed into culture media will not result in growth in every tube of medium and it is not possible to predict which cell or tube will show growth."

6. Models: a guide to underlying physiology

6.1. Types of models

Many scientists, including biologists, have a penchant for classification of organisms and the properties and characteristics of those organisms to provide a framework for comparative studies. Classification of predictive models is based on the population behaviour that they describe and includes growth models, limits of growth (interface) models and inactivation models. A further classification was proposed by Whiting and Buchanan (1993) as primary, secondary and tertiary. Primary models describe how population density changes with time in a specified environment and are depicted as microbial growth or death curves; secondary models indicate how the parameters of primary models change with environmental factors and involve a transformation of response time data, for example, Arrhenius-type models in which the natural logarithm of rate or Bélehrádek-type models in which the square root of rate is related to temperature; tertiary models are application tools such as nomograms, software packages and expert systems. Models may also be based on the kinetics of microbial population behaviour or examine the probability that an organism will respond by growing, dying or producing a metabolite in a given period of time. An extension of probability modelling is to define the absolute limits for growth of an organism confronted with an environment containing more than one stressor (growth/no growth interface modelling) (Ratkowsky and Ross, 1995).

Another classification of models as mechanistic or empirical describes those with or without a theoretical basis. However, in reality, models commonly used in predictive microbiology are not purely mechanistic and some (to be avoided) are simply curve-fitting exercises with obvious deficiencies such as the number of degrees of freedom equal to or greater than experimental observations. In such cases, usually based on a small number of points, a perfect fit of the model to the data used to generate it is not surprising. Each of the model types described above and the patterns that become evident when they are visualised by plotting the variables concerned provide information on population behaviour that, in turn, may suggest the underlying physiological basis.

6.2. Primary models

Let us consider a primary model relating the logarithm of microbial numbers to temperature or water activity or acid pH in the suboptimal region. Sigmoidal curves at each level of the independent variable indicate that, with progressively harsher conditions, lag phase duration increases and growth rate decreases. Growth rate decreases progressively, but cell yield (maximum population density) is not markedly affected by reduced temperature or water activity until conditions approach those that preclude growth. However, under increasingly stringent acid conditions, cell yield decreases progressively whilst growth rate is unaffected over a relatively wide pH range. This level of information suggests that the physiological basis for the effect of pH on microbial development differs from that of temperature and water activity that may, but not necessarily, exert their influence in a similar manner.

6.3. Secondary models

When this information is translated into a secondary model, the dependence of growth rate on temperature or water activity or pH is revealed, as is the existence of specific effects attributable, within the general pattern established for temperature/water activity or pH, to different humectants or acidulants.

Two major models are used to describe the temperature dependence of bacterial response times. As indicated above, Arrhenius-type models are transformed to the natural logarithm of rate and plotted against the reciprocal of absolute temperature, and with Bélehrádek-type models, a square root of rate transformation is plotted against temperature. The latter plot is characterised by:

- a linear response as indicated by the form of the equation
- a minimum observed growth temperature (T)
- a theoretical minimum observed growth temperature (T_{min}) where extrapolation of the square root of rate plot crosses the temperature axis, that is, where the growth rate is zero
- usually the actual minimum temperature (T) is several degrees higher than the theoretical minimum temperature (T_{min}).

The form of the Van't-Hoff-Arrhenius equation, originally derived for chemical kinetics, also predicts a linear response with temperature. However, typically Arrhenius plots of microbial growth are continually downward sloping curves. To introduce appropriate curvature to fit the data, additional terms are added as in the Schoolfield et al. (1981) equation or the Davey (1989) model.

Detailed arguments about the appropriateness of the competing model types are beyond the scope of this paper. However, despite that both, when applied to microbial growth kinetics, are essentially empirical, their characteristics may provide clues to underlying physiological processes. Support for this hypothesis is derived from analysis of thermodynamically based models developed in Chapter 10 of McMeekin et al. (1993) and by Ross (1999). These include terms for enthalpy and entropy of thermal denaturation of a rate-limiting enzyme and the difference in heat capacity between its normal and denatured state. These models indicate that the apparent activation energy remains almost constant in a region below the optimum temperature for growth and eventually deviates significantly from the predicted linear relationship. This region has been termed the normal physiological region (NPR) (Ingraham et al., 1983) and attributed with physiological significance.

Turning to Bélehrádek-type models, the term $(T - T_{min})$ provides an algebraic basis for reconcili-

ation of the Arrhenius and Belehradek equations (McMeekin et al., 1988) and a link to the energetics of growth through the relationship

$$E_{app} = \frac{2RT^2}{(T - T_{min})}$$

Derived from the differential form of both equations, the apparent activation energy (E_{app}) at any temperature is dependent on the distance ($T - T_{min}$). The consistent observation of reaching a fixed minimum temperature for growth at greater than zero rate that is not changed by extended incubation suggests that there may be a critical activation energy for growth (Krist, 1997).

The concept of a theoretical T_{min}, in addition to providing information on the energetics of the temperature dependence of microbial growth, also has utility as a reference point in that:

- it roughly characterises microorganisms as psychrophiles, psychrotrophs, mesophiles or thermophiles in the continuum of temperature relationships (McMeekin et al., 1988),
- it allows ready conversion of temperature dependence data into a relative rate function which is the basis for much practical application of predictive models (McMeekin et al., 1993).

6.4. The normal physiological range

Focus on physiological processes at the extremities of the NPR is also suggested by a fascinating observation made by Nichols et al. (2000). These workers studied the growth of a psychrophilic bacterium, *Shewanella gelidimarina*, isolated from Antarctic sea ice at close temperature intervals (~ 1 °C) and two water activities over the entire biokinetic range. In addition to estimating growth rates to produce a temperature/growth profile, membrane lipid composition was determined at each temperature. The estimated NPR for growth was a function of water activity and could be manipulated by changing the concentration of NaCl. In addition, growth temperatures at the boundaries of the NPR (at $a_w = 0.977$ low 2 °C, high 15 °C) were characterised by increased variability in fatty acid composition and a similar observation was made at $a_w = 0.993$ (low 1 °C, high 12 °C).

Deviation of the growth rate of *E. coli* from classical Arrhenius kinetics that characterises the NPR has led to the suggestion that transition of a "master" enzyme(s) from an active to an inactive state may define the NPR (Ross, 1999). The observations of Nichols et al. (2000) may indicate, for the first time, a physiological basis for the NPR for bacterial growth. Similar observations on disturbance of the fatty acid composition of two bacilli (Suutari and Laakso, 1992) and a yeast (Suutari et al., 1996) were observed in narrow temperature ranges near the growth/no growth interface and a perturbation in the fatty acid composition of *L. monocytogenes* at the ends of the NPR has now been reported by Nichols et al. (2002).

6.5. Secondary models for more than one constraint

So far we have considered the effects of individual factors, but in many situations, it is necessary to consider how different factors interact to restrict microbial growth. When the combined effects of two factors are modelled, for example, temperature and water activity, a common observation is that the actual minimum temperature for growth increases with decreasing water activity. That is, energy required to deal with the water activity hurdle is unavailable to overcome the temperature hurdle, and, as a result, the minimum temperature for growth must increase. McMeekin et al. (2002) argued that this explanation was not consistent with the observation of nearly constant cell yields over a wide range of temperature or water activity values. However, the primary models relating the logarithm of cell numbers to temperature or water activity must be interpreted carefully as an apparently small reduction in cell numbers or biomass on a logarithmic scale may represent a significant percentage reduction in population size when considered arithmetically. Nevertheless, when compared to reductions in cell mass occasioned by reduced pH, the cell's ability to withstand temperature/water activity constraints is much less energy demanding (Krist et al., 1998a). Further insight into the energetics of growth may be obtained by modelling the dependence of activation energy on

temperature and water activity. This type of plot reveals that the NPR, where activation energy values are approximately constant, spans a wider temperature range in the absence of a water activity constraint than in its presence. The effect of truncating the NPR can be partially ameliorated by addition of a compatible solute, glycine betaine (Krist et al., 1998b).

6.6. Growth/no growth interface models

A recent trend in predictive modelling has been the development of growth/no growth interface models that predict the probability of growth occurring when a population is faced with more than one constraint. A procedure to develop interface models was reported by Ratkowsky and Ross (1995) and commentary on developments in this facet of modelling was presented by McMeekin et al. (2000, 2002). Several common features appear to characterise interface models.

(i) Experimental observations of conditions just preventing growth suggest that the interface is sharply defined (Presser et al., 1998; Salter et al., 2000; Tienungoon et al., 2000; McKellar and Lu, 2001). The latter authors commented ''We observed an abrupt transition between growth and no growth; of 1820 combinations of experimental conditions with five replicates of each, all replicates either grew or did not grow''. This consistent observation lends support to the concept of a critical activation energy under a given set of conditions.

(ii) Tolerance to low water activity and pH is not optimal near the optimum temperature for growth rate. For example, with *E. coli* growth at the lowest, a_w (0.948) occurred at 25–30 °C with a minimum a_w of 0.951 at 37 °C.

(iii) The synergistic interaction of temperature and water activity and/or pH can be quantified and combinations preventing growth specified. For example, with *E. coli*, at 20 °C, a water activity of $\sim$ 0.95 is required, but at 10 °C, a water activity of $\sim$ 0.97 will suffice to prevent growth.

Location of the interface between growth and no growth maps precisely an area of intense physiological interest. Many of the events occurring in this region will not be intuitive and may well be reversed when the interface is crossed. Reversal of consequences is illustrated by the effect of compatible solutes. For growing cultures of *E. coli*, these increase the rate and temperature range for growth, but in non-growing cultures, the death rates may be enhanced (Krist, 1997). Presumably, this occurs because uncoupled biosynthetic and energy-yielding enzymes continue to operate in an uncoordinated way as a result of the protective effect of compatible solutes. Further, when the growth of *E. coli* is inhibited by reducing a_w, populations decline more rapidly at 25 °C than at 4 °C (Shadbolt et al., 1999). Thus, it appears that the interface may be a region where the physiological coin flips. However, whilst the growth/no growth interface may be the point at which biosynthetic functions cease, it represents an end point of progressively decreasing biosynthetic functions and increasing survival functions. The pattern depicted by an Arrhenius curve leads to the suggestion that this cascade starts at the end of the NPR.

6.7. Death models

While traditional thermal death models are well established in the food industry, attention is now being focussed on mild heat processes designed to produce foods, the safety and shelf life of which are assured by distribution at chill temperatures or in combination with other hurdles.

Models are also required to describe the effects of using alternative physical treatments such as high-pressure processing or pulsed electric fields. Several non-thermal death models are available, but for the most part, non-thermal death kinetics remain at the 'pattern' stage. Nevertheless, these patterns provide clues to areas on which physiological studies should concentrate and from which preservation strategies might be developed. Non-thermal death models are a logical extension in the continuum from growth and interface models, but will require considerable effort in experimental design and execution. Whilst indirect methods, such as turbidimetry, have been valuable adjuncts to viable counts in the development of growth and interface models, it will be difficult to circumvent the need for time-consuming viable counts as the standard method for construction of non-thermal death models.

7. Preservation, microbial physiology and predictive microbiology

7.1. The paradox

Whilst predictive microbiology provides invaluable information for the production of safe food with adequate shelf life, truly innovative food preservation initiatives will only be derived by combining quantitative microbial ecology with improved understanding of physiological responses to environmental constraints used in food processing.

In considering the origins of predictive models in the food industry, we cited the botulinum cook of Esty and Meyer (1922) as an early, perhaps the first, example of a model to find widespread utility. Because of the serious consequences of botulism, the thermal process proposed incorporated a large safety factor. Thus, the probability of a "fail-dangerous" outcome was zero for a properly controlled thermal process and this, despite complicating factors such as deviations from log-linear death kinetics, manifested as shoulders and tails. Today's food industry, however, services "sophisticated" consumers who demand products that are minimally processed and contain less additives thereby retaining superior nutritional and sensory properties and maintaining these characteristics for an extended period. Herein lies the paradox of balancing "clean, green" aspirations versus the risk (to safety or shelf life) of removing traditional barriers to microbial development. It is our hypothesis that the paradox of designing minimally processed, yet microbiologically safe foods can only be resolved by detailed knowledge of the ecology and physiology of food-borne microorganisms.

The requirement for ecological and physiological knowledge to underpin developments in preservation is, of course, not new and one finds a call to link ecology and physiology in the writings of Monod (1949). A reference point emphasising the nexus between advances in preservation technology and physiology is found in a special issue of the International Journal of Food Microbiology [28 (2) (1995)] reporting on the work of a European Union research program on the physiology of food poisoning microorganisms.

In that volume, the general thrust of the Concerted Action Program was addressed by Gould et al. (1995) using the key words food poisoning, food industry and microbial physiology. Connections were also made to predictive modelling and HACCP but not to QMRA consistent with the chronology of development of these food safety initiatives. The ecology/physiology/preservation triangle was further emphasised by Brul and Klis (1999) who addressed mechanistic and mathematical inactivation studies of food spoilage fungi.

In the preceding section, we drew attention to modelling studies, suggesting fertile areas for physiological investigations, and below we speculate on the potential of these observations to be used in the development of preservation methods.

7.2. Energy depletion

Energy depletion has been proposed as a central mechanism to control microbial populations (Knøchel and Gould, 1995). This suggestion is supported by the results of Shadbolt et al. (2001) who demonstrated that the sequence in which hurdles are applied has an important influence on death kinetics. Specifically, applying acid stress before osmotic stress is much more effective in killing *E. coli* than osmotic followed by acid stress. We hypothesise that this is due to the energetic burden imposed by maintaining internal pH homeostasis that renders the cell susceptible to imposition of a second hurdle (osmotic stress). Energy availability considerations were also featured prominently by Brul and Coote (1999) in their excellent review "Preservative agents in foods" when they concluded that:

> Ultimately, the amount of available energy will determine the extent to which a given microbial cell can have various stress response pathways activated. Stress responses leading to adaptation are energetically expensive and a microbial cell must strive to maintain a balance between the induction of energy consuming mechanisms used to restore homeostasis and retaining sufficient energy to continue general housekeeping functions that allow growth. The link between macroscopic bioenergetic parameters (growth rate, yield) and microscopic bioenergetic parameters (substrate utilisation, ATP levels, ATP/ADP ratios, intracellular redox balance), and the

molecular reactions in stress response mechanisms, is a field of research that is only just emerging.

7.3. The normal physiological range

The observation of Nichols et al. (2000) of a significant perturbation in membrane fatty acid composition of *S. gelidimarina* has now been reported for *L. monocytogenes* (Nichols et al., 2002) and is worthy of further investigation for food-borne pathogens. Not only does it provide, in part, a physiological explanation for the extent of the NPR, but potentially identifies an area where the organism could be vulnerable to the application of a second membrane disturbing constraint. Studies are also required to determine if similar perturbations occur in other aspects of biosynthesis at the ends of the NPR.

7.4. The cell membrane as a target

As indicated above, modelling studies have identified combinations of environmental conditions that progressively limit and eventually prohibit growth and beyond which death may ensue as a result of energy depletion within the cell. In turn, this and NPR observations identify the cell membrane, a major site of energy-yielding metabolism, as a target for inimical process. The maintenance of homeostasis requires an intact cell membrane; therefore, any treatments that disrupt membranes or interfere with energy conservation will hinder or abolish the homeostatic capacity and will eventually lead to cell degeneration and death (Mackey, 1999). Thus, it is not surprising that many emerging preservation treatments, both chemical (nisin) and physical (high pressure), target the cell membrane.

Membrane transport processes are central to adaptive responses and as such demand intensive investigation in the search for effective minimal processing technologies. A promising avenue of research involves use of the microelectrode flux estimation system (MIFE), originally designed for use with plant cells (Shabala et al., 1997; Shabala, 2000). That system has recently been adapted to study microbial films immobilised on a glass surface (Shabala et al., 2001). MIFE provides, in real time, information on the cascade of ion transport into and out of the cell in response to stresses applied. It markedly decreases the time required to obtain information on the efficacy of inimical processes compared with conventional microbiological techniques and powerfully complements other real-time observations such as those providing information on internal pH changes by fluorescence ratio imaging microscopy (FRIM) (Shabala et al., 2002).

A myriad of opportunities exists to study microbial stress responses using the MIFE system. These may be characterised on the basis of the type of stress imposed and the target organism. Examples of stresses include acidic and osmotic stresses and those induced by antimicrobial agents such as sanitisers, detergents, nisin and other membrane active antimicrobials. Each of these stresses could be characterised in relation to its magnitude, the rate of change of environmental conditions and the sequence of application of stresses. The overall outcome will be to optimise the hurdle combinations necessary to specify minimal processing conditions consistent with food safety. Where the target organisms are food-borne pathogens, or spoilers, the aim will be to determine susceptibility to stress. Conversely, the outcome may be to select strains for biotechnological use or as probiotics that display enhanced tolerance to environmental stress (e.g. *Lactobacillus* strain *Shirota*, the Yakult™ organism).

In addition, MIFE has the potential to provide insights into several seemingly intractable problems in food microbiology, viz. membrane-associated events leading to resolution of the bacterial lag phase and the viable but non culturable (VBNC) state, aspects of which were reviewed by Mackey (1999), and the appearance of stress-resistant populations and methods to delay this phenomenon. Particular advances are projected in knowledge of the functional genomics of microbial adaptability and stress tolerance when MIFE studies of appropriately selected mutants are combined with other techniques in cell and molecular biology.

7.5. The hurdle concept and growth/no growth interface models

For many years, Professor Leistner and his colleagues at the Federal Centre for Meat Research in Kulmbach, Germany, have advocated food preserva-

tion by combined methods (the Hurdle Concept). The essence of this approach is that foods can remain stable and safe even without refrigeration, and are acceptable organoleptically and nutritionally due to mild processes (Leistner, 1978). The mode of action of combined hurdles may be additive or even synergistic with the latter deserving particular attention as a means to select constraints that best achieve microbial stability and safety (Leistner, 1992). That synergism is anticipated, derives from the effect of hurdles on separate targets within the cell which disturb homeostasis by different mechanisms. Whilst the Hurdle Concept is widely accepted as a food preservation strategy, its potential has still to be fully realised as it is largely a qualitative concept, the application of which is often empirical. The intelligent selection of hurdles in terms of the number required, the intensity of each and the sequence of application to achieve a specified outcome provides significant potential to approach the edge of the "food safety cliff" with certainty (McMeekin et al., 2002). This widely used analogy is attributed to Dr. M.B. Cole in a presentation to the 1994 Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians (Zwietering, 2002).

A practical outcome of bacterial growth/no growth interface models is to define that set of hurdles that will ensure product stability or preclude the possibility of pathogen development. In other words, a quantitative description of the hurdle concept is now available on which to balance the competing claims of a reduction in preservative levels versus microbial activity.

7.6. Non-thermal death kinetics

The development of non-thermal death models is consistent with the desire for minimal processing and reduced additives in foods. As indicated earlier, we currently have access to patterns of death rather than models, but from these, for example, the studies of Shadbolt et al. (1999, 2001) and Brown (2002), we can conclude that:

1. The kinetics, and by inference the mechanisms, of inactivation of *E. coli* differ for water activity and pH.

2. Severe osmotic shock induces biphasic behaviour in which a rapid initial decline in viability (phase 1) is followed by a phase of gradual decline that may extend over several days (phase 2).

3. When exposed to low pH (<4), *E. coli* populations exhibit three distinct phases of inactivation. Phases 1 and 2 are similar to those observed with a_w stress. Phase 3 is a phase of rapid decline with rates similar to that observed in phase 1.

4. The magnitude and rate of phase 1 decline is affected by the severity of water activity and to a lesser extent by the pH shock imposed on the population.

5. The rate of decline of the population in phase 2, whether the growth suppression is caused by water activity or pH, is not greatly affected by the severity of the water activity or pH stress imposed.

6. Temperature potentiates the rate of death in phase 2.

7. Triphasic pH death kinetics are observed in "exponential" growth phase cultures as judged by conventional methods in which the inoculum used for death experiments is derived from rapidly dividing cultures. In reality, these "exponential" cultures contain a proportion of stationary phase cells.

8. When special precautions are taken to prepare totally exponential phase cultures, the pattern of decline changes with phase 2 eliminated or greatly reduced. This observation confirms that stationary phase cells display greater resistance to low pH treatments than exponential phase cells.

9. When 'older' stationary phase cells are used as the inoculum, resistance to acid conditions decreases. Maximum tolerance is shown by cells after $\sim$ 24 h in the stationary phase.

10. The sequence of application of hurdles affects the pattern and extent of decline. When cultures initially exposed to lowered a_w (0.90) experienced a pH of 3.5, a second rapid period of rapid inactivation was observed before a second phase of gradual decline. When the hurdles were reversed (pH 3.5/a_w 0.90), the population was rapidly inactivated to levels below the limit of detection.

The observations reported above may have practical implications for the design and evaluation of preservation techniques, for example, in the manufacture of fermented meats.

The rapid third phase of decline in response to reduced pH, observed in laboratory media by Brown (2002), requires examination in salami batters at pH values appropriate to the fermentation process.

The effect of sequence and timing of water activity (a_w) and pH constraints (Shadbolt et al., 2001) may have relevance in processes where pH and a_w hurdles are imposed. This avenue of investigation represents a new opportunity, as hurdle sequence effects have not been previously reported.

Temperature, water activity and pH are major factors impacting on the rate of decline of *E. coli* during the production of uncooked, fermented meats and the potential of increased, non-lethal temperatures to enhance the rate of decline is clear from the results of Shadbolt et al. (1999). Using this data, Ross and Shadbolt (2001) concluded that, in the range of conditions likely to be experienced in preparation of uncooked fermented meats that variation in temperature will have a much larger effect on the rate of inactivation than variation in water activity. The difference in inactivation rate due to temperature at 10 °C compared to 40 °C is of the order of 400-fold at any water activity, while the difference due to water activity in the range 0.85–0.95 is ~ 2–3-fold at any temperature.

Patterns of non-thermal death also have significant implications for the design and conduct of survival experiments. The greater susceptibility of exponential versus stationary phase cultures to inimical conditions is conventional wisdom. However, the observation of Brown (2002) that "overall" population resistance reaches a maximum at ~ 24 h post-inoculation and declines in older stationary phase cultures, indicates that a standard procedure for preparation of inocula for death kinetics experiments is necessary. This is reinforced by a report on sensitivity of *Staphylococcus aureus* to disinfectants in a suspension test by Luppens et al. (2002).

The decline in resistance of older stationary phase cultures and the discovery of mixed physiological states within apparently exponential phase cultures should also be considered when interpreting the significance of non-thermal death kinetics studies reported in the literature.

7.7. Do predictive models work in practical situations?

The question of whether predictive models work in practice has been the subject of numerous verbal and written commentaries and debates. Advocates point to various successful applications (for reviews, see McMeekin and Ross, 1996; Ross and Olley, 1997), while others have demonstrated that models can fail. While so-called 'fail-dangerous' predictions, in which the extent of microbial growth is underpredicted or the extent of death overpredicted, have been reported (Walls et al., 1996), most reports indicate that models are usually too conservative (Walls and Scott, 1996, 1997b; Dalgaard and Jørgensen, 1998; Lebert et al., 2000; Kleer and Hildebrandt, 2001; Wilson et al., 2002). From both producers' and consumers' perspectives, overprediction of growth (or risk) is undesirable because the losses incurred in incorrectly discarding acceptable products are ultimately borne by consumers.

Objective measures to assess model performance have been proposed (Ross, 1996; Baranyi et al., 1999), and critical values of these measures suggested as criteria for acceptance of models (see Ross et al., 2000). Many models currently available do satisfy these criteria when compared to appropriate observations.

As yet, however, there is no universally accepted set of criteria by which a model is considered to be "valid". Nonetheless, these measures enable differentiation of models that occasionally predict poorly due to variability in microbial responses, from models that consistently predict poorly. To date, most models have considered relatively simple systems in which temperature, pH, water activity, amount of nitrite and organic acid levels are the main factors controlling growth (or other microbial responses), but which do not consider the effect of other microbiota, critical levels of other agents, or the physical structure of the food, on the potential for growth of the modelled organisms. This has been termed model "completeness error".

A craftsman may require many variations of the same basic tool for specific tasks. By analogy, completeness error simply indicates that the wrong tool is being used to perform a task—not that the task cannot be done or that the tool does not work! Among several other sources of error (discussed in Ross et al., 2000), completeness error is the largest source of error encountered in the application of most predictive microbiology models. When completeness error limits model performance, the appropriate response may be to adopt the iterative approach (Dalgaard et al., 1997, 2002), that is, to develop a model that is specific to the

intended application, not to abandon the predictive microbiology concept. When applied situations in which model performance is not compromised by completeness error, model performance can be very good. Rasmussen et al. (2002) provides an excellent example, in which a model developed to describe the growth of pseudomonads in the dairy industry and shown to work well for liquid milk products (Neumeyer et al., 1997a,b), was applied to characterisation of the shelf life of fish fillets. The agreement between model predictions, and those observed, including the effects of variability was remarkably good.

It is noteworthy that identifying avenues for change does not necessarily require the construction of a fully validated predictive mathematical model. Indeed, in the examples cited above for non-thermal death kinetics, the *patterns* of population responses are sufficient to suggest modification of processing times and/or temperature will more effectively enhance the rate *E. coli* inactivation than changing product formulation. Fully validated models may follow and allow precise optimisation of those changes, but the response patterns identified the opportunity.

7.8. Application software: the key to using predictive microbiology to manage change

We have suggested, in general terms, that predictive microbiology has a role to play in supporting food safety initiatives such as HACCP and microbial risk assessment. As such, the systematic accumulation and storage of knowledge embodied in predictive models has contributed to and facilitated the changed philosophy of managing food safety issues proactively rather than by retrospective end-product testing. We have also drawn attention to the role of the systematic nature of predictive microbiology studies in revealing unexpected physiological responses in narrow regions that may be exploited to develop novel preservation procedures or to allow refinement of existing methods.

However, successful use of models by industry for specific applications depends on the development of appropriate application technologies. Whilst chemical, enzymic and physical indicators have been proposed, applications have largely been based on computer software that automates predictions, or the integration of software with temperature recording devices (for reviews, see McMeekin et al., 1993; Dalgaard et al., 2002).

There are a number of free commercial software programs that provide predictions of microbial growth or growth rate and lag time under defined conditions. These include the USDA's Pathogen Modeling Program (Buchanan, 1993) which is available without charge from: http://www.arserrc.gov/mfs/pathogen.htm. A similar suite of software (Food MicroModel, sponsored by the UK government) was developed by a consortium of industry and government researchers (McClure et al., 1994; Panicello and Quantick, 1998); see also: http://www.lfra.co.uk/micromodel/index.html. Seafood Spoilage Predictor was developed to predict shelf life of seafood at constant and under fluctuating temperature storage conditions (Dalgaard et al., 2002). The software can read data from different types of loggers and in this way evaluate the effect of fluctuating temperatures on shelf life of seafood using several models for specific spoilage organisms of seafoods. It is available from http://www.dfu.min.dk/micro/ssp/. Food Spoilage Predictor is similar commercial software (see http://www.hdl.com.au/html/products.htm) that models the effect of water activity and fluctuating temperature on the growth of psychrotolerant pseudomonads (Neumeyer et al., 1997a). Food Spoilage Predictor can also read and evaluate temperature profiles collected by electronic temperature loggers and its performance is discussed further below. While various intrinsic and extrinsic factors can be critical to the safety of foods (e.g. a critical pH or water activity are used to control safety of low acid foods or of fermented meats), it is generally conceded that temperature is the factor that is most likely to change, and to affect the potential for pathogen growth and product safety, along the farm-to-fork chain. The influence of time is also critical and an additional benefit of temperature loggers is that they record time so that a full record of the age of the product, and the temperatures to which it has been exposed, is acquired.

The most widespread application of predictive models has been to assess the hygienic efficiency of meat-processing operations, a use in large part derived from the work of Gill and colleagues in New Zealand and Canada allowing comparison of processes through use of the process hygiene index or, where shelf life was the criterion to be predicted, the process

spoilage index. Specific applications from this work include: validation of meat-thawing procedures (Lowry et al., 1989); offal cooling (Gill and Phillips, 1990; Gill and Jones, 1992a,b); conventional carcass cooling (Gill et al., 1991a); spray cooling (Gill et al., 1991b); hot boning processes (Reichel et al., 1991). A good example of continued application of Gill's work was given by Jones and Phillips (1999) who examined the ability of three lamb processes (carcass chilling, leg processing and tenderloin processing) to allow bacteria to contaminate sterile meat and then to proliferate during subsequent cutting and cooling operations. For each process, 'hazard' sites and points of hazard control were identified and incorporated into a descriptive flow diagram. Product temperature histories were then used to illustrate the microbial growth potential of each process phase. In the Antipodes, this type of application is also evident in the Australian meat industry (Sumner and Krist, 2002) and is used by regulatory authorities (Armitage, 1997; Sumner and Krist, 2002).

While temperature loggers and interpretive software may be the "public face" of predictive microbiology applications, a great deal of activity is hidden within the food industry as part of expert systems and decision support systems. These types of applications technology were described in the literature a decade ago (Zwietering et al., 1992; Adair and Briggs, 1993) and are now widely used by major food manufacturers (M. Cole, pers. comm.).

Finally, there are two criteria that must be satisfied if any form of application software, incorporating a predictive model, is to be used effectively. The first is a properly developed and validated model and the second the ability of an operator to interpret correctly the microbiological significance of the results. Poorly performing models coupled with poor interpretation continue to be the greatest threat to widespread use of predictive microbiology as a technology with the potential to assure the microbiological quality and safety of foods.

8. Conclusions

The value of predictive microbiology models is becoming increasingly recognised in HACCP and risk assessment where quantitative information on microbial behaviour in foods is required for effective application of those approaches to ensure and evaluate food safety. This obvious role for predictive models is complemented by the value of quantitative information to indicate parameter values for the development of mild preservation techniques. Combining predictive microbiology with studies of microbial physiology offers the prospect of a mechanistic understanding of cell and population behaviour. In turn this may provide the opportunity to exploit microbial responses to change as a means to resolve the paradox of minimal preservation requirements consistent with safety and stability.

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