Control of vegetative micro-organisms in foods

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Microbes share our food whether we want them to or not. We need to control microbial proliferation in foods in order to avoid spoilage, to enhance flavour and, most importantly, to reduce the risk of food-borne illness. A broad spectrum of interventions are available to control microbial growth, but the most widely used is temperature. The use of temperature to control metabolically active bacteria is discussed briefly in the context of current practices. The marketing and legislative climate has provided an impetus to develop an ever-widening range of systems for microbiological control. This short review highlights some of the problems associated with such novel control systems, including selection of new spoilage agents or food-borne pathogens, and the difficulties of monitoring the efficiency of microbial control in the light of a better understanding of bacterial physiology.

Micro-organisms are inextricably linked with the food we eat. We may sometimes employ rigorous processing procedures to eliminate them but in the main we tend to accept their presence and have decided to adopt a strategy of containment rather than elimination. We, therefore, allow for the presence of undesirable micro-organisms in raw materials but attempt to control the microbial population of foodstuffs that are offered for sale or consumption. Control of vegetative (*i.e.* metabolically active) micro-organisms in food-stuffs is exercised for a number of reasons including prolonging shelf-life by minimising spoilage, or encouraging the growth of organisms that have a desirable trait, such as imparting a particular flavour to the food. However, the primary reason for control is to enhance safety by preventing the growth or activity of disease causing organisms.

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Food-borne bacterial pathogens and disease

Periodic reviews indicate that diseases caused by food-borne bacterial pathogens are a world-wide, and increasing, public health problem[1–4],

varying with demographics, industrialisation and centralisation of food production and supply, travel and trade, and microbial evolution and adaptation. Symptoms vary greatly from mild to severe or occasionally fatal gastro-enteritis. Food-borne disease is usually acute, but can also become chronic with long-term sequelae.

In spite of the ubiquitous nature of food-borne illness, relatively few bacteria are recognised as significant food hazards. Until the mid-1990s, in many countries the combined annual total number of outbreaks of illness attributed to salmonellae, *Staphylococcus aureus* and *Clostridium perfringens* often represented 70–80% of the reported bacterial disease outbreaks. With improved refrigerated storage during food distribution and use, *Staph. aureus* and *C. perfringens* now cause illness only when the food has been temperature abused. Improved refrigeration has also lengthened the shelf-life of foods leading to concerns that psychrotrophic pathogens may increase to dangerous levels without spoilage being evident to the consumer. Micro-organisms of most concern are non-proteolytic strains of *C. botulinum* types B, E and F, *Listeria monocytogenes* and *Yersinia enterocolitica*, all of which cause little or no deterioration of the food supporting their growth.

Botulism is the result of ingesting pre-formed toxin, usually the result of spores of *C. botulinum* surviving a process, germinating and growing under conditions in the food that do not prevent multiplication of vegetative cells. While botulism is relatively infrequent, it remains a serious concern because of its life-threatening nature and the impact on trade in the incriminated product type. Over many years, home-canned or home-prepared foods have mainly been responsible for the disease. In recent years, commercially prepared products have been implicated due to faulty processing and/or inappropriate storage temperatures and examples include: inadequately heat-processed hazelnut yoghurt[5], unheated chopped garlic-in-oil, containing no acidulants or preservatives[6], commercially roasted eggplant (aubergine)[7], and a commercial cheese sauce[8].

Disease caused by *L. monocytogenes*, although not common, can be severe with a high mortality rate. Listeriosis is most common in at-risk populations such as pregnant women, infants and the immunocompromised. The organism is ubiquitous in agricultural and food processing environments. Foods implicated in outbreaks have included raw milk products, ready-to-eat meat products, frankfurters, sausages, smoked fish and vegetables. Disease caused by *Y. enterocolitica* occurs world-wide and is often associated with the consumption of raw or undercooked pork.

Salmonellae have long been considered the most important food-borne pathogen in many countries. Foods commonly implicated in outbreaks of salmonellosis include meat and poultry, eggs and egg products, milk and milk products, seafood, fresh produce and spices. In recent years, the incidence of disease caused by *Campylobacter jejuni/coli* has

exceeded that due to salmonellae in several countries[9]. Outbreaks of campylobacteriosis are most commonly associated with undercooked poultry as well as cross-contamination of various foods from raw poultry. Other foods and untreated water have also been implicated.

Escherichia coli strains are a common part of normal microbial flora of animals, including man. Most strains are harmless, but some strains cause diarrhoea, while strains bearing particular virulence properties have emerged as a serious hazard, consumption of low numbers causing life-threatening illness. During the last two decades enterohaemorrhagic *E. coli* (EHEC), especially *E. coli* O157:H7, has emerged as a serious food-borne hazard. EHEC infection can be severe causing bloody diarrhoea, haemolytic uraemic syndrome (HUS), and even death. The disease typically affects children, and the infective dose is low. The reservoir of *E. coli* O157:H7 is the intestinal tract of ruminants and a common source of infection is undercooked ground (minced) beef. However, other foods have been implicated, including fresh vegetables, unpasteurised milk and recreational waters. Outbreaks have also been traced to unpasteurised apple juice, vegetable sprouts, yoghurt, fermented sausage, and contact with farm animals[10].

Bacillus cereus and other *Bacillus* spp. have been responsible for food-borne disease, but normally cause problems only after poor handling and/or temperature abuse. Some strains are able to multiply and produce enterotoxin at domestic refrigeration temperatures.

Where raw fish is a major part of the diet, disease caused by *Vibrio parahaemolyticus* is frequent. However, disease caused by *V. parahaemolyticus* and other *Vibrio* spp. has been reported in western countries, but the vehicle of transmission is usually processed rather than raw sea-foods. *Vibrio cholerae* is endemic in many tropical countries and water plays a major role in the epidemiology of cholera.

Shigella spp. Also represent an important public health problem in many third-world countries. Cases of shigellosis reported in developed countries are often associated with travelling. As the reservoir of *Shigella* spp. is restricted to humans, the source of infection is food or water contaminated by human carriers.

Microbial contamination of food and food processing

Most food preservation processes developed empirically, *e.g.* converting milk into cheese, curing pork, salting and drying fish. Micro-organisms were first observed and described by Leeuwenhoek in 1683, and only in 1837 did Pasteur first associate bacteria with food spoilage. The demonstration that diseases are transmitted via foods also came in the

19th century. Hence, for most of man's history, food spoilage and disease transmission have been dealt with in ignorance of the responsible agents.

Raw agricultural products carry a wide range of microbes making up the 'primary contamination', varying with commodity, geographic region, and production and harvesting methods. Crop and animal diseases are controlled stringently because losses affect producers, but the two major concerns for the food industry attributable directly to microbes are: (i) losses of products before consumption due to spoilage; and (ii) costs associated with food-borne disease.

From the point of view of the food processor, the primary microbial contamination of animals and plants is rarely under such control that complete freedom from particular hazardous microbes can be guaranteed. Nor does mechanization necessarily improve hygiene or the microbiological conditions of foods. Modern mechanized slaughter-houses have not provided raw meat that is less heavily contaminated with bacteria, partly because the emphasis remains on high through-put rather than high standards of hygiene.

Efforts continue to minimize the occurrence of microbes able to cause illness, but attempting to control this initial flora is often less cost-effective than applying well-established preservative measures later in the food-chain. With available technology there seems little prospect that complete freedom of particular microbes from new foods will ever become possible; indeed, intensive animal production has in some cases worsened the situation considerably, *e.g.* with respect to salmonellae.

Hence food processors identify the microbes of concern that could be present and consciously take measures to either kill them, or to ensure that they are unable to multiply in their products. Further processing (*e.g.* slicing and repacking) often recontaminate meat products with bacteria of human origin, commonly *Staph. aureus* and occasionally salmonellae and *L. monocytogenes*.

Animals have long been recognised as a major source of several pathogens, faeces being the origin of salmonellae, campylobacters, enterotoxigenic *E. coli*, *C. perfringens*, *C. botulinum* and *L. monocytogenes*. Although it has long been recognised that fruit and vegetables can be contaminated with soil, only in recent years has the association of produce with food-borne illness become obvious and frequent[11].

Control strategies: inactivation of vegetative cells

Brown (this issue) deals with control of bacterial spores. It should be emphasised that, if spores survive a heat process, control of that micro-organism during food storage is by preventing multiplication of its vegetative cells.

The most certain means of controlling bacteria in foods is to inactivate them by heating. Their resistance to heat varies with the species and with the environment in which they are heated. Products of low pH can be rendered stable and safe by lower heat processes than those of neutral pH, because bacterial spores are more sensitive to heat in acid conditions. Some bacteria of concern are unable to grow at acid pH values (*e.g. C. botulinum* will not grow below pH approximately 4.5 except under

Table 1 Growth-limiting conditions for common food-borne bacterial pathogens

	Growth temp min (°C)	Growth temp opt (°C)	Growth temp max (°C)	°C	Heat D-value minimum	pH min	pH opt	pH max	Inhibited by % NaCl	a_w	Ref
Aeromonas spp	>0<4	26–35	>42<45	55	0 2	<4 5	7 2	?	>5<6	0 96	[53]
**B cereus*	10†	30–40	55	Spores		5	6–7	8 8	ca 10 5	0 93	[53]
**B cereus*	10†	28–35	50	Spores		4 35			ca 10	0 912	[54]
Campylobacter spp	32	42–43	45	60	0 2–0 4	4 9	6 5–7 5	ca 9 0	–	?	[53]
Campylobacter spp	32	42–45	45	55	0 74–1 0	4 9	6 0–8 0	9 0–9 5	>1 5<2 0	0 99	[54]
**C botulinum* (prot)	ca 10	35	ca 47	Spores		4 6	6 5–7 0	–	ca 10	0 935	[53]
**C. botulinum* (prot)	ca 12	30–40	48	Spores		4 6	7	9	ca 10	0 935	[54]
**C. botulinum* (non-pr)	ca 3 0	27–30	ca 33	Spores		ca 5 0	6 5–7 0	?	ca 5 0	0 97	[53]
**C perfringens*	12	43–47	ca 52	Spores		5 5–5 8	7 2	8 0–9 0	ca 10	0 935	[53]
**C perfringens*	12	43–45	50	Spores		5	6 0–7 5	8 3	ca 6 0	0 964	[54]
E coli	ca 7 0–8 0	35–40	44–46	60	0 75	4 4	6 0–7 0	9	ca 8 0	0 95	[53]
L monocytogenes	ca 0	37	45	70	0 1–0 2	4 4	7	9 4	ca 11 5	0 92	[53]
L monocytogenes	0	25 3	45	70	0 14–0 27	4 4–4 6	6 5–8 0	9 5	ca 10	0 935	[54]
Salmonellae	4 8–5 2‡	35–43	46	60	0 2 (a)	3 8	7 0–7 5	9 5	9 5	0 94	[53]
				90	40–80 (b)						[53]
Salmonellae	5 1	37	45–47	60	0 06–0 1	4	6 5–7 0	9	ca 8 0	0 95	[54]
Shigellae	ca 6 0	47	?	–	–	4 9	–	9 4	ca 5 0	0 97	[53]
Staph aureus	ca 7	37	48	60	5 0–15 0	4	6 0–7 0	10	ca 14 0	0 83	[53]
Staph aureus	11	37	48	61 7	20	4	6 0–7 0	9 8–10	ca 14 0	0 83	[54]
V parahaemolyticus	5	37	43	55	2 5	4 8	7 8–8 6	11	ca 10 0	0 935	[53]
V parahaemolyticus	12 8	37	42–43	60	ca 1 0	4 5–5 0	7 5–8 5	11	ca 8 5	0 948	[54]
Y enterocolitica	–1	25–37	42	55	ca 2 0	4 2	7 2	>9 6<10 0	> 5<7	0 964	[53]
Y. enterocolitica	0 to –1	32–34	44	62 8	0 1–1 0	4 6	7 0–8 0	9	>5<7	–	[54]

Tabulated values are for single controlling factors when other factors are close to optimal In reality control is achieved by combinations of sub-optimal growth conditions

*Spores if spores survive a process, control during food storage is by preventing multiplication of the vegetative cells

†Most strains ca 10°C, some strains grow at 4–5°C

‡Salmonellae most serotypes fail to grow below 7°C

(a) Salmonellae when heated in 'wet' environments

(b) Salmonellae when heated in 'dry' environments such as chocolate

prot = proteolytic; non-pr = non-proteolytic, max = maximum, opt = optimum, min = minimum

unusual circumstances not normally found in foods). Hence pH also plays an important role in food preservation.

Inactivation occurs more rapidly with increasing temperature. The death rate of vegetative bacteria increases 10-fold for every (approximately) 5°C increase in temperature within the lethal range. Most vegetative pathogens are inactivated almost instantly above about 70°C unless heating occurs at low water activities, *e.g.* as occurs in chocolate, when the heat resistance increases very substantially (Table 1).

Thermal treatment processes are designed around a limited number of parameters, *e.g.* time, temperature, pH, and a_w. They are calibrated with selected test organisms to achieve a particular reduction in numbers (*e.g.* 4-decimal [4-D] reductions of *L. monocytogenes*). For example, the process for milk pasteurisation in the US is 71.7°C for 15 s[12]. This combination of temperature and time will assure the destruction of *Coxiella burnetii*, as well as other non-sporeforming pathogens that are known to occur in raw milk. In other foods, specific heat treatments may be required. For example, in the US, minimum heat processes (any one of 16 time and temperature combinations equivalent to instant heating to 63°C) targeted at killing salmonellae must be used for the manufacture of pre-cooked roast beef joints for chilled or frozen distribution[13,14]. A similar requirement (equivalent to instant heating to 70°C) is mandatory for fully cooked meat patties[15], aimed at destruction of *E. coli* O157:H7.

Design of thermal treatment processes must also take into account subsequent storage conditions such as use of modified atmospheres and vacuum systems, *etc.* Whilst *L. monocytogenes* is the target organism for 'short shelf-life' chilled foods, psychrotrophic strains of *C. botulinum*, which are able to multiply and produce neurotoxin even at 4–5°C, are the main organisms of concern in perishable, 'extended shelf-life' chilled foods (shelf-life at chill longer than 10 days), including the so-called '*cuisine sous-vide*' products.

The UK Advisory Committee on the Microbiological Safety of Foods (ACMSF), in its report on vacuum packaging and associated processes[16], made a series of recommendations on the safety of 'extended shelf-life' vacuum-packed foods. These were the use of a heat treatment such that all components receive a minimum heat process of 10 min at 90°C (6-D reduction of group II [psychrotrophic] strains of *C. botulinum*); or to reduce the a_w of all components to 0.97 (equivalent to 3.5% w/w sodium chloride) or less; or to reduce the pH to 5.0 or less; or to use a combination of preservation treatments capable of giving equivalent security against group II strains of *C. botulinum*[17]. Similar requirements to these are incorporated in the guidelines recently published by the European Chilled Foods Federation[18]. National guidelines or regulations for vacuum-packed and other extended shelf-life meat products exist in many countries including the US[19], Canada[20], Australia[21] and Europe[22,23].

The cooking process given to industrially produced cooked meats will effectively destroy all vegetative bacterial pathogens, viruses, parasites and most vegetative spoilage micro-organisms. 'Faecal streptococci' (enterococci) and certain lactobacilli are relatively heat resistant and often survive commercial pasteurisation processes and multiply slowly, even under good refrigeration.

Recognising that such processes will not destroy all organisms, specific cooling requirements are also specified. For meat joints this requires them to be rapidly and continuously cooled such that the time between temperatures 48.9°C and 12.8°C does not exceed a total of 6 h, with cooling continuing until a temperature of 4.4°C is reached; this is intended to prevent the growth of spore-forming bacteria. In the UK, there are Department of Health guidelines for the manufacture of pre-cooked chilled and frozen foods for catering[24], and trade guidelines for pre-cooked chilled meals and meal components sold through retail outlets[25]. These guidelines specify both cooking and cooling requirements and also hygienic practices to prevent contamination of the cooked product. Similar guidelines have been developed by other countries and can be useful sources of information[26,27]. The cooking process required for products with a shelf-life of 10 days or less is a heat process equivalent to heating to a minimum of 70°C for 2 min. This will give at least a 6 decimal [6-D] kill of *L. monocytogenes*[28].

Control strategies: growth limitation

Vegetative bacteria multiply less rapidly as the temperature falls and maintaining the storage temperature at, or below, the minimum temperature for growth is the most common means of preventing their growth. Storing foods in vacuum packs or under modified atmospheres, commonly containing carbon dioxide, extends shelf-life.

Meat and meat products represent a group of foodstuffs associated with a wide variety of growth limitation strategies and a good understanding of the microbiological processes involved is being developed. Vacuum packing meat extends the shelf-life by preventing the aerobic Gram-negative portion of the flora (*e.g. Pseudomonas*, *Acinetobacter*, *Psychrobacter* spp.) multiplying and allowing the facultative anaerobes (*e.g. Lactobacillus*, *Carnobacterium*, *Leuconostoc* spp.) to dominate. The extent of microbial growth is determined by the food pH, film permeability to oxygen and carbon dioxide, temperature and the microbes' tolerance of carbon dioxide. On meat of pH 6.0 and above,

Brochothrix thermosphacta grows anaerobically to about $10^7/cm^2$, and *Shewanella putrefaciens* grows when the pH is 6.0 and above, but not when the pH is 5.8 or below. Different mixtures of oxygen, nitrogen and carbon dioxide are used for a wide range of foods.

Spoilage of meat by psychrophilic clostridia has only relatively recently been recognized and is characterised by the production of hydrogen and carbon dioxide causing pack distension, and of butanol, butanoic acid, ethanol, acetic acid and a range of sulphur containing compounds[29,30]. Thus new sets of growth limiting storage conditions are selecting for novel spoilage agents. The isolate/strain of Dainty *et al.* was shown by 16S rRNA sequencing to be a new species *C. estertheticum*[31]. Yet another new psychrotrophic species, *C. algidicarnis*, spoiled vacuum-packed cooked pork[32]. The use of microbiological population analysis based upon interrogation of 16S rRNA databases has proved to be exceedingly useful in identifying novel spoilage organisms and some pathogens. Such technology will be at the forefront of the future food microbiology research as it will allow for rapid recognition of emerging hazards based upon changing food products or processes.

Spoilage of meat stored in air is due to formation of a complex mixture of esters, branched-chain alcohols, sulphur-containing compounds, amines, unsaturated hydrocarbons and ketones[33]. Meat of normal pH, vacuum packed and stored refrigerated develops a relatively inoffensive sour acid odour, while high pH meat tends to develop sulphydryl, putrid and faecal odours[33]. 'Greening' of meat is caused by the formation of sulphmyoglobin from hydrogen sulphide, formed by *Shew. putrefaciens*, *Enterobacteriaceae* or *Lactobacillus sake*, reacting with oxymyoglobin.

Freezing prevents growth of vegetative bacteria, but should not be relied upon to eliminate pathogens. Most vegetative bacterial pathogens survive for many months in frozen foods. Thawing frozen foods is a crucial stage because bacteria at the surface of the frozen food multiply at the thawing temperature. Ideally thawing should be under refrigeration, but the time taken to thaw large items, *e.g.* a large Christmas turkey, often tempts consumers to try to accelerate thawing by holding the food under warm conditions. In the case of chicken and turkeys, thawing without refrigeration, followed by inadequate cooking, has led to outbreaks of salmonellosis. Another cause of illness has been cooking a large turkey that has not thawed in the centre.

Cured meats have an extended shelf-life because sodium chloride and curing salts, *e.g.* sodium nitrite, combine to prevent multiplication of spoilage bacteria and slow greatly the growth of pathogens. Many fermented meats have a low pH value due to production of lactic acid during the fermentation[34], although in those that are subsequently mould-ripened, the pH can rise again during ripening to near 7.0.

Control strategies: some future developments

At every stage of food processing, temperature plays a critical role. Increasingly, traditional products and processes are changed to accommodate the wish for improved textural properties after lower heat processes, curing with reduced levels of salt and nitrite, and foods from which established preservatives have been removed. Developments in other methods of food preservation are dealt with elsewhere in this issue. While many new techniques show promise as alternatives to heat, few are yet in widespread commercial use. Thus, greater and greater reliance is being placed on temperature control as the main factor controlling the growth of spoilage microbes and those able to cause food-borne illness.

In recent years, changing eating patterns and technological developments in food production, processing and preservation have increased enormously the variety of products available. Many are sold 'ready-to-eat' and are not, therefore, subjected to heating immediately before consumption which would kill the occasional vegetative pathogenic bacteria[35]. Some products spend weeks in distribution and storage before being offered for sale alongside the fresh product. The food industry has developed novel products, modified formulations and devised alternative means of packaging (vacuum, modified atmosphere). There are pressures to extend even further the shelf-life of foods held under chill and continuing demands for longer shelf-life products, coupled with minimal heating and reduced, or no preservatives/additives.

The development of microbes on foods has long been recognized to be a response to physical and chemical conditions such as pH, available water, gaseous atmosphere, temperature, preservatives and numerous other factors. Much effort has been directed to defining conditions that limit growth, since understanding those conditions appeared to proscribe conditions that would extend shelf-life and minimise the growth of microbes associated with food-borne illnesses. As a consequence tables of 'minimum' values are readily available for key spoilage and pathogenic bacteria (Table 1). In most instances, these data have been generated when other controlling factors are near optimal, *e.g.* the minimum water activity when the pH value is near 7 and incubation temperatures are optimal, which is unrealistic if the concern is safe food storage.

Stable and safe foods are the consequence of preservative factors acting in combination, often at levels which singly would not be inhibitory. Our understanding of the relative contributions of factors to give safe and shelf-stable food products remains surprisingly poor.

Biological control systems

There is no doubt that the development of rigorous physical and chemical methods of food preservation has been one of the most important

contributions to food safety in the latter half of the 20th century. At this stage, one might reasonably conclude that, within a properly controlled environment, application of appropriate techniques would result in microbiologically safe foods. This may well be the case if the range of foods available were to remain static, but in a consumer-led industry considerable pressure is applied to replace traditional preservation methods particularly the use of chemical additives. There has been considerable interest in the use of a class of bacteria-specific toxins known as bacteriocins. These are usually low molecular weight proteins that are synthesised by a particular strain of bacteria to be active against related strains. The spectrum of activity can vary quite widely. Some strains of *E. coli* can produce bacteriocins (called colicins) that are only active against other *E. coli* strains[36]. On the other hand, many lactic acid bacteria produce bacteriocins with a much broader spectrum of activity, some even targeting food borne pathogens such as *L. monocytogenes*. In principle they would make ideal food additives as they are natural bacterial products released by organisms, such as lactic acid bacteria, that would be present in many foods. They also have not been demonstrated to have toxic effects on mammalian cells and would, therefore, behave as 'magic bullets' only attacking specific groups of undesirable bacteria[37,38].

There are few general characteristics shared by bacteriocins as a group. There is still continuing debate on nomenclature and it is common to have molecules with different names reclassified when nucleic acid or protein sequencing studies reveals them to be identical. As a working system, many researchers utilize a four group classification for these low molecular antimicrobial proteins.

Group I

This group is characterised by proteins with post-translationally modified serine and threonine amino acids which allow the formation of unusual thioether lanthionine rings. There are many examples of these bacteriocins but the best known is the 'lantibiotic' nisin.

Group II

These are very low molecular weight, heat stable proteins. They are characterised by the presence of a consensus leader sequence (Gly–Gly^{-1}–Xaa^{+1}) that is essential for the correct export and processing of the molecule. Pediocin AcH is probably the best known member of this group.

Groups III and IV

These are less well characterised than the previous sets but they generally contain higher molecular weight proteins (up to 30 kDa) and

are normally heat labile. Group III molecules can be distinguished from Group IV by the absence of carbohydrate or lipid moieties.

In spite of an extensive scientific literature on the antimicrobial efficacy of many bacteriocins in laboratory systems, there have been few commercial applications to date. This is a reflection of the difficulty in obtaining large quantities of sufficiently well-characterised preparations that would be suitable for use as a food additive, and the absence of toxicological evaluation. Nisin has been added to a range of foods particularly dairy products[39]. It is active against vegetative bacteria such as *L. monocytogenes* and also has an effect against endospore formers such as clostridia. It has been demonstrated to permeabilize cell membranes in a time- and concentration-dependent manner suggesting an oligomerisation of inserted monomers to form hydrophilic diffusion channels[40]. The mechanism of action against spore-forming bacteria is less well understood and the process is less efficient.

Nisin and pediocin AcH have been demonstrated to have synergistic effects with both thermal[41] and non-thermal antimicrobial processes[42,43]. The immediate future for bacteriocins looks to be in combination with other processes. However, the genetics of these proteins are well known and recombinant organisms expressing heterologous bacteriocins (with a wider range of bacteria attacked) have been described. However, in the current climate of hostility towards the use of any genetic manipulation associated with food, it is unlikely that recombinant organisms will be a commercial strategy for some time to come.

Control – future challenges

Food microbiology developed via studies on the main commodities such as milk, meat, poultry, fish, fruit and vegetables, without appreciation of the main factors controlling microbial growth. Factors are sometimes claimed to be important without convincing evidence of their contribution, *e.g.* competition between pathogens and the spoilage flora is less widespread than believed. Most foods are nutrient rich, and it has been shown that microbial growth is largely determined by a relatively small number of factors, *e.g.* pH, a_w, temperature, atmosphere, particular organic acids. This realisation has led to efforts to model microbial death and growth responses as a function of those factors. The resulting models give a quick 'first estimate' of the microbial response and are being used in many aspects of product design and development, shelf-life assessment, HACCP and risk assessment. The greatest advantage of models is that they can be used to test the consequences of changing a number of factors at the same time and, with the power of modern

computers, the answers are almost instantaneous. A disadvantage is that the predictions may not be precise and may only indicate a trend, but knowing that trend quickly is highly advantageous when reformulating or modifying or evaluating storage conditions. Care should be taken that the controlling factors included in the model are those relevant to the foods in question, and models should be validated by comparing predictions from the model with microbial responses in different foods before reliance is placed on them[44–47].

In order to improve control methods, it is necessary to have standard, reproducible assays to assess the impact of a treatment on microbial cell viability. As knowledge of microbial physiology has gradually increased, some of the most basic concepts, such as what actually constitutes a dead cell, have been revisited. The discipline of food microbiology has centred on the manipulation of various environmental parameters to influence microbial populations in foods. This has been measured by traditional culture techniques relying on organisms forming colonies on solid nutrient media. The assumption is that each viable bacterial cell will always give rise to a single colony. This central tenant of microbiology has been increasingly challenged with evidence pointing towards the generation of sub-populations of injured organisms following sublethal administration of physical or chemical control methods. Injury to cells will prevent their growth in simple enumeration assays and, as such, the efficacy of certain processes can be significantly overestimated. An injured cell retains the capacity for multiplication and there are numerous procedures for reviving such cells[48,49]. The current trend in food processing is towards the use of milder treatment conditions. This has meant that evaluation of sublethal injury is now more pertinent than ever to the critical analysis of all processes aimed at controlling vegetative micro-organisms.

A second major challenge to the traditional concept of cell viability has come from the description of a 'viable but non-culturable' (VBNC) state. A major taxonomic subdivision amongst bacteria continues to be the use of the Gram stain providing the distinction: Gram-positive and Gram-negative. Gram-positive organisms constitute a very diverse group also contains a small subset of species that can form a desiccated, metabolically dormant and highly heat-resistant stage called the endospore. Endospore forming bacteria such as *C. botulinum* and *B. cereus* are well known food-borne hazards and control measures based around rigorous, moist heat treatment (such as canning) are well established. The VBNC state has been best defined for Gram-negative bacteria[50], but has also been noted among some Gram-positive organisms.

Many important food-borne Gram-negative bacteria such as *Campylobacter*, *Salmonella*, *Vibrio*, and *Escherichia* spp. have been demonstrated to enter the VBNC state. This in normally induced in

response to nutrient limitation, but certain thermal and chemical stresses have produced a similar effect. Cells in this state will not form colonies on hitherto appropriate culture media. The media contain all the necessary nutrients to support bacterial growth, yet once the bacteria enter the VBNC state they no longer undergo normal cell replication. Even though the bacteria no longer reproduce they can be demonstrated to be metabolically active using stains for RNA. By definition RNA is only produced by metabolically active bacterial cells since it is the information carrier in protein production and protein production is an absolute requirement for metabolism. Since RNA also has a very short half-life in bacteria, its detection means that cells are displaying metabolic activity. Recovery of cells from a viable but non-culturable state, *i.e.* a restoration of the ability to grow and divide in culture, has also been reported but the signals controlling the process are incompletely understood. The concern is that if we do not know how the entry and exit from this state is effected we have little chance of controlling it, in particular if our current controls are targeted against vegetative organisms that are in a very different physiological state.

Difficulties in accounting for the contribution of sublethally injured cells and viable but non-culturable cells to overall risk assessment has highlighted the limitations of some current methods of microbiological analysis. The realisation that the application of control methods could push vegetative cells into states that we do not understand physiologically and cannot easily be detected by current analytical methods is of concern. This suggests that with some of the advances in control methods outlined below we should also improve methods of microbiological assay, and perhaps move away from heavy reliance on cultural methods to some of the tools afforded by advances in molecular biology. A discussion of this area is beyond the scope of this short article, but recent reviews have identified the gradual transfer of molecular technology from medical to food applications[51,52].

Conclusions

The food industry has come to terms with the control of some long established hazards, *e.g.* group I (proteolytic) strains of *C. botulinum*, *C. perfringens*, *Staph. aureus*, via designed and controlled cooking and temperature control during subsequent storage. However, the nature of microbiological hazards is constantly changing in response to changes in food processing, consumer demands for new and more 'natural' products, changes in purchasing and eating habits and to the introduction of a wider variety of products into traditional markets. Of the more recently

identified hazards, *V. parahaemolyticus* and *B. cereus* are increasingly prevalent world-wide, but are still relatively unimportant in the UK, while *C. jejuni/coli* has become the major cause of bacterial gastro-enteritis. Other recent hazards include *L. monocytogenes*, psychrotrophic strains of *Bacillus* spp, *S. enteritidis*, antibiotic-resistant *S. typhimurium*, *V. vulnificus*, *E. coli* O157:H7 and other verocytotoxic strains. Systems that have been designed to protect foods from currently recognised pathogens should be checked for their performance against the most recently identified food-borne pathogens.

The microbiological lessons that have been learned re-inforce the view that the future safety of foods cannot be assured by any realistic amount of end-product testing. It must rely heavily on an integrated strategy combining: (i) a structured approach to understanding hazards and their control, such as HACCP; (ii) the development of specific and rapid methods for identification and characterising microbes; (iii) a full understanding of the factors leading to microbial death, survival and growth, *e.g.* via mathematical modelling; and (iv) control of all the factors affecting microbial survival and growth during processing and through the food-chain.

In many cases, we currently have only a basic knowledge and significant investment in research will be required to provide the information necessary to ensure food safety in a rapidly changing consumer society.

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