BASIC MICROBIOLOGY

EDITOR: J. F. WILKINSON

VOLUME 8

A Modern Introduction to Food Microbiology

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Preface

This book on food microbiology is intended to be an introductory text for students of microbiology, food science, food technology and related disciplines. It has been assumed that such students will have received or be receiving an adequate introduction to microbiology *per se*. As emphasis was given at the planning stage to the introductory role of this book, it differs from most, if not all, current textbooks on food microbiology in giving emphasis to general principles rather than cataloguing information about the major categories of man's food. To provide a theme, food microbiology has been considered to be a facet of ecology, a concept that has pervaded both the theory and practice of the discipline in the 25 years since the classic review by Mossel and Ingram.

The current text is probably unique in its genre in citing, when appropriate, the day-to-day problems that have to be tackled by the practising food microbiologist. At the same time, an attempt has been made to set such examples against a wide perspective so that the would-be food microbiologist recognizes from the outset that success in his/her chosen profession depends on attention to detail as well as a thorough knowledge of the discipline and an awareness of the insults to which ingredients, as well as finished products, may be exposed. Moreover, the opportunity has been taken, when pertinent, to stress that the successful application of food microbiology in a factory is based on the collaborative efforts of persons from diverse backgrounds and training—the successful food microbiologist is simply a member of a team of successful specialists.

I should like to express my sincere thanks to Charlie Davidson, Martyn Brown, John Abbiss, Jeff Banks, Howard Tranter, Nick Sparks, Hilary Dalton, George Nychas and Chris Leads who through their research efforts have provided me with a deeper appreciation and understanding of the interplay of pure and applied microbiology, and to Mrs Rita Pratt who has managed quite spectacularly to type the manuscript from notes lacking calligraphic grace.

R. G. Board

Introduction

This book deals with only one of the many topics included under the heading, industrial or applied microbiology (Table 1)–food microbiology*. This table highlights an apparently immutable trait, the cataloguing of materials destroyed by microorganisms or products synthesized by them. This approach tends to compartmentalize the knowledge of the student and to isolate the specialists in one field of applied microbiology from those in others. Thus at a fairly early stage the study of applied topics can become divorced from that of fundamental aspects of genetics, biochemistry and microbiology. Although this introductory book surveys only one area, by emphasizing general principles rather than describing a few subjects in detail it provides a much broader introduction than the title might imply.

Table 1. Topics included in industrial microbiology.

Biodeterioration of	Timber and paper
	Paints and coating materials
	Metals
	Petroleum products
	Pharmaceutical products
	Wool
	Rubber
	Leather
	Paper
	Food and beverages
Biosynthesis of	Pharmaceutical products
	Foods and feeds
	Alcoholic beverages
	Industrial solvents and other chemicals
	Amino acids and other organic acids
	Industrial polymers, e.g. dextran
Reclamation of	Metals from low grade ores
	Water from domestic and industrial wastes
	Petroleum products other than by distillation
Assay of	Antibiotics
	Amino acids and vitamins in foods
	Industrial preservatives and disinfectants
	Pollution of the environment with fungicides, herbicides, etc.

What is the theme that can unify industrial microbiology? In the author's opinion it is the study of those factors which govern the growth of microorganisms. If in this approach, the substrate being attacked or the product being synthesized is considered as but one component in an environment, attention can be given to rate of attack or synthesis and thereby to the overall environment in which the changes are occurring. If, for example, one is dealing with the conservation of a food then the emphasis is given to the means whereby the rate of change due to microbial activity is minimized. On the other hand, a rapid rate of change is sought when microorganisms play an important role in the processing of a food, as with the fermentation of milk. This unifying concept of applied microbiology is discussed in Chapter 1 and provides the theme for the other chapters dealing with foods.

In selecting topics for discussion, care has been taken to select examples of ancient as well as modern methods. Such a choice emphasizes another feature of applied microbiology. Through the thousands of years that man has developed methods in which microorganisms are of cardinal importance, many tricks have often been included in a process and even today detailed analysis is required to identify those having a direct bearing on the process. Discussions of modern fermentation technologies highlight two features. A technology may well be based on the analysis of an old craft, as with the fermentation of milk and vegetables where the processes are based on those few facets which have a direct bearing on the performance of the fermentative organisms. In contrast, the processes used to produce single-cell proteins are based on a thorough knowledge of large-scale fermentation technology and the organisms tend to be selected for their capacity to exploit the optimal conditions offered by modern techniques.

These differences in approach are of relevance when one is considering the basic training required for a career in industrial microbiology. It is obvious that there needs to be an adequate coverage of biochemistry, genetics and microbial physiology so that the unifying theme noted previously has a firm theoretical basis. Allied to this is the requirement for skill in the enrichment, isolation and characterization of microorganisms and a sound understanding of the principles of ecology. These considerations dictated the choice of subjects discussed in the following chapters.

* For a detailed discussion of training for food microbiology see: Changing roles of food microbiologists in the 1980's, Feature articles (1982) *Food Tech.* 36, 58–77.

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1 Ecology and Food Microbiology

As noted in the Introduction, the absence of a common theme impeded the development of food microbiology as a discipline in its own right. The classic review by Mossel and Ingram (1955) provided a conceptual framework for the development of food microbiology in the past two decades. In practice the study of food preservation is the study of the means used to destroy or to inhibit the growth of those microorganisms which have the potential to cause deterioration of food. Three factors can be considered to be involved (Fig. 1.1):

1 *intrinsic factors*—the physicochemical properties of the food;
2 *extrinsic factors*—the physical and chemical properties of the environment, either bulk or micro, surrounding the food;
3 *implicit factors*—the physiological properties that enable particular organisms to flourish because of the interaction of (1) and (2).

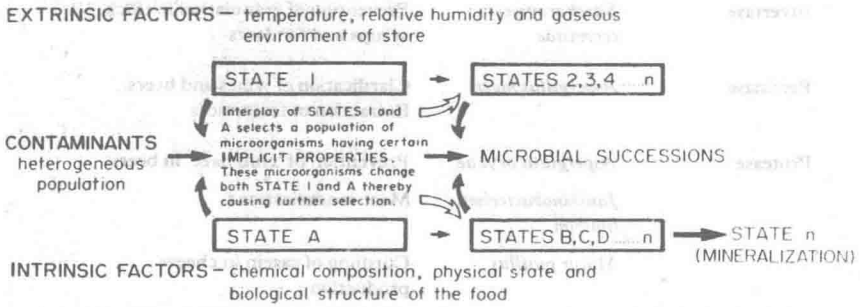

Fig. 1.1. Food spoilage and, eventually, its mineralization results from the selection of microorganisms having *implicit* properties which allow them to acquire numerical dominance (form a 'bloom') in the niches that develop from an interplay of the physicochemical properties of the food (*intrinsic factors*) and storage conditions (*extrinsic factors*). See Chapter 6 for further details.

There are two major but opposing strategies in the preservation of food. (a) *The inhibition/destruction of microorganisms in or their removal from a food.* It has to be accepted that sterilization or aseptic production is applicable to particular foods only. The food microbiologist, therefore, becomes involved with the problems of hindering the growth and activities

of organisms associated with the degradation and, ultimately, the mineralization of foods. It is important also to ensure that potentially pathogenic microorganisms do not survive in large numbers, multiply or leave toxic products in a food. A complete inhibition of microbial activity need not ensure stability of a food; chemical attack, e.g. fat oxidation, and degradation by enzymes, either of food or microbial origin, can lead to undesirable changes in flavour and texture (Fig. 2.5), processes which can be slowed by blanching (see p. 69), chilling, freezing, or the addition of antioxidants, for example, the addition of sulphite to apple slices in order to prevent browning due to polyphenol oxidases (Fig. 2.8).

(b) *The fostering of those organisms whose activities enhance and/or endow flavour, texture and colour; they may also inhibit the growth of potentially harmful organisms.* Today the successful outcome of many processes is helped by the use of specially selected microorganisms ('starter' cultures) or enzymes of microbial origin (Table 1.1).

Table 1.1. Microbial enzymes used in food manufacture.

Enzyme	Source	Use
Amylase	*Aspergillus niger* *Bacillus subtilis*	Manufacture of syrups
Glucose oxidase	*Aspergillus niger*	Removal of glucose from egg white that is to be dried
Invertase	*Saccharomyces cerevisiae*	Prevention of granulation in thick syrups and fondants
Pectinase	*Aspergillus niger*	Clarification of wines and beers. Extraction of fruit juices
Protease	*Aspergillus oryzae*	Prevention of 'chill haze' in beers
	Janthinobacterium lividum	Meat tenderization*
	Mucor pusillus	Curdling of casein in cheese production

* Used only in trials to date.

Setting aside the organization of the efforts of production workers, many of whom will be semi-skilled, by management, the well-being of a food in a developed country is determined by the application of principles by a relatively few technologists who factor the 'food' at some or all of the stages from harvest through production and storage until offered to the consumer (see Fig. 9.4). Indeed it is becoming more and more evident that little of value is achieved in food microbiology unless a complete production and distribution chain is considered.

To apply either of the strategies of food preservation noted on page 1, the conditions required for microbial growth must be understood. In the following section, some features of microbial growth will therefore be considered.

ENVIRONMENTAL GRADIENTS

In plant ecology, the concepts of environmental gradients are used to account for the distribution of species or communities along a latitude or longitude or from sea level to a mountain top. The emphasis is given to space because of its importance relative to geological time and evolution. With some foods, such as meat having easily defined portions of fat and lean, different communities or associations of organisms are selected because of the intrinsic, chemical properties of the two substrates. A layer of dilute syrup on the top of thick syrup is an example of a practical situation which would select facultative osmophiles (at the top) and obligate ones in the depths of the syrup. Thus, with fast-growing moulds and bacteria, there are situations in food microbiology when it is useful to consider the environmental gradient concept in the sense of 'a defined space for unlimited time', when attempting to predict or interpret the distribution of different microorganisms in a solid food or viscous liquid.

In many other situations, emphasis can be given equally to *time* and *space*. In other words, attention is directed at the *rate* at which a change occurs at a point within a food. This concept can be applied to: a food gaining or losing heat; a product in which a change in redox controls microbial selection and growth; the diffusion of salt, syrup or preservatives through a solid food—as with the diffusion of brine into meat; the production of acid by fermentative bacteria in products such as sauerkraut. Thus, for practical purposes, the organism introduced, either intentionally or unintentionally, to a food during the preliminary stages of processing can be considered to be 'stuck' at a point and, providing that the natural, implicit factors of the food will support its growth, it will be capable of growth for only as long as the imposed environmental stress is within its tolerance; for example, if temperature was being used as the environmental stress, the sluggish loss of heat could, say, result in a mesophile being provided with a temperature decreasing from 30 to 15 °C over five hours, during which time it might well achieve upwards of ten generations, whereas if the drift from 30 to 15 °C was achieved within three minutes, then no growth would occur (see Fig. 9.7).

Heat and milk

When attempting to achieve rapid heating or chilling, the bulk and form of the food to be treated is important as well as changes caused by processing. Thus with milk, the original method of pasteurization in a large tank (at 62.8 °C for 30 min) was replaced by the application of a thin film of milk

to an efficient heat exchanger (71–73 °C for 15–17 s). Latterly, milk has been rendered germ free by treatment at 135–150 °C for up to 4 s, a process referred to as ultra-high temperature treatment (UHT). In practice UHT can be considered to be nothing other than a special case of a general approach to the heat treatment of foods (High Temperature Short Time (HTST)), where the objective is to achieve a germ-free state by the application of high temperatures (130 °C and above) for short times, i.e. a few seconds to 6 minutes.

Pasteurization of milk was adopted initially in the United Kingdom to kill *Mycobacterium tuberculosis*, but was subsequently used for short-term preservation. The UHT method gives 'commercial sterility' without inducing a cooked flavour in the milk because the temperature coefficient (Q_{10}) for change in reaction rate (Fig. 4.5) is greater for the destruction of the bacterial spore (Q_{10}, 8–30) than for chemical change (Q_{10}, 2–3). Although such heat treatments will kill the psychrotrophic flora that may develop in refrigerated milk, they will not inactivate completely thermostable enzymes secreted by the organisms. On storage, the processed milk can acquire a rancid flavour, due to lipases hydrolysing triglycerides, or bitterness, due to breakdown of casein by proteases, particularly if the numbers of microorganisms in the raw milk were large. Indeed should such pasteurized milk be used for cheese production, the carry-over of thermostable lipases of microbial origin can cause rancidity during the ripening process. Thus although technological innovation allows the production of milk with a good shelf-like and no 'cooked flavour', an undesirable feature of milk in glass bottles sterilized in tanks of boiling water, it is successful only when the production and storage of the raw material have been rigorously controlled. Moreover, the success of a process such as UHT is only assured by aseptic dispensing into germ-free containers. As the latter are commonly formed from heat labile materials, novel methods of sterilization have been introduced so that the container is rendered germ-free without leaving a residue that could cause chemical taint. H_2O_2, UV light, Cl and hydrogen chloride have been selected for these reasons. Indeed, recent studies have indicated that when H_2O_2 and UV light are used in combination, the rate at which microorganisms are killed is greater than when either is used alone.

Tolerance profiles

Figure 1.2 illustrates the response of an organism to temperature, pH, salt or sugar concentration. Since it has been composed from data obtained from separate experiments using, for example, a psychrophile, a mesophile and a thermophile, the figure is probably spurious in a natural situation. From a theoretical viewpoint, it is probable that with foods containing a heterogeneous flora, competition or synergism between organisms could give profiles of the type shown in Fig. 1.3. The growth of an organism can be controlled by the qualitative or quantitative deficiency or excess of one or more factors which approach the limits of tolerance of the organism. An

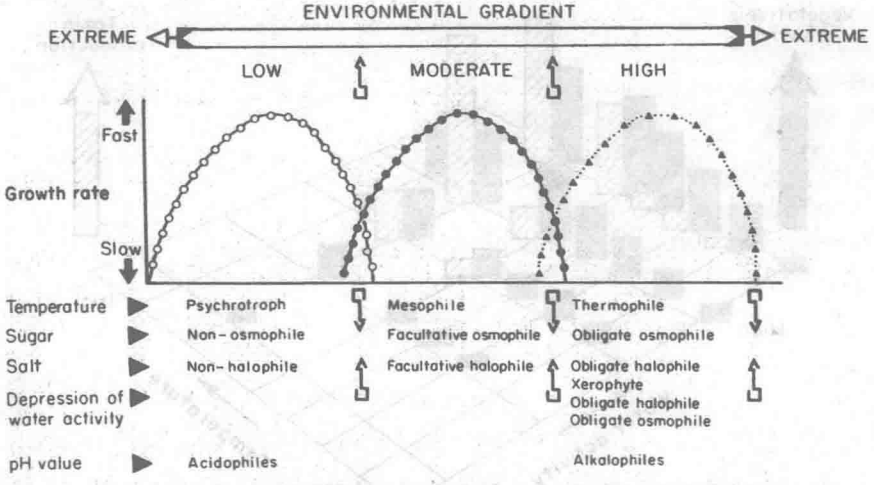

Fig. 1.2. The profiles obtained in experiments in which pure cultures of microorganisms are used to determine growth rate in a medium containing a range of concentrations of H^+, salt, sugar, etc., or incubated over the termperature range from *c.* −5–55 °C.

organism may have a wide range of tolerance of one factor, but a narrow range for another. When conditions for a species with respect to one extrinsic or intrinsic factor are approaching the limits of tolerance, the limits of tolerance may be reduced with respect to another ecological factor. In nature, and probably in foods also, an organism rarely lives at the optimum range as determined by laboratory studies of a particular factor, thus indicating that another, possibly unsuspected, component is growth limiting.

In the discussions of methods of preservation (see p. 21), examples are given of traditional foods (butter, cheese, bacon, etc.) whose stability has been shown to be dependent upon an interplay of factors, none of which was known to those who contributed to the evolution of the craft. Although current research is demonstrating the subtle nature of the interplay of pH,

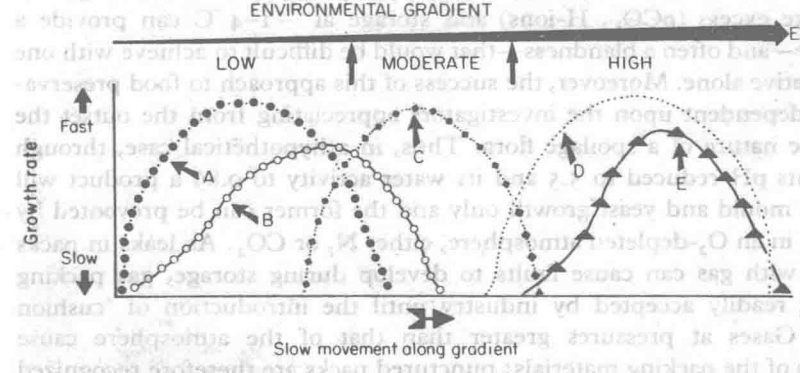

Fig. 1.3. Hypothetical growth rates of contaminants in a food subjected to temperatures over the range *c.* −5–55 °C or changing concentrations of an additive such as sugar or salt.

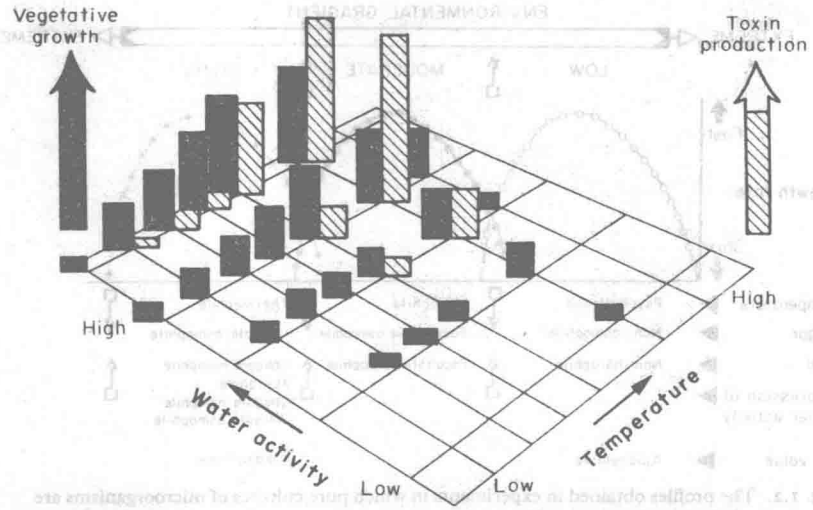

Fig. 1.4. The interplay of two factors on microbial growth in a food or a model system simulating a food are commonly displayed as 'sky-scraper' figures. This figure shows the influence of temperature and water activity on growth and toxin production by *Penicillium cyclopium* (redrawn from *Nestle Research News* (1980/81) Safety aspects of food fermentation, by M. von Schothorst).

NO_2^-, NaCl, pO_2, etc., in the preservation of ham and bacon, the complexities of the interactions are such that mathematical modelling (see Chapter 9) is only just beginning to replace the presentation of results as models based on three-dimensional histograms (Fig. 1.4).

Interplay of factors

It is from an appreciation of the factors involved in tolerance that food microbiologists are beginning to make foods whose stability under defined conditions of storage for known times can be assured. Thus, for example, a slight to moderate deficiency (water, pO_2) together with a slight to moderate excess (pCO_2, H-ions) and storage at -1–$4\,^{\circ}C$ can provide a stability—and often a blandness—that would be difficult to achieve with one preservative alone. Moreover, the success of this approach to food preservation is dependent upon the investigators appreciating from the outset the probable nature of a spoilage flora. Thus, in a hypothetical case, through having its pH reduced to 5.5 and its water activity to 0.89 a product will support mould and yeast growth only and the former can be prevented by packing in an O_2-depleted atmosphere, either N_2 or CO_2. As leaks in packs flushed with gas can cause faults to develop during storage, gas packing was not readily accepted by industry until the introduction of 'cushion packs'. Gases at pressures greater than that of the atmosphere cause inflation of the packing materials; punctured packs are therefore recognized easily. The stability of grain having more than its alarm water content

(see p. 31) is assured if the pO_2 in the void spaces is reduced either by sparging with CO_2 or by being denied exchange with the bulk atmosphere, the O_2 present originally being depleted by the activity of the grain and microbial contaminants. In the case of hay, drying in the field to low levels of water can be avoided and stability assured by adding fatty acids, e.g. butyric acid.

Although nomograms can be devised so that food manufacturers can choose appropriate combinations of low pH and sugar concentration for preservation, care must be taken in the uncritical application of such systems. Thus, the storage of olives in mineral acid instead of the fermentation products of lactic acid bacteria can cause spoilage by pectinolytic yeast, although the H ion concentration is about 1000-fold higher in the former case. In this and many other cases, the organic acid anion has itself an antimicrobial action, probably due to its lipophilic properties, which is more effective than the H ions themselves.

Detailed microbial analysis of a solid food which has had its intrinsic properties changed slightly and even one which has spoiled, results in the isolation of a wide range of organisms (Table 1.2). This suggests that such foods offer a variety of environmental niches or that the spatial relationship between contaminants, and thus the microcolonies formed by them, ameliorates the competition between organisms having similar implicit properties; alternatively, some type of synergism or symbiotic relationship between the organisms may be established such that a microbial succession is favoured or a stable mixed culture or consortium formed. It has been claimed that proteolytic pseudomonads may be essential pioneers in the succession leading to the dominance of non-proteolytic pseudomonads on meat and poultry. A complex example of synergism is provided by the fermented milk product, kefir, in which fermentation is brought about by lactic streptococci, leuconostocs and yeasts associated in large aggregates or 'grains'. If an environmental extreme, such as that resulting from relatively high quantities of salt or sugar, is used to preserve a food, the resident flora consists of a very few species, namely the obligative halophiles, osmophiles, or xerophytes. In other words, preservation can be considered to be a form of enrichment culture unless sterility is achieved (see Chapter 4).

Microbiological analysis

When attempting to interpret the information gathered from a detailed examination of a spoiled food which had not been exposed to some extreme of an environmental gradient, it is difficult to provide an explanation for those organisms which have not achieved dominance. Is their failure to grow merely a function of the inadequacy of the nutrients—either in variety or amount—in the food, due to antagonism by another organism or do they need to live synergistically with some other organism in the food? In recent years, the demonstration in the laboratory of one isolate from a food inhibiting

Table 1.2. The microorganisms associated with meat and meat products.

Process and product	*Pseudomonas* spp.	*Acinetobacter* spp.	Enterobacters	*Clostridium* spp.	*Bacillus* spp.	*Micrococcus* spp.	*Staphylococcus* spp.	*Vibrio* spp.	*Brochothrix thermosphacta*	*Lactobacillus* spp.	*Streptococcus* spp.	*Leuconostoc* spp.	*Pediococcus* spp.	*Kurthia zopfii**	Yeasts	*Cladosporium herbarum*	Moulds general
Joints																	
Freshly butchered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stored at 0–4 °C in normal, moist atmosphere	+ +	+															
Stored at 0–4 °C in impermeable films—reduction of pO_2, increase in pCO_2	+ +	+						+									
Stored at 0–4 °C in atmosphere enriched with CO_2								+ +									
Stored at −1 °C in atmosphere enriched with CO_2											+ +						
Stored at 0–4 °C and surface allowed to dry															+ +		
Stored at 10–25 °C in normal, moist atmosphere	+ +	+			+ +				+ +	+ +					+ +	+	+

Stored at >25 °C in normal, moist atmosphere			++	++	++											
Stored at >40 °C in normal, moist atmosphere				++	++											
Comminuted (ground) meat																
Stored at 0–4 °C in normal atmosphere	++	+							++	+				++		
Stored at 0–4 °C in atmosphere enriched with CO_2	+	+							+	++						
Containing sulphite									++							
Glucose added						++	++			++	++	++	++			
With biscuit crumb and sulphite									++	++				++		
Cured meats																
Whole side of pork cured (NaCl, NO_3^-, NO_2^-) and heavily smoked—surface growth only																++
Pork cured (as above), lightly smoked, sliced and put into vacuum packs						++	++	++		++	++			++		
Pieces of pork cured, cooked, sliced and packed										++						

(continued)

Table 1.2. (*continued*)

Process and product	*Pseudomonas* spp.	*Acinetobacter* spp.	Enterobacters	*Clostridium* spp.	*Bacillus* spp.	*Micrococcus* spp.	*Staphylococcus* spp.	*Vibrio* spp.	*Brochothrix thermosphacta*	*Lactobacillus* spp.	*Streptococcus* spp.	*Leuconostoc* spp.	*Pediococcus* spp.	*Kurthia zopfii**	Yeasts	*Cladosporium herbarum*	Moulds general
Cured meats																	
Large pieces of meat cooked and then allowed to cool slowly				+ +	+ +												
Meat treated experimentally with tetracyclines															+ +		
Meat, with bones removed, placed in bag of impermeable film, atmosphere reduced in pressure ('vacuum pack') and stored at 0–4 °C	+ +								+ +	+ +							

* Although common in abattoirs and on meat, it rarely achieves numerical dominance on any product.
In all except freshly butchered meat, + + = extensive growth; + = moderate growth.