THE MICROBIOLOGY OF SAFE FOOD

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Blackwell Wissenschafts-Verlag GmbH
Kurfürstendamm 57
10707 Berlin, Germany

Blackwell Science KK
MG Kodenmacho Building
7–10 Kodenmacho Nihombashi
Chuo-ku, Tokyo 104, Japan

First published 2000

Set in 10.5/12.5 pt Garamond Book
by DP Photosetting, Aylesbury, Bucks
Printed and bound in Great Britain at
the University Press, Cambridge

DISTRIBUTORS

Marston Book Services Ltd
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Carlton, Victoria 3053
(*Orders:* Tel: 03 9347 0300
Fax: 03 9347 5001)

A catalogue record for this title is available from the British Library

ISBN 0-632-05487-5

Library of Congress
Cataloging-in-Publication Data
Forsythe, S.J. (Steve J.)
The microbiology of safe food/
S.J. Forsythe.
p. cm.
Includes bibliographical references and index.
ISBN 0-632-05487-5 (pb)
1. Food—Microbiology. I. Title.
QR115+
00-029784

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PREFACE

Throughout the world, food production has become more complex. Frequently raw materials are sourced globally and the food is processed through an increasing variety of techniques. No longer does the local farm serve the local community through a local shop, nowadays there are international corporations adhering to national and international regimes. Therefore approaches to safe food production are being assessed on an expanding platform from national, European, transatlantic and beyond. Against this backdrop there have been numerous highly publicised food safety issues such as BSE and *E. coli* O157:H7 which has caused the general public to become more vociferous concerning food issues. The controversy in Europe over genetically modified foods is perceived by the general public within the context of 'food poisoning'.

This book aims to review the production of food and the level of microorganisms which humans ingest. Certain circumstances require zero tolerances for pathogens, whereas more frequently there are acceptable limits set, albeit with statistical accuracy or inaccuracy depending upon whether you subsequently suffer from food poisoning. Microbes are traditionally ingested in fermented foods and this has developed into the subject of pre- and probiotics with disputed health benefits. Whether engineered 'functional foods' will be able to attain consumer acceptance remains to be seen.

Food microbiology covers both food pathogens and food spoilage organisms. This book aims to cover the wide range of microorganisms occurring in food, both as contaminants and deliberate inoculation. Due to the heightened public awareness over food poisoning it is important that all companies in the food chain maintain high hygienic standards and assure the public of the safety of the produce. Obviously over time there are technological changes in production methods and in methods of microbiological analysis. Therefore the food microbiologist needs to know the effect of processing changes (pH, temperature, etc.) on the microbial load. To this end this book reviews the dominant foodborne

microorganisms, the means of their detection, microbiological criteria as the numerical means of interpreting end-product testing, predictive microbiology as a tool to understanding the consequences of processing changes, the role of Hazard Analysis Critical Control Point (HACCP), the objectives of Microbial Risk Assessment (MRA) and the setting of Food Safety Objectives which have recently become a focus of attention. In recent years the Web has become an invaluable source of information and to reflect this a range of useful food safety resource sites is given in the back to encourage the reader to boldly go and surf. Although primarily aimed for undergraduate and postgraduate courses, I hope the book will also be of use to those working in industry.

The majority of this book was written during the last months of 1999, a time when France was being taken to the European Court over its refusal to sell British beef due to BSE/nvCJD and there had been riots in Seattle concerning the World Trade Organisation. While large organisations were wondering about the impact of the 'millennium bug', in the UK the public were waiting to see the impact of the BSE 'bug' (a few hundred or a few thousand cases?).

As usual no book can be achieved without assistance and special thanks are due to Phil Vosey concerning MRA, Ming Lo for considerable help with the computer packages, Alison Howie at Oxoid Ltd for the invaluable information on microbiological testing procedures around the world, Pete Silley and Andrew Pridmore at Don Whitley Scientific Ltd for the RABIT diagrams and Garth Lang at Biotrace Ltd for the ATP bioluminescence data. Not forgetting of course Debbie and Cathy for reading through the draft copy; nevertheless all mistakes are the author's fault.

This book is especially dedicated to Debbie, James and Rachel, Mum and Dad for their patience while I've been burning the midnight oil.

Steve Forsythe
June 2000

1

INTRODUCTION TO SAFE FOOD

This chapter is largely an overview of the book in order for the reader to understand the approach being taken. The key topics will be covered in greater depth in each of the specific chapters. Definition of terms will be found in the glossary at the end of the book, where there is also a listing of useful hypertext links.

1.1 What is safe food?

The increasing number and severity of food poisoning outbreaks worldwide has considerably increased public awareness about food safety. Public concern on food safety has been raised due to well publicised incidences such as food irradiation, BSE, *E. coli* O157:H7 and genetically modified foods.

'What is ''safe'' food?' invokes different answers depending upon whom is asked. Essentially, the different definitions would be given depending upon what consitutes a significant risk. The general public might consider that 'safe food' means zero risk, whereas the food manufacturer would consider 'what is an acceptable risk?'. The opinion expressed in this book is that *zero risk is not feasible* given the range of food products available, the complexity of the distribution chain and human nature. Nevertheless, the risks of food poisoning should be reduced during food manufacture to an 'acceptable risk'. Unfortunately there is no public consensus on what constitutes an acceptable risk. After all how can one compare the risk of hang gliding with eating rare beef? Also hang gliding has known risks which can be evaluated and a decision 'to glide or not to glide' taken. In contrast, the general public (rightly or wrongly) often feels it is not informed of relevant risks. Table 1.1 shows the possible causes of death in the next 12 months. Many of these risks are acceptable to the general public; people continue to drive cars and cross the road. This table can be compared with Tables 3.2 and 3.3 in Chapter 3 which give the recent data

Table 1.1 Risk of death during the next twelve months.

Event	Chance of one in
Smoking 10 cigarettes a day	200
Natural causes, middle-aged	850
Death through influenza	5 000
Dying in a road accident	8 000
Dying in a domestic accident	26 000
Being murdered	100 000
Death in a railway accident	500 000
Struck by lightning	10 000 000

for the USA and UK on the chances of food poisoning. The USA data indicate that each year 0.1% of the population will be hospitalised due to food poisoning. Food scares cause public outcries and can give the industry an undeserved bad reputation. Whereas, in fact, the majority of the food industry has a good safety record and is in the business to stay in business, not to go bankrupt due to adverse publicity. See Table 1.2 for some business philosopy on consumer confidence.

Table 1.2 Loss of consumer confidence in your product (adapted with permission from Corlett, 1998 *HACCP User's Manual*, Aspen Publishers Inc.).

- In the average business, for every customer who bothers to complain, 26 remain silent.
- The 'wronged' customer will tell 8 to 16 people of the problem, and more than 10% will tell 20 more.
- Of the unhappy customers, 91% will never purchase the offending goods or service again.
- If 26 people tell 8 people = 208 lost customers.
- If 26 people tell 16 people = 416 lost customers.
- It costs five times as much to attract a new customer as it costs to retain an old one.

A difficulty that arises in manufacturing 'safe' food is that the consumer is a mixed population with varying degrees of susceptibility and general life style. Additionally, food with 'high' levels of preservatives to reduce microbial growth are undesirable by the consumer and perceived as 'over processed' with 'chemical additives'! The consumer pressure is for greater varieties of fresh and minimally processed foods, natural preservatives with a *guarantee of absolute safety*.

The manufacture of safe food is the responsibility of everyone in the food chain, and food factory, from the operative on the conveyor belt to the higher management. It is not the sole responsibilty of the food

microbiologist. Nevertheless, the food microbiologist in industry will need not only to know which food pathogens are likely to occur in the ingredients, but also the affect of the food matrix and processing steps on cell survival in order to give the best advice on the most appropriate manufacturing regimes. The best methods for microbiological analysis are still being developed. It is obvious from the plethora of differing methods adopted by different countries that food poisoning statistics cannot be directly compared between countries due to the differing methods of analysis applied.

1.2 The manufacture of hygienic food

Is there a way forward for the manufacture of food which is nutritious and appetising, yet meets the expectations of the consumer regarding risk? This book aims to cover the microbiological aspects of safe hygienic food manufacture. It is only one aspect of the whole jigsaw, but should direct the reader to sources of supplemental information where necessary. It also points towards areas of future expansion such as functional foods.

The production of safe food requires:

- Control at source;
- Product design and process control;
- Good hygienic practice during production, processing, handling and distribution, storage, sale, preparation and use;
- A preventative approach because effectiveness of microbial end-product testing is limited.

Taken from CAC Alinorm 97/13 (Codex Alimentarius Commission 1995).

Control of foodborne pathogens at source is not always easy. Many pathogens survive in the environment for long periods of time (Table 1.3). They can be transmitted to humans by a variety of routes (Fig. 1.1).

Production processes can be very complicated. A general flow diagram for poultry processing is given in Fig. 1.2. This is a relatively simple flow diagram with only one branching. It does not indicate the temperature and time at each step which affect microbial growth. Therefore safe food production requires an all-encompassing approach involving the food operatives at the shop floor through to the management. Hence a number of mangement safety tools such as Good Hygienic Practices (GHP), Good Manufacturing Practice (GMP), Total Quality Management (TQM) and Hazard Analysis Critical Control Point (HACCP) need to be implemented. Only an outline of some of these tools are given in this book as detailed

Table 1.3 Survival of pathogenic microorganisms in sewage sludge, soil and on vegetables.

Organism	Conditions	Survival
Coliforms	Soil	30 days
Mycobacterium tuberculosis	Soil	up to 2 years
	Soil	5–15 months
	Radish	3 months
Salmonella spp.	Soil	72 weeks
	Potatoes at soil surface	40 days
	Vegetables	7–40 days
	Beet leaves	21 days
	Carrots	10 days
	Cabbage/gooseberries	5 days
	Apple juice, pH 3.68	>30 days and multiplies
	Apple juice pH <3.4	2 days
S. typhi	Soil	30 days
	Vegetables/fruit	1–69 days
	Water	7–30 days
Shigella spp.	Tomatoes	2–7 days
Vibrio cholerae	Spinach, lettuce, non-acid vegetables	2 days
Enterovirus group (polio, echo, coxsackie)	Soil	150–170 days
	Cucumber, tomato, lettuce, at 6–10°C	>15 days
	Radish	>2 months

descriptions are outside its scope. Regarding legislation, the reader should always seek the appropriate regional authority.

For information on hygienic food production with an emphasis on the factory layout, equipment design and staff training the reader is recommended Forsythe & Hayes (1998) *Food Hygiene, Microbiology and HACCP* and Shapton & Shapton (1991) *Principles and Practices for the Safe Processing of Foods*. Detailed information on individual microorganisms can be found in the International Commission on Microbiological Specifications for Foods (ICMSF) book series, especially *Microorganisms in Foods 5: Characteristics of Microbial Pathogens* (1996) and *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (1998), which should be regarded as essential requirements on the bookshelf (or library) of any food microbiologist.

The whole issue of safe food manufacturing comes within the umbrella of quality control and quality assurance. Hence it requires the hygienic

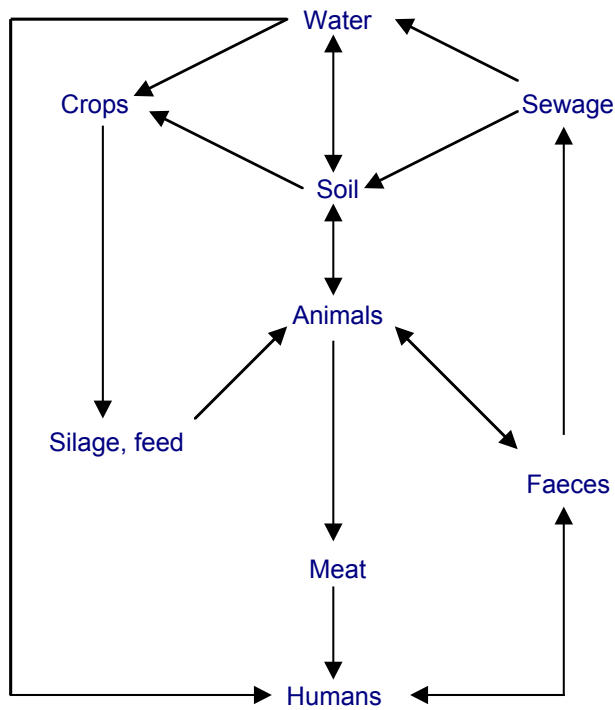

Fig. 1.1 Routes of enteric pathogen transmission to humans.

design of equipment and factory, and managerial commitment to safety and quality. Diagrammatically this can be perceived in Fig. 1.3. The current issue concerning food safety is microbiological risk assessment and the development of *food safety objectives*. These are governmental activities that eventually may decide the permissible level of foodborne pathogens, etc. (Fig. 1.4). This issue is covered in Chapter 9.

There are some foods which are currently difficult to produce without a significant risk of foodborne infection. Outbreaks of *Salmonella* spp. and *E. coli* O157:H7 associated with raw seed sprouts have occurred in several countries and currently the elderly, children and those with compromised immune systems are advised not to eat raw sprouts (such as alfalfa) until effective measures to prevent sprout-associated illness are identified (Taormina & Beuchat 1999).

1.3 Functional foods

In direct contrast to the general concern on the presence of bacteria in food, there are in fact a number of foods which deliberately contain

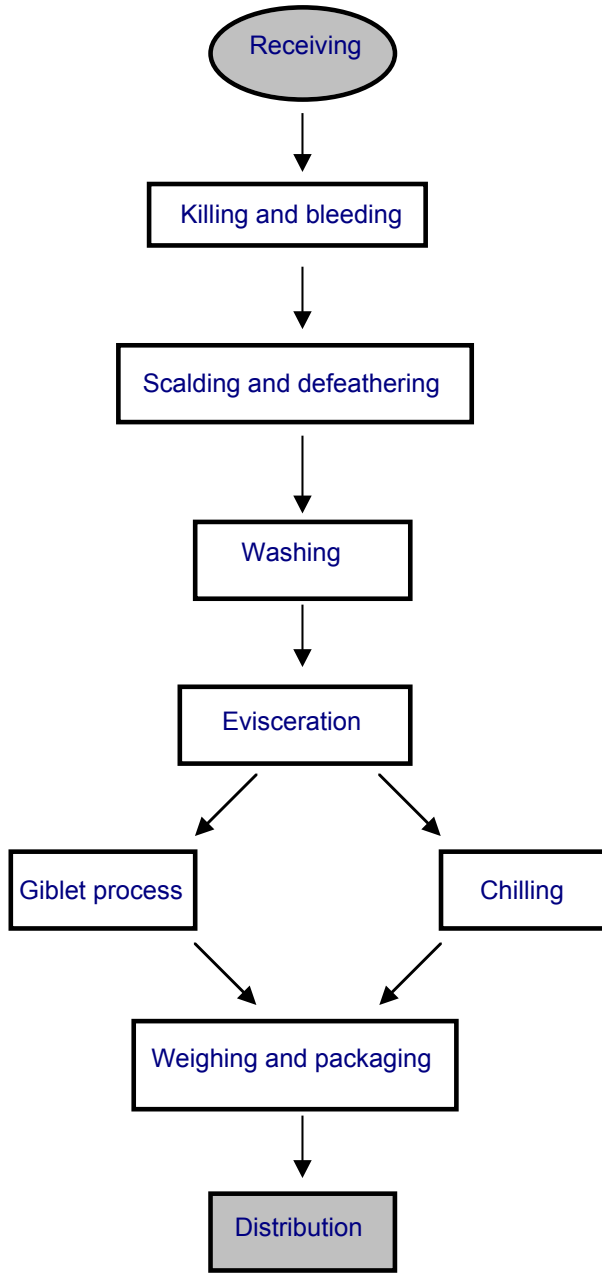

Fig. 1.2 Flowsheet for poultry processing.

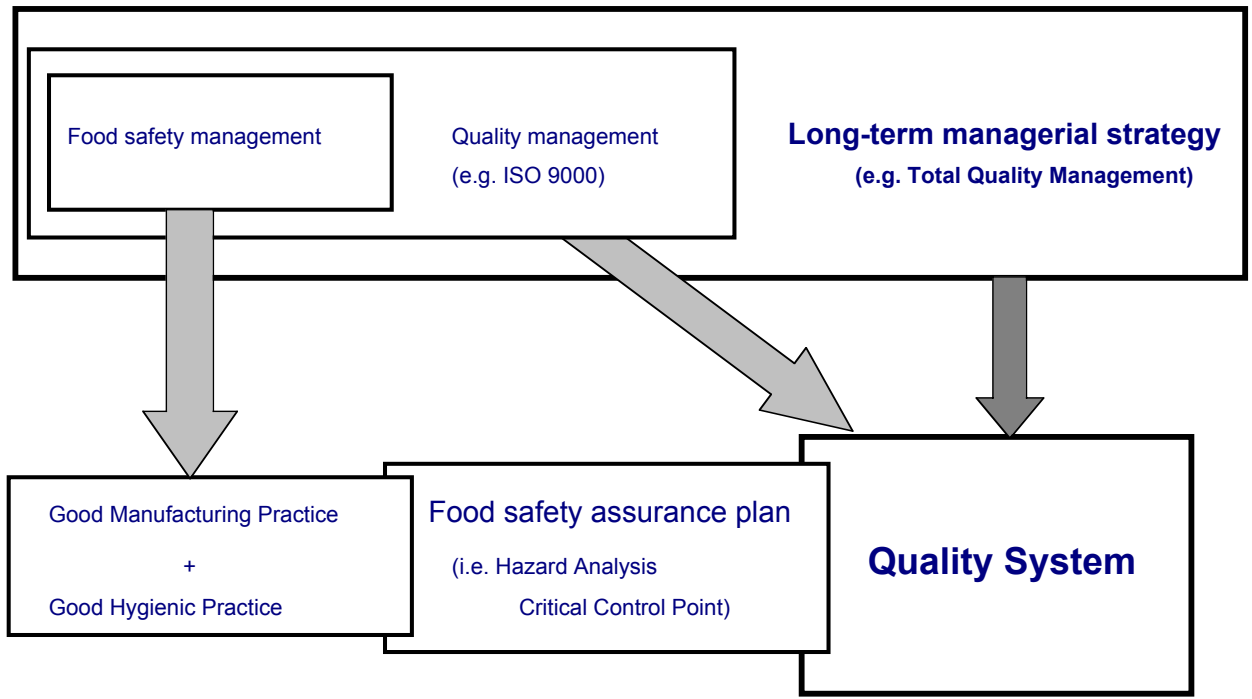

Fig. 1.3 Food safety management tools (adapted from Jouve *et al.* 1998).

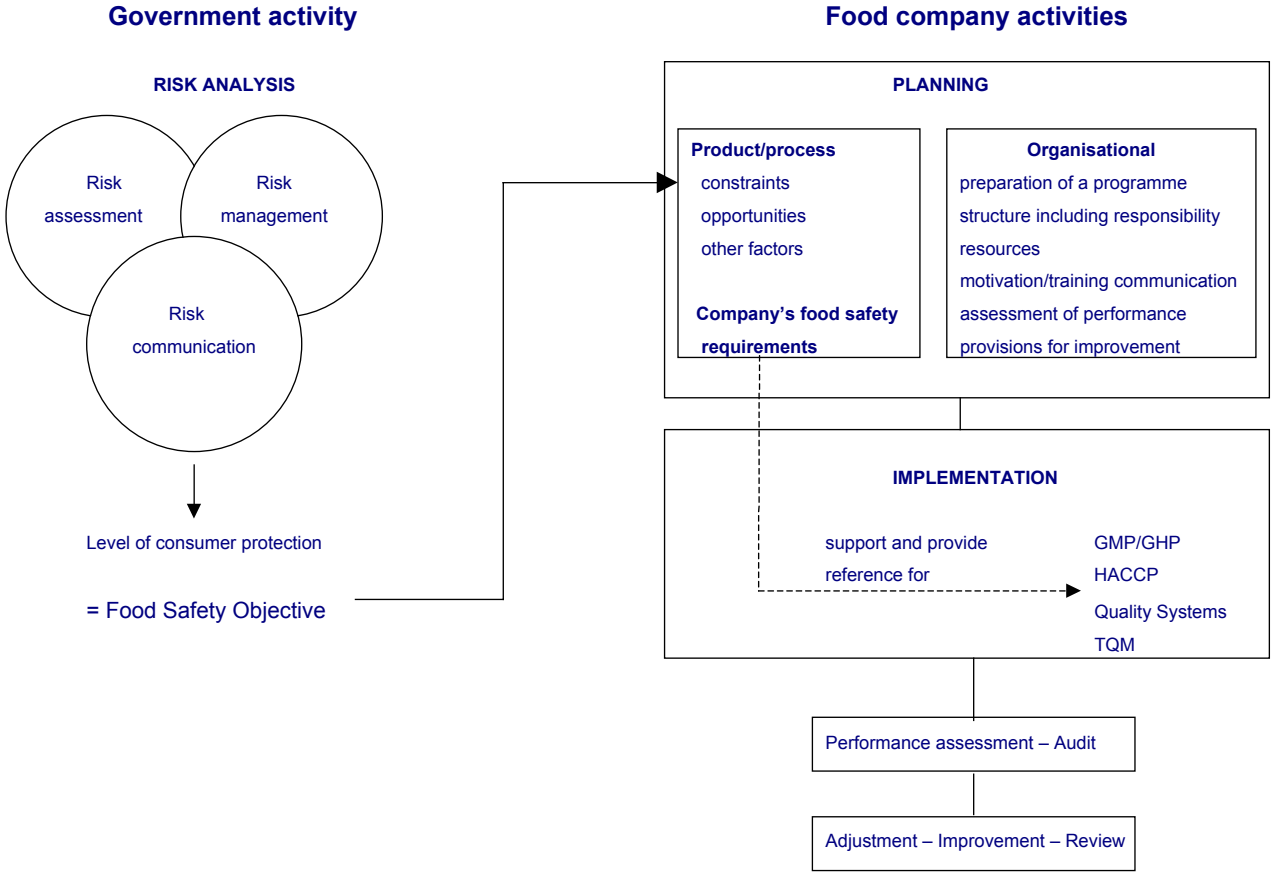

Fig. 1.4 Interaction between governments' and companies' food safety activities (adapted from Jouve *et al.* 1998).

bacteria and fungi. These are the 'fermented' foods (Section 4.4) and these foods have been produced since the early era of civilisation. The taste, texture and flavour of the food are due to microbial metabolism. Hence they are regarded as microbiologically safe. These foods now form the basis over the past 10–12 years for the development of 'functional foods'.

In Japan, and to a lesser extent in the USA, research into functional food has expanded greatly in recent years. Functional foods are expected to have a health-related or physiological effect, such as reduce the risk of disease. Most functional foods currently approved contain either oligosaccharides or lactic acid bacteria for promoting intestinal health. In 1998 the Food and Drug Adminsitration (FDA) had recognised 11 foods or food components as showing correlation between intake and health benefits (Diplock *et al.* 1999). A significant portion of function foods is concerned with lactic acid bacteria ingestion ('probiotics'). Therefore this book will review probiotics (Section 4.5) as an extension of the age-old practice of fermented foods. The legislation regarding genetically modified foods will not be covered as there are more suitable texts available (IFBC 1990; WHO 1991; OECD 1993; Jonas *et al.* 1996; FAO 1996; SCF 1997; Tomlinson 1998; Mosely 1999).

2

BASIC ASPECTS

2.1 The microbial world

The world of microbiology covers a wide range of life. Using the definition that microbiology studies life forms that are not clearly visible to the naked eye means that it includes protozoa, fungi, bacteria, viruses and prions. The major organisms studied have been bacteria, because of their medical importance and because they are easier to cultivate than other organisms such as viruses, and the more recently recognised 'prions' which are uncultivatable infectious organisms. Despite the predominance of bacteria in our understanding of microscopic life there are numerous important organisms in the other microbial categories.

The cell structure reveals whether an organism is 'eucaryotic' (also spelt eukaryotic) or 'procaryotic' (also spelt prokaryotic). Eucaryotes contain cellular organelles such as mitochondria, endoplasmic reticulum and a defined nucleus, whereas procaryotes have no obvious organelle differentiation and are in fact similar in size to the organelles of eucaryotes. Analysis of the genetic information in the ribosome (16S rRNA analysis) has revealed a plausible relationship and evolution of life from procaryotes to eucaryotes through intracellular symbiotic relationships.

A brief survey (see Table 2.1) of food poisoning microorganisms can start with eucaryotic organisms such as helminths. These include the cestode worms that are responsible for taeniasis, *Taenia solium*, the pork tapeworm, and *T. saginata*, the beef tapeworm. Both have worldwide distribution. Infection results from the ingestion of undercooked or raw meats containing the cysts. The mature worms can infect the eye, heart, liver, lungs and brain. A third tapeworm is *Diphyllobothrium latum* which is found in a variety of freshwater fish including trout, perch and pike. Contaminated water supplies can carry infectious organisms including pathogenic protozoa.

Fungi (a term which includes yeast and moulds) are eucaryotic. They are members of the plant kingdom that are not differentiated into the

Table 2.1 Microbiological contaminants.

	Where found	Sources
Viruses		
A wide range which cause diseases, including hepatitis A	Most common in shellfish, raw fruit and vegetables	Associated with poor hygiene and cultivation in areas contaminated with untreated sewage and animal and plant refuse
Bacteria		
Includes *Bacillus* spp. *Campylobacter*, *Clostridium*, *Escherichia coli*, *Salmonella*, *Shigella*, *Staphylococcus* and *Vibrio*	Raw and processed foods: cereal, fish and seafood, vegetables, dried food and raw food of animal origin (including dairy products)	Associated with poor hygiene and unclean conditions generally: carried by animals such as rodents and birds, and human secretions
Moulds		
Aspergillus flavis and related fungi	Nuts and cereals	Products stored in high humidity and temperature
Protozoa		
Amoebae and Sporidia	Vegetables, fruits and raw milk	Contaminated production areas and water supplies
Helminths		
A group of internal parasites including *Ascaris*, *Fasciola*, *Opisthorchis*, *Taenia*, *Trichinella* and *Trichuris*	Vegetables and uncooked or undercooked meat and raw fish	Contaminated soil and water in production areas

usual roots, stems and leaves; they also do not produce the green photosynthetic pigment chlorophyll. They may form a branching mycelium with differentiating cells producing hyphae to dispere spores or exist as single cell forms commonly referred to as yeast. Yeasts are very important in food microbiology. In fact the brewing and bakery industries are dependent upon yeast metabolism of sugars to generate ethanol (and other alcohols) for beer and wine production, and carbon dioxide for bread manufacture.

Mycotoxicoses are caused by the ingestion of poisonous metabolites (mycotoxins) which are produced by fungi growing in food. Aflatoxins

are produced by the fungi *Aspergillus flavus* and *A. parasiticus*. There are four main aflatoxins designated B1, B2, G1 and G2 according to the blue (B) or green (G) fluorescence given when viewed under a UV lamp. Ochratoxins are produced by *A. ochraceus* and *Penicillium viridicatum*. Ochratoxin A is the most potent of these toxins.

The bacteria are procaryotes and are divided into the eubacteria (true bacteria) and archaea organisms (old term 'Archaebacteria') according to 16S rRNA analysis and detailed cell composition and metabolism studies. There are few *Archae* organisms of importance in the food industry, the vast majority are eubacteria. As a generalisation, the size of a rod-shaped bacterium is about 2 μm × 1 μm × 1 μm. Although they are very small, even 500 cells of *Listeria monocytogenes* can be an infectious dose to a pregnant women resulting in a stillbirth. Familiar foodborne pathogens such as *Salmonella* spp., *Escherichia coli* and *Campylobacter jejuni* are eubacteria which are able to grow at body temperatures (37°C) and damage human cells resulting in 'food poisoning' symptoms such as diarrhoea and vomiting.

Viruses are very much smaller than bacteria. The large ones, such as the cowpox virus, are about 0.3 μm in diameter; the smaller ones, such as the foot and mouth disease virus, are about 0.1 μm in diameter. Because of their small size, viruses pass through bacteriological filters and are invisible under the light microscope. Bacterial viruses are termed bacteriophages (or 'phages'). They can be used to 'fingerprint' bacterial isolates which is necessary in epidemiological studies; this is termed phage typing.

Prions (short for proteinaceous infectious particles) have a very long incubation period (months or even years) and resistance to high temperature, formaldehyde and UV irradiation. With regard to sheep and cattle the isomer of the normal cellular protein PrP^{C}, termed PrP^{SC}, accumulates in the brain causing holes or plaques. This leads to the symptoms of scrapie in sheep and BSE in cattle. The equivalent disease in humans (new variant Creutzveld Jacob Disease, nvCJD) is probably due to ingestion of infectious agents from cattle.

2.2 Bacterial cell structure

2.2.1 Morphology

Bacteria are characteristically unicellular organisms. Their morphology can be straight or curved rods, cocci or filaments depending upon the organism concerned. The morphology of the organism is consistent with the type of organism and to a lesser extent to the growth conditions. Bacteria in the genera *Bacillus*, *Clostridium*, *Desulphotomaculum*,

Sporolactobacillus and *Sporosarcina* form spores in the cytoplasm under certain environmental conditions (stress related). The spore is more resistant to heat, drying, pH, etc. than the vegetative cell and hence enables the organism to persist until more favourable conditions when the spore can subsequently germinate and grow into a vegetative cell.

2.2.2 Gram stain

Christian Gram was a Danish microbiologist who wanted to visualise bacteria in muscle tissue. He tried a range of histological staining protocols and eventually came up with the, nowadays ubiquitous, 'Gram stain' procedure. It was noted by Christian Gram that the bacteria stained either dark blue or red. This was due to the precipitation of crystal violet with Lugol's iodine in the cell cytoplasm which could not be extracted using solvents such as ethanol or acetone from certain organisms (Gram positives) but was extracted from others (Gram negatives). Since the latter cells were no longer stained by the crystal violet, counterstaining was required with safranine or basic fuschin. More recently, the reason for the differential extraction of the crystal violet–iodine complex was found to be due to differences in the cell wall structure (Fig. 2.1).

Gram-positive organisms have a thick cell wall surrounding the cytoplasmic membrane. This is composed of peptidoglycan (also known as murein) and teichoic acids. The Gram-negative organisms have a thinner cell wall which is surround by an outer membrane. Hence Gram-negative organisms have two membranes. The outer membrane differs from the inner membrane and contains the molecule known as lipopolysaccharide (LPS). Since peptidoglycan is the site of action for the original penicillin antibiotic (penicillin G) this explains why the antibiotic was initially so effective against streptococci and staphylococci (both Gram positives) rather than Gram negatives such as *Escherichia coli*. Consequently the semi-synthetic penicillins were developed to widen the range of sensitive organisms to penicillin.

2.2.3 Lipopolysaccharide (LPS, O antigen)

The outer membrane of Gram-negative organisms contains the molecule lipopolysaccharide (LPS). It is composed of three regions: lipid A, core and O antigen. Lipid A anchors the molecule in the outer membrane and is toxic (Fig. 2.2). It is a virulence factor for organisms such as *Salmonella* and *Chlamydia*. The core region is composed of sugar molecules, the sequence of which reflects the organism's identity. The O region is more variable. In some organisms the O region may only contain a few sugar residues, whereas in others there are repeating units of sugars. Isolates of a

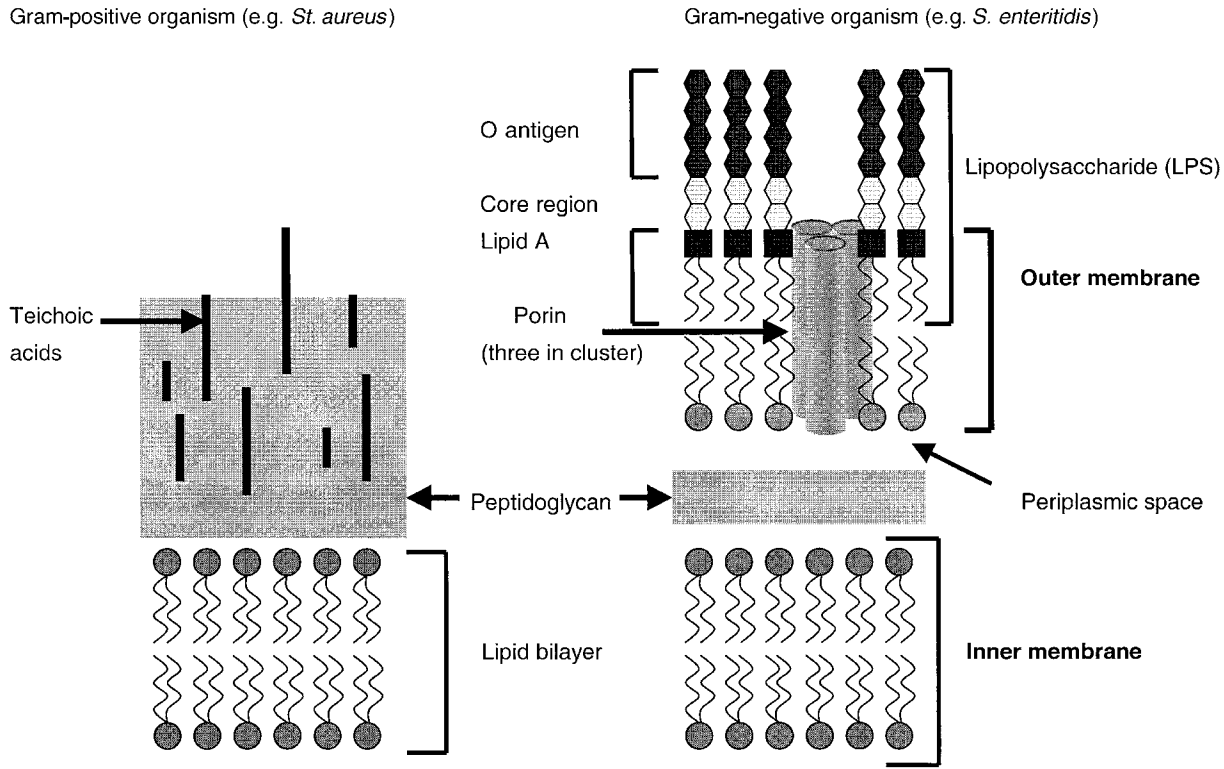

Fig. 2.1 Structure of the bacterial cell wall.

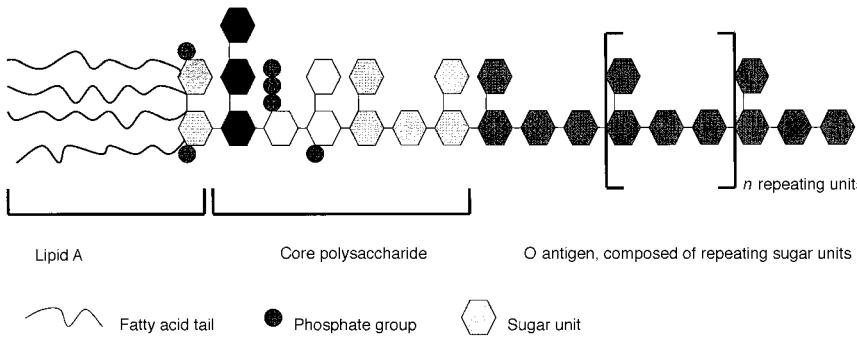

Fig. 2.2 Structure of the lipopolysaccharide molecule of Gram-negative organisms.

single species vary in the amount of O region present and hence this gives rise to the terms 'smooth' and 'rough' variants. The antigens are on the body of the organism and are known as the 'somatic' or 'O antigens'. 'O' is for 'ohne', the German for 'without', which originally referred to non-swarming or non-flagellated forms. The LPS structure is resistant to boiling for 30 minutes and is therefore also referred to as a 'heat-stable' antigen.

One of the factors causing the fatality of Gram-negative septicaemia is that the presence of LPS causes the overproduction of tumour necrosis factor (TNF), which leds to overstimulation of nitric oxide synthase.

2.2.4 Flagella (H antigen)

Most rod-shaped (and a few coccoid) bacteria are motile in liquid media. Motility occurs due to the rhythmic movement of thin filamentous structures called flagella. The cell may have a single flagellum (monotrichus) or a tuft of flagella (lophotrichus) at one or both poles, or many flagella (peritrichus) over the entire surface. The proteinaceous nature of the flagellum gives rise to its antigenicity, called the H antigen. 'H' is from the German word 'hauch' meaning 'breath' which originated from a description of the appearance of *Proteus* swarming on moist agar plates being similar to the light mist caused by breathing on cold glass. Flagella are denatured by heat (100°C, 20 minutes) and therefore the H antigen is referred to as heat-labile. Flagella are also denatured by acid and alcohol.

Salmonella species may express two flagellar antigens: phase 1, which is possessed by only a few other serotypes of *Salmonella*, and phase 2, which is less specific. Phase 1 antigen is represented by letters, phase 2 antigen by numbers. A culture may entirely express one phase (monophasic culture) which will give rise to mutants in the other phase

(diphasic culture), especially if the culture is incubated for more than 24 hours. The serotyping of some *Salmonella* serovars is given in Table 2.2.

E. coli has a total of 173 different O antigens and 56 different H antigens. *E. coli* O157:H7 is a very pathogenic strain which is recognised by serotyping the O and H antigens.

Table 2.2 *Salmonella* serotyping.

Serotype	Group	O antigen	H antigen	
			Phase 1	Phase 2
S. paratyphi A	A	(1), 2, 12	a	—
S. typhimurium	B	(1), 4, (5), 12	i	1, 2
S. paratyphi C	C_1	6, 7, Vi	c	1, 5
S. newport	C_2	6, 8	e, h	1, 2
S. typhi	D	9, 12, Vi	d	—
S. enteritidis	D	(1), 9, 12	g, m	
S. anatum	E_1	3, 10	e, h	1, 6
S. newington	E_2	3, 15		
S. minneapolis	E_3	(3), (15), 34		

Parentheses indicate antigen determinant which may be difficult to detect. Dominant antigenic determinants are underlined.

2.2.5 Capsule (Vi antigen)

Some bacteria secrete a slimy polymeric material composed of polysaccharides, polypeptides or polynucleotides. If the layer is very dense then it may be visualised as a capsule. The possession of the capsule endows a resistance to white blood cell engulfment. Subsequently the capsule's antigenicity is called the Vi antigen and was originally thought to be responsible for the virulence of *S. typhi* (see Table 2.2).

2.3 Microbial growth cycle

The microbial growth cycle is composed of six phases (Fig. 2.3):

(1) Lag phase
Cells are not multiplying, but are synthesising enzymes appropriate for the environment.

(2) Acceleration phase
An increasing proportion of the cells are multiplying.

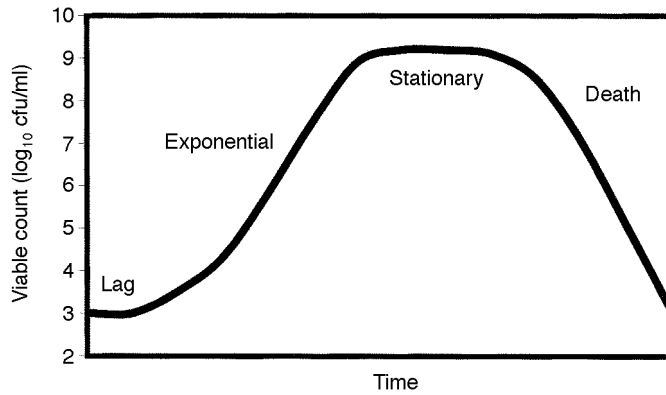

Fig. 2.3 The microbial growth curve.

(3) Exponential (or log) phase
The cell population are multipying by doubling (1–2–4–8–16–32–64, etc.). The cell numbers are increasing at such a rate that to represent them graphically it is best to use exponential values (logarithms). This results in a straight line, the slope of which represents the μ_{max} (rate of maximum growth), and the doubling time t_d (time required for the cell mass to increase two-fold).

(4) Deceleration phase
An increasing proportion of cells are no longer multiplying.

(5) Stationary phase
The rate of growth equals the rate of death, resulting in equal numbers of cells at any given time. Death is due to the exhaustion of nutrients, the accumulation of toxic end products and/or other changes in the environment, such as pH changes. The length of the stationary phase is dependent upon a number of factors such as the organism and environmental conditions (temperature, etc.). Spore forming organisms will develop spores due to the stress conditions.

(6) Death phase
The number of cells dying is greater than the number of cells growing. Cells which form spores will survive longer than non-sporeformers.

The length of each phase is dependent upon the organism and the growth environment, temperature, pH, water activity, etc. The growth cycle can be modelled using sophisticated computer programs and leads to the area of microbial modelling and predictive microbiology (see Section 2.8).

2.4 Death kinetics

2.4.1 Expressions

There are a number of expressions used to describe microbial death:

- D value: decimal reduction time. Defined as the time at any given temperature for a 90% reduction (=1 log value) in viability to be effected.
- Z value: temperature increase required to increase the death rate 10-fold, or in other words reduce the D value 10-fold.
- P value: time at 70°C. A cook of 2 minutes at 70°C will kill almost all vegetative bacteria. For a shelf life of 3 months the P value should be 30 to 60 minutes, according to other risk factors.
- F value: this value is the equivalent time, in minutes at 250°F (121°C), of all heat considered, with respect to its capacity to destroy spores or vegetative cells of a particular organism.

Since these values are mathematically derived they can be used in predictive microbiology and Section 2.8 and Chapter 9, Microbial Risk Assessment (MRA).

2.4.2 Decimal reduction times (D values)

In order to design an effective heating treatment time and temperature regimes it is imperative to have an understanding of the effects of heat on microorganisms. The thermal destruction of microorganisms (death kinetics of vegetative cells and spores) can be expressed logarithmically. In other words for any specific organism, in a specific substrate and at a specific temperature, there is a certain time required to destroy 90% (=1 log reduction) of the organism. This is the decimal reduction time (D value). The rate of death depends upon the organism, including the ability to form spores, and the environment (Table 2.3). Free (or planktonic) vegetative cells are more sensitive to detergents than fixed cells (biofilms or slime). The heat sensitivity of an organism at any given temperature varies according to the suspending medium. For example, the presence of acids and nitrite will increase the death rate whereas the presence of fat may decrease it.

Plotting the D values for an organism in a substrate against the heating temperature should give a straight-line relationship (Fig. 2.4). The straight-line relationship does not always occur due to cell clumping. The slope of the line (in degrees Fahrenheit or Celsius) can be used to determine the change in temperature resulting in a 10-fold increase (or decrease) in the D value. This coefficient is called the Z value (Fig. 2.5). The integrated