MICRO-FACTS

Seventh Edition

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FOREWORD

The previous six editions of 'Micro-Facts' have proved a useful ready reference for all those who are concerned with food safety. In this seventh edition, we have extensively updated the book with some sections significantly expanded, and some new chapters. We have also updated the book for currency of information. Our emphasis continues to be to serve the needs of the food industry, whether a manufacturer, retailer or caterer.

Key changes in the seventh edition are the inclusion of a new chapter on factors affecting microbial growth and a glossary of microbial terms. The bacterial pathogens chapter now includes a section on rapid methods. The chapter on moulds and mycotoxins, and yeasts and moulds as spoilage organisms which was new for the sixth edition, has been extensively expanded, with 28 additional key mould species, and 12 key yeast species. In many cases, product recalls are initiated by excessive growth of spoilage organisms, not foodborne pathogens; it is important to be aware of these organisms.

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Several factors related to the environment and the conditions in which food is stored influence the growth of micro-organisms in food. These factors can be divided into intrinsic and extrinsic elements. Growth is possible over a wider range of temperatures than that of toxin production. For example, *Aspergillus flavus* can grow from 10 - 12 °C to 43 - 48 °C, whereas aflatoxins are produced from 13 - 15 °C up to 37 °C. The minimum water activity (a_w) for *A. flavus* is 0.78 - 0.80, but aflatoxins are produced above a_w 0.82 - 0.83

Some organisms have the ability to produce spores when exposed to conditions outside their typical growth range. These organisms pose difficulties for the food industry as the spores are more resistant to the intrinsic and extrinsic factors that are lethal to vegetative cells. Unless a factor or treatment is targeted at destruction of the spores, they can survive in the product, and when the environmental conditions return to suitable levels, the spores are able to germinate and grow.

The issue is compounded by the fact that spores sometimes germinate earlier than would be expected as a result of heat shock if they are exposed to temperatures outside their growth range but less than their lethal limit, and can make the spores more resistant to other factors than normal. It is for this reason that foods which are subject to contamination with spore-forming organisms are often subjected to high temperature processing.

INTRINSIC FACTORS

The inherent physical, chemical and biological properties of the food, such as pH, redox potential, water activity and the presence of antimicrobial substances have the capacity to either stimulate or retard the growth of micro-organisms. Some intrinsic factors are interlinked with some extrinsic factors. For example, water activity rises with increasing temperature; there is an increase in water activity of 0.03 with a 10 °C rise in temperature.

pH

The intracellular pH of any organism must be maintained above the pH limit that is critical for that organism. The control of intracellular pH is required in order to prevent the denaturation of intracellular proteins. Each organism has a specific requirement and pH tolerance range; some are capable of growth in more acid conditions than others. Most micro-organisms grow best at neutral pH (7.0). Yeasts and moulds are typically tolerant of more acidic conditions than bacteria but several species of bacteria will grow down to pH 3.0. These species are typically those that produce acid during their metabolism such as the acetic or lactic acid bacteria. Bacterial pathogens are usually unable to grow below pH 4.0. The type of microbial growth typically seen in a particular food is partly related to the pH of that product. Fruits are naturally acidic, which inhibits the growth of many bacteria, therefore spoilage of these products is usually with yeasts and moulds. Meat and fish however have a natural pH much nearer neutral and they are therefore susceptible to the growth of pathogenic bacteria. Individual strains of a particular species can acquire acid resistance or acid tolerance compared to the normal pH range for that organism. For example acid-adapted *Salmonella* have been reported that are capable of growth at pH 3.8.

There is a broad distinction between high and low acid foods; with low acid foods being those with a pH above 4.6, and high acid, below this. This is because pH 4.6 is the lower limit for the growth of mesophilic *Clostridium botulinum*. Foods with a pH greater than 4.6 must either be chilled, or if ambient stored, undergo a thermal process to destroy *C. botulinum* spores, or have a sufficiently low water activity to prevent its growth.

Different foods tend to spoil in different ways. For example, carbohydrate-rich foods often undergo acid hydrolysis when they spoil; this usually reduces the pH, and tends to reduce the risk of pathogen growth. This principle is used in the fermentation of dairy and lactic meat fermentations. In contrast, protein-rich foods tend to increase in pH when they spoil, making them possibly less safe, as the pH rise to the zone where more pathogens can grow.

Redox Potential

Also known as the oxidation-reduction potential or Eh, the redox potential of a food has an impact on microbial growth. Aerobic organisms require a

food to have a positive redox potential (an oxidised state) whereas anaerobes require a negative potential (a reduced state) for growth. It should be noted that the presence of oxygen is not an absolute requirement for oxidation-reduction reactions as other compounds can accept electrons. Different foods have distinct redox potentials and these influence the type of microbial growth typically seen in that food. Foods of plant origin typically have a redox potential of +300 to 400 mV thereby favouring the growth of aerobic bacteria and moulds. Solid meat typically has a redox value of -200 mV and therefore anaerobic organisms are associated with this food type.

Water Activity

Water activity (a_w) is a measure of the amount of freely available water within a food. The a_w of a food can be expressed as the ratio of the water vapour pressure of the food to the water vapour pressure of pure water at the same temperature. Equilibrium relative humidity values can be converted to a_w by dividing by 100. Water is required for microbial growth; therefore foods with low water activities cannot support the growth of micro-organisms. Pathogenic and spoilage bacteria do not grow in food with a water activity of less than 0.85. Many yeasts and moulds however are capable of growth at much lower water activities than this; some can even grow at a_w 0.60. The water activity of a food can be altered from the value typical for a food type in order to prevent microbial growth via the addition of solutes or ions or by freezing or drying. It is as a result of water activity that dry foods such as crackers or dried pasta can have a shelf life of many months and not be spoilt by micro-organisms. Foods such as jams and parmesan cheese (a_w 0.60 - 0.85) will show signs of mould growth over time but no bacterial growth, and foods such as meat and milk (a_w 0.98 - 0.99) are associated with food poisoning causing bacteria.

Antimicrobials

Certain foods naturally contain antimicrobial substances that will exhibit an inhibitory action on the growth of micro-organisms. Examples are essential oils contained within cloves, garlic, mustard and thyme, lactoferrin in cows' milk and lysozyme in eggs. Some plants release isothiocyanates which have antibacterial and antifungal effects.

EXTRINSIC FACTORS

The characteristics of the environment in which the food is maintained, such as the temperature, atmosphere and relative humidity can affect the properties of the food as well as the potential for the growth of micro-organisms.

Temperature

As temperature influences enzymic reactions it has an important role in promoting or preventing microbial growth. Micro-organisms can be categorised into one of four groups depending on their optimum growth temperature and the temperature range at which they will grow.

- Thermophiles have optimum growth *ca*. 55 °C and a growth range of 30 - 75 °C
- Mesophiles have optimum growth *ca*. 35 °C and a growth range of 10 - 45 °C
- Psychrotrophs have optimum growth *ca*. 20 - 30 °C and a growth range of 0 - 40 °C
- Psychrophiles have optimum growth *ca*. 15 °C and a growth range of -5 - 20 °C

At temperatures higher than an organism's optimum growth range, cells die rapidly. Lower temperatures still result in cell death but at a slower rate. Temperature can therefore be used to eliminate or control the growth of micro-organisms. Heat treatments (pasteurisation or sterilisation) eliminate contaminating micro-organisms via the application of heat for a specific time period (time and temperatures used being dependent upon the target organism). Refrigeration of a food can prevent spoilage by controlling the growth of thermophilic or mesophilic organisms. Most pathogens are capable of growth at refrigeration temperatures and therefore cannot be controlled via refrigeration alone. Some, for example *Listeria*, can grow at very low temperatures.

Atmosphere

As all micro-organisms have specific requirements for oxygen and carbon dioxide, by altering the atmosphere within a food package the growth of micro-organisms can be controlled. Vacuum packing food removes available oxygen and thereby prevents the growth of aerobic organisms; it does however still allow the growth of anaerobes such as *C. botulinum*. Modified atmosphere packaging (MAP) allows the food producer to select the atmosphere within the package using varying combinations of oxygen, carbon dioxide and nitrogen, depending upon the product type and target micro-organisms. The majority of MAP foods have varying combinations of carbon dioxide and nitrogen.

Relative Humidity

The relative humidity in which a food is stored can have an influence on the water activity of that product and an influence on the growth of micro-organisms on the surface of a product. If the growth of micro-organisms in a food is controlled by the water activity of a product then it is very important that the food be stored under relative humidity conditions which will not allow the uptake of moisture from the air, and therefore an increase in water activity. Packaging can be used to limit the migration of moisture into the product.

HURDLE CONCEPT

There is an increasing demand from consumers for minimal processing of the food that they eat. Therefore the hurdle concept has increasingly been used to preserve foods. The hurdle concept controls food safety and spoilage by ensuring that a number of factors are in place that prevent the growth of micro-organisms rather than one single controlling factor at a level beyond the range of the target organism. The benefit of hurdle technology is that the controlling factors can be at sub-optimal limits for the micro-organisms concerned rather than at lethal limits, and that when used in combination they can control microbial growth, the concept being that by placing small barriers against micro-organism growth by the various intrinsic and extrinsic influences, the micro-organisms are unable to overcome all the small ‘hurdles’ and are unable to grow. Also as cells are injured by the conditions of one hurdle, they become more sensitive to the other hurdles and are killed

in their presence. Therefore mild but reliable preservation can be achieved. Combinations of water activity, temperature and pH can be used. For example, *Listeria monocytogenes* is inhibited by 15% salt, pH 4.1 or a temperature at 0 °C or below. Whereas if a product has a combination of 5% salt and a pH of 5.5, then a temperature of 14.1 °C is sufficient.

FOODBORNE BACTERIAL PATHOGENS

INTRODUCTION

Food Poisoning – a Brief Overview

Foodborne illness is a major public health concern worldwide. The costs in terms of human illness and economic losses to individual companies and to the public health sector can be immense. Recalls, factory closure, legal proceedings and adverse publicity to food companies involved in 'food scares' can result in both acute and long-term losses, with repercussions that may continue for many years. For example, sales of corned beef after the 1964 typhoid outbreak in Aberdeen, Scotland, were not restored to pre-outbreak levels for about twenty years. More recently, an outbreak of *Escherichia coli* O157 infection in the UK caused the closure of a meat processor and was the subject of major headlines in the media, following the death of a five year old boy as a result of eating a contaminated product.

We can see by observing the scientific literature and reviewing the media that foodborne illness is a worldwide problem, not restricted to any particular country. Solutions to problems will often have to be considered from outside the European community if they are to be effective. Nevertheless, there was a real increase in food poisoning in the UK in the 1980s and 1990s, reaching a peak of approximately 100,000 reported cases in 1997/1998. Generally, levels of foodborne illness are now on the decline in the UK following improvements in disease prevention and an increased awareness of the issues of foodborne illness.

There were many different causes for this increase, and the 'Richmond' Committee on the Microbiological Safety of Food concluded in 1990 that poultry and their products were the most important source of human gastrointestinal infections arising from food, both from carcass meat contaminated with *Salmonella* and *Campylobacter*; and shell eggs that could also be a source of *Salmonella*. They considered that many factors were involved in this increase, with no factor more important than the next.

More recently, the number of recorded cases of food poisoning in the UK has fallen significantly, and this is thought to be due principally to action taken by the poultry industry to eliminate *Salmonella* from laying flocks and broilers by vaccination programmes and improved hygiene. In contrast, however, reports of *Campylobacter* infection have risen over the same period, and the organism is now the most important cause of bacterial foodborne disease in the UK. The same trends are noted in other European countries and the United States, and much attention is now being directed to the control of *Campylobacter* in poultry. Some countries remain free of *Salmonella* and *Campylobacter*, by the effective application of bio-security measures designed to contain the organism. Generally levels of foodborne illness from other organisms are now also in decline in the UK following improvements in disease prevention and an increased awareness of the issues of foodborne illness.

The global nature of the food chain itself can cause problems, with a lack of control over the ingredient supply chain resulting in large-scale chemical contamination issues. Several recent large food poisoning outbreaks in the UK, caused by *Salmonella*, have had, at their root, the purchase of (presumably) cheaper eggs from outside the UK, where there is no vaccination policy.

'Emerging' pathogens are the subject of much research and discussion. They continue to present new challenges for identification and control. Changing consumer demands mean that some unlikely organisms may emerge over the next 10 years, and it is the job of microbiologists to anticipate and control these new organisms. It is important to remember that less than 20 years ago, *L. monocytogenes* was an obscure species, almost unknown outside the veterinary field. *Campylobacter* has emerged more recently as a significant cause of food poisoning from the consumption of poultry, and *E. coli* O157 as a cause of food poisoning in ground beef.

An explanation of the term 'food poisoning'

For the purposes of Micro-Facts, the rather loosely applied term 'food poisoning' encompasses all those types of illness that are caused by the consumption of food contaminated with pathogenic micro-organisms and/or their toxins. In this context, therefore, *Campylobacter* has been included as a 'food poisoning' bacterium, despite the fact that it does not grow in food - food acts only as a vehicle for the organism.

In most cases food poisoning involves gastroenteritis, vomiting and/or diarrhoea; but in the cases of botulism and listeriosis, the main symptoms are caused by effects on other ('extra-intestinal') parts of the body.

Intoxication versus infection

Food poisoning can be split further into three types: 'intoxication', 'infection', or 'intermediate'.

Infection is caused by the *in vivo* multiplication of bacteria that are taken in with food; subsequently, live food poisoning organisms (usually many) need to be ingested and there is normally a delayed response (typically involving diarrhoea) reflecting the time needed for an infection to develop. Examples of food poisoning bacteria that cause infection are *Salmonella*, *Campylobacter* and *Vibrio parahaemolyticus*.

Intoxication is caused by the ingestion of toxin(s) that has (have) been pre-formed in the food. Hence, there is no requirement for live organisms to be present, and the onset of symptoms (usually starting with vomiting) is soon after the toxic food is ingested. Examples are *Bacillus cereus* and *Staphylococcus aureus* intoxication.

The 'intermediate' type of food poisoning is caused by the formation of toxin(s) in the bowel as a consequence of consumption of contaminated food, as in the case of *Clostridium perfringens* food poisoning. However, for the purposes of Micro-Facts, the 'intermediate type' is included under 'infection'.

In each individual section, descriptions are provided of all the main food poisoning bacteria, including those that cause intoxication, infection, or the intermediate type of food poisoning. Micro-organisms that are known to be occasionally associated with foods, but not to cause 'food poisoning' symptoms (viz. the causative agents of tuberculosis, typhoid, brucellosis, Q-fever, etc.), are not included. Other foodborne - or potentially foodborne - pathogens are also mentioned, as an aid to awareness.

Summaries of the key characteristics of the main organisms considered are to be found in the convenient tables to follow.

INFECTIONS
Food-poisoning characteristics

	Incubation time*	Symptoms*	Duration*	Mortality rate	Infective dose
Salmonella	8-72 h	Abdominal pain diarrhoea, nausea, fever, (vomiting)	2-5 d	Rare but important exceptions	Usually high (10^{6}+), low in some foods (10-100 cells)
Campylobacter	1-11 d Usually 2-5 d	Fever, diarrhoea (sometimes with blood and mucus), nausea, abdominal pain	1-7 d+	Rare	Low, 50-500 cells
L. monocytogenes	3-70 d ‡	Flu-like symptoms, meningitis, septicaemia, meningoencephalitis	Variable	30-40%	Not known
Y. enterocolitica	1-11 d	Abdominal pain,† diarrhoea, fever, others, incl. pharyngitis	1-3 weeks	Rare	Not known probably high >10^{4}
V. parahaemolyticus	4-96 h Usually 12-24 h	Diarrhoea (in severe cases with blood and mucus), abdominal pain, nausea	1-7 d Usually 3-5 d	Rare	10^{5}+
C. perfringens	8-22 h	Diarrhoea, abdominal pain	1-2 d	Rare	High: 10^{5}/g+
Verocytotoxigenic *E. coli*	1-14 d Usually 3-4 d	Diarrhoea, abdominal pain	5-10 d	1%**	Very low, between 2 and 2,000 cells
C. sakazakii	18-30 d (premature babies)	Ventriculitis, brain abscess hydrocephalus, neonatal necrotising enterocolitis		40-80% in neonates	Not known

* Typical - can vary quite widely.
‡ Depends on dose, immune state of the host, and virulence of the strain.
† Can resemble appendicitis.
** VTEC-associated HUS infection.

INFECTIONS
Growth/survival characteristics

	Temperature (°C)		Heat resistance	Min pH*	Min a_w*	Aerobic/anaerobic
	Min*	**Optimum**				
Salmonella	4	35-37	Heat-sensitive† $D_{60\ °C}$ 1-10 min.	3.8‡	0.93	Facultative
Campylobacter	30	42-43	Slightly more heat sensitive than *Salmonella* $D_{55\ °C}$ 0.74-1 min.	4.9	0.98	Fastidious micro-aerophile – capnophilic
L. monocytogenes	-0.4	30-37	Slightly less heat sensitive than *Salmonella* $D_{60°\ C}$ 5-8 min.	4.3	0.9 in glycerol	Facultative
Y. enterocolitica	-2	28-29	Heat-sensitive $D_{60°\ C}$ 27 sec	4.2	0.95	Facultative
V. parahaemolyticus	5	30-35	Heat-sensitive $D_{41°\ C}$ 0.8-65.1 min	4.8	0.92 **	Facultative
C. perfringens	15	43-45	Forms heat-resistant spores	5	0.93	Anaerobic
Verocytotoxigenic *E. coli*	7	37	Heat sensitive $D_{63\ °C}$ 0.5 min	4.0 - 4.4	0.97	Facultative
C. sakazakii	5.5	37-40	Thermo-tolerant $D_{60\ °C}$ 2.5 min	3	0.2	Facultative

* Under otherwise optimal conditions - limits will vary according to strain, temperature, type of acid (in the case of pH), solute (in the case of a_w) and other factors. They will normally be higher in foods. However, variabilities in measurement, etc., must be allowed for - a margin of error must be incorporated.
† *S. Senftenberg* 775W is much more heat-resistant at high a_w. D-values for other strains of *Salmonella* increase at lower a_w, approaching or exceeding those for *S. Senftenberg* 775W.
‡ Most *Salmonella* serotypes will not grow below pH 4.5.
** Has a minimum requirement for salt – it is a halophile

INTOXICATIONS
Growth/survival characteristics

	Temperature (°C)		Heat resistance		Min pH*	Min a_w*	Aerobic/anaerobic
	Min*	**Optimum**	**Spores**	**Toxins**			
C. botulinum							
Group I	10	37	$D_{121\,°C}$ 0.21 min.		4.6	0.94	
				Destroyed by 5 min at 85 °C			Anaerobic
Group II	3	25-30	$D_{100\,°C}$ <0.1 min.		5	0.97	
Staph. aureus	7	30-37	NA	Heat-resistant $D_{56\,°C}$ 1-2 min in phosphate buffer	4.2†	0.86**	Facultative
B. cereus	4	30-35	$D_{95\,°C}$ 1.2-36 min.	Emetic toxin, extremely heat-resistant	4.3	0.95x	Facultative

* Under otherwise optimal conditions – limits will vary according to strain, temperature, type of acid (in the case of pH), solute (in the case of a_w) and other factors. They will normally be higher in foods. However, variabilities in measurement, etc., must be allowed for – a margin of error must be incorporated.
† Minimum for enterotoxin production » pH 5.2, under aerobic conditions.
** Range from 0.86 - >0.99
x Possibly as low as 0.91 or less.

INTOXICATIONS
Food-poisoning characteristics

	Incubation* time	Symptoms*	Duration*	Mortality rate	Infective dose
C. botulinum	12-36 h	Impaired vision, dryness of mouth, nausea, vomiting paralysis†		Formerly 30-65%, now much lower (<10%)	0.005-0.5 mg of toxin
Staph. aureus	1-7 h	Nausea, vomiting, abdominal pain, diarrhoea		Rare	10^5+ cells needed to produce toxin <1 mg of toxin
B. cereus					
emetic	1-5 h	Nausea, vomiting	6-24 h	Nil	10^5+ cells (+ toxin)
diarrhoeal	8-16 h	Abdominal pain	12-24 h		10^5+ cells

* Typical - can vary widely

† Affects the nervous system

Methods for Detecting/Counting Foodborne Pathogens in Foods

The sections in Micro-Facts that describe different foodborne pathogens include certain references to methods for their isolation. These references have been selected, in the main, on the basis that they either describe well-verified and established methods, or they review a number of methods.

The selection and use of detection methods - whether traditional or 'rapid' - should be carefully considered. Microbiological testing alone - especially end-product testing - cannot ensure food safety (you cannot 'test in' safety), and should be done as part of a HACCP scheme. Unfortunately, many people still seem to feel that end-product testing can be a useful means of determining overall safety of food products, coupled with 'positive release'.

Before embarking on any microbiological examination of foods, the questions should be asked: "What are we testing for and why? What will the results mean? What action will need to be taken on finding a particular result?"

Account also needs to be taken of the uneven distribution of micro-organisms in non-liquid foods, and appropriate sampling plans should be used (see, for example, the sampling plans described by the International Commission on Microbiological Specifications for Foods (ICMSF) 1986).

Furthermore, the detection of low numbers of foodborne pathogens may require the careful selection of appropriate resuscitation, enrichment, isolation and confirmatory tests. Pathogens may be damaged, and may be greatly outnumbered by other, competitor, organisms. These facts need to be taken into account when considering methods to be used.

Many standard methods are described by the British Standards Institution (BSI) and the International Standards Organization (ISO) (available through BSI). Other standard methods, relating to dairy products or chocolate and confectionery, are available from the International Dairy Federation (IDF) (through the United Kingdom Dairy Association), or the International Office of Cocoa, Chocolate and Sugar Confectionery (IOCCC), respectively (see chapter on Addresses of Authorities/Sources of Further Information, etc.). The ICMSF and the Association of Official Analytical Chemists (AOAC) also describe recommended/standard isolation procedures. Certain books, such as 'The Microbiological Safety and Quality of Food: Volume 2' (by Lund, Baird-Parker and Gould), and 'Foodborne Pathogens: An Illustrated Text' (by Varnam and Evans), review or describe many methods for various pathogens, and major media suppliers provide useful guidance on methods, through their catalogues and their technical support.

American standard methods are described in the FDA Bacteriology Analytical Manual (BAM) and/or in the APHA Compendium of Methods for the Microbiological Examination of Foods.

Advice concerning the use of different isolation procedures, whether 'traditional' or rapid, can be obtained through Leatherhead Food Research and other research associations.

Rapid Methods for Detection/Enumeration of Foodborne Pathogens

The field of rapid detection methods has emerged over the last few decades and has provided many faster, more specific and sensitive and sometimes more accurate methods for the isolation, detection, enumeration or characterisation of micro-organisms. These methods are often less labour intensive than traditional methods but often involve expensive equipment or techniques that require a high degree of knowledge or experience from the operator.

Immunological techniques, diagnostic kits, genetic and molecular techniques and most recently biosensors are all examples of rapid methods. The following section aims to highlight the major methods available for rapid detection/enumeration with a brief overview of the technology and the brand names or manufacturers.

Biochemical (Enzymatic) Methods

Miniaturisation tests use biochemical methods to facilitate the characterisation of various species of bacteria. Typically, wells are used that contain lyophilised substrates. Once the organism has been added, if a positive reaction occurs, colour develops or changes. Numerous wells can be incorporated into one panel and therefore many tests can be conducted at the same time. Identification is then made according to results pattern. Examples of these are; API, Vitek (Biomerioux), Biolog (Hayward), Enterotube, Minitek, Crystal ID, MicroID, RapidID.

Metabolism Methods

Electrical techniques work on the basis that the growth and metabolism of micro-organisms will change the electrical conductivity and resistance of a liquid. These changes can be expressed as impedance, conductance or

capacitance changes. When population numbers reach 10^5 cfu/ml these parameters will change. The time taken for the curve to accelerate upwards (detection time of test sample) is inversely proportional to the initial concentration of micro-organisms; results are read according to a standard curve. Examples are; Bactometer (BioMerieux), Rabit (Don Whitley Scientific), Malthus (Crawley).

Colorimetric techniques use a colour-changing system, which is applied to monitor changes in media due to microbial growth. For example, BacT/Alert (Organon Technika) detects CO_2 production, BioSys (BioSys) utilise colour changes of media during culture growth.

Immunological Methods

Specific antibodies are used in these techniques that are designed to either detect many cellular targets (polyclonal antibodies) or specific targets (monoclonal antibodies).

Latex agglutination test kits contain latex particles that are coated with antibodies; contact with the target antigen produces visible agglutination. Tests for *S. aureus*, *C. perfringens*, *B. cereus*, *Vibrio cholerae* and *E. coli* are available.

Immunomagnetic separation works via the addition of magnetic beads coated with antibodies to sample. The target cells then bind to the antibodies and thus the beads. The bead/antibody/cell complex is then isolated using a magnet. These beads can then be used to inoculate a broth, be plated onto agar, or used in PCR or ELISA techniques. Dynabeads are available for *E. coli* O157:H7, *Listeria* and *Salmonella*.

Immunodiffusion is based upon the migration of an antibody-antigen system through agar, for example the 1-2 Test (BioControl) is used for the detection of motile *Salmonella*. An alternative is the migration of a sample along a chromatographic strip: VIP (BioControl), Reveal (Neogen Corp), Safe Path (Safe Path Laboratories LLC), PATH–STIK (Lumac). Tests of this type are available for *E. coli* O157:H7, *Listeria*, *Salmonella*, *Campylobacter* and a variety of toxins.

Enzyme-Linked Immunosorbent Assay (ELISA) is a multi-step procedure whereby an enzyme-labelled antibody is added to wells containing attached micro-organisms, the well is incubated, washed and a substrate added. The products of the breakdown of the substrate by the enzyme can then be detected visually or with a spectrophotometer. TECRA or UNIQUE (TECRA) for *Salmonella*, *Campylobacter*, *E. coli* O157:H7,

Listeria-TEK (Organon Teknika), VIDAS (BioMerieux) Assurance (BioControl), DETEX (Molecular Circuitry). Several variations of ELISA exist depending on the techniques used: Indirect, Sandwich, Competitive and Reverse ELISA.

Immunofluorescence assays are similar to ELISA, in that they use labelled antibodies and a fluorescent substrate.

Molecular Methods

Molecular methods are based upon DNA or RNA assay systems and enable identification of an organism. Examples are:

1) In the Hybridisation Method, hybridisation of isotopic or enzymatic labelled DNA probes with specific pathogen DNA occurs. The stages involved are pre-enrichment, enrichment, lysis, hybridisation, signal development. Examples are GeneTrak (Framingham) for *E. coli* O157:H7, *Listeria*, *Salmonella*, *Campylobacter*

2) The Polymerase Chain Reaction (PCR) method is a three-step process based on amplification of a specific DNA fragment. First, the double-stranded DNA is denatured (95 °C), secondly, primers are added which attach to a single strand DNA (55 °C), finally amplification is made by a thermostable DNA polymerase (70'C) using a repeating cycle of temperatures. Examples are the BAX system (Qualicon) for *E. coli* O157:H7, *Listeria*, *Salmonella*; TaqMan (Applied Biosystem).

3) Pulsed-Field Gel Electrophoresis allows bacterial typing using a pure culture, the cells are lysed, the genetic material is fragmented using restriction enzymes, electrophoresis is run on the fragments, and the results are compared using the PulseNet system.

4) DNA Fingerprinting enables the identification of an organism to genus, species and subspecies level. The cells are lysed, the genetic material is fragmented using restriction enzymes, electrophoresis is run on the fragments, and a form of signal development is used. An example is the RiboPrinter (Qualicon)

Flow Cytometry

Flow cytometry is a rapid technique for the enumeration of cells. The cells are labelled with an appropriate fluorescent label and passed rapidly in suspension on a cell-by-cell basis through a tunnel where they are detected by an electronic device.

ATP Methods

These work on the basis that in the presence of firefly enzymes (the luciferase and luciferin system), oxygen and magnesium ions, ATP will facilitate a reaction to generate light. The amount of light is proportional to the amount of ATP, therefore to the amount of 'living' cells in the sample. This method is used mainly to validate cleaning efficiency and hygiene monitoring. Dirty surfaces have more ATP, regardless of whether from micro-organisms or other 'living' cells. The surface is swabbed, the reaction is activated by placing the swab into an enzyme solution, the swab and tube are inserted into the chamber of a luminometer in order to obtain a reading in relative light units (RLU). Many tests are available, Lumac (Landgraaf), BioTrace (3M), Lightning (BioControl), Hy-Lite (EM Science), Charm 4000 (Charm Sciences) System SURE (Hygiena).

Colilert and Colisure

Colilert and Colisure are rapid tests used for detection and enumeration of coliform and *E.coli* bacteria in water. They are produced by IDEXX Laboratories. They work on the principle that coliform and *E.coli* bacteria possess the enzyme β-D-galactosidase that degrades *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG), producing yellow-coloured *o*-nitrophenol. *E. coli* also has the ability to cleave methylumbelliferyl-β-glucuronide (MUG), producing fluorescent 4-methylumbelliferone. Colisure uses yellow CPRG (chlorophenyl red ß-D-galactopyranoside) instead of ONPG, and produces magenta chlorophenyl red. Colilert is able to detect these bacteria at 1 cfu/100 ml within 24 hours with as many as 2 million heterotrophic bacteria/100 ml present. An 18-hour version is also available. Colisure provides results within 48 hours and has the advantage of obvious end point colours.

Biosensors

Biosensors are analytical devices incorporating a biological or biologically-derived component specific to the analyte and a physical component, which is able to transduce the biological signal to a physical one. For example enzymes, antibodies or cells can be used as the biological components. The signal (electric, optical or mass-changing) can be detected in many ways. Most biosensors for detecting pathogens use the antibody-antigen complex as biological component. Detectors utilise optical or electrochemical signals.

Biochips

Biochips are essentially DNA microarrays. They are a system of as many as 50,000 individual spots, each one containing millions of copies of a specific DNA probe that are immobilised on a slide. These chips are essentially miniaturised laboratories that can perform hundreds or thousands of simultaneous biochemical reactions to enable the detection of micro-organisms. Biochips can be designed to detect all kinds of pathogens by using a variety of antibodies or DNA probes against specific pathogens on one chip, thereby enabling the detection of many organisms at the same time.

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Please note: There is no shortage of literature relating to food poisoning bacteria! For Leatherhead Food Research Members, access to such material can be gained through our database 'Foodline Science' and Leatherhead Food Research's Food Safety Department. Members can also access the extensive collection of books and journals in the Leatherhead Food Research library, and utilise the rapid document delivery service now available.

A Few Words about the Bibliographies

The choice of literature to include in these bibliographies was not always an easy one. For the purposes of brevity, some deliberate bias was shown in certain cases to UK and USA publications and to those that are of more practical (rather than academic) interest. The authors apologise for any inevitable important omissions.

BACILLUS CEREUS

Bacillus cereus is now recognised as a significant cause of foodborne illness in humans. It can cause two distinct forms of food poisoning. These are the so-called "diarrhoeal" type and the "emetic" (vomiting) type, caused by toxins produced by *B. cereus*. The emetic type is considered to be most common in the United Kingdom (UK).

The Organism *B. cereus*

B. cereus is a large Gram-positive, endospore-forming, catalase positive, usually oxidase negative, motile rod-shaped bacterium with peritrichous flagellae belonging to the family Bacillaceae. It grows best in the presence of oxygen, but also grows well anaerobically. Sporulation readily occurs only in the presence of oxygen. It tends to grow in long chains. The cell width is ≥0.9 μm and it produces central to terminal ellipsoid or cylindrical spores that do not distend the sporangia. Under anaerobic conditions both growth and toxin production are reduced.

The genus *Bacillus* encompasses a wide variety of species and strains. Although other *Bacillus* species have been associated with food poisoning, *B. cereus* is the species of most importance to food microbiologists. These other species are covered in more detail at the end of this section.

B. cereus Food Poisoning

B. cereus has been known to be a cause of food poisoning since the early 1950s. *B. cereus* food poisoning occurs after the ingestion of foods in which the organism has grown and formed its toxin(s). Emetic food poisoning arises from the ingestion of emetic toxin that has been pre-formed in the food (i.e. it is intoxication). The emetic toxin has been characterised as a ring form peptide (cereulide) and is thought to be associated with sporulation (1). The less common diarrhoeal type of *B. cereus* poisoning arises from the

formation and release of enterotoxin in the small intestine, although the enterotoxin can also be pre-formed in food (i.e. it is an intermediate type of food poisoning) (2). At least two different enterotoxins have been found, but their role in food poisoning is unclear.

Incubation time

The emetic type of illness, characterised by nausea and vomiting has a short onset period of between 1 and 5 hours. The diarrhoeal illness, characterised by watery diarrhoea, typically has a longer incubation time of approximately 8-16 hours (more usually 10 - 12 hours) (3, 4).

Symptoms

Emetic food poisoning symptoms, which usually last for between 6 and 24 hours (3, 4), include nausea, vomiting, general malaise, and occasionally diarrhoea. This type of food poisoning closely resembles that of *Staphylococcus aureus*.

Predominant symptoms of diarrhoeal food poisoning are diarrhoea and abdominal cramps, with only occasional nausea and vomiting. Symptoms usually persist for between 12 and 24 hours (3, 4). However, body aches, fever, chills, and longer incubation and duration times were reported in a large outbreak in the United States of America (USA) (5). This type of food poisoning resembles that of *Clostridium perfringens*.

Mortality

Although recovery from *B. cereus* food poisoning is normally within 24 hours with no complications, there have been reported fatalities due to ingestion of emetic toxin (6).

Infective dose

Large numbers of *B. cereus* are required to cause food poisoning. Numbers found in foods implicated in *B. cereus* poisoning are typically within the range of 10^5 - 10^9/g, although smaller numbers have occasionally been reported. *B. cereus* food poisoning may also occur after ingestion of food containing the pre-formed heat-stable emetic toxin, but which, as a result of

further processing or reheating, contains few viable organisms (1, 5). Any food containing more than 10^3 *B. cereus* /g cannot be considered completely safe for consumption.

Foods involved

Emetic food poisoning is almost exclusively associated with farinaceous foods, especially boiled or fried rice, and cooked pasta and noodle dishes. Most of the outbreaks in the UK have been associated with cooked rice from Chinese restaurants and take-away outlets. In Chinese restaurants, cooked rice (prepared in bulk) is allowed to cool at room temperature overnight, after which it may be reheated/fried for serving. These practices result in the survival and growth of strains of *B. cereus*, producing the pre-formed toxin in the food (2, 7).

The diarrhoeal type of *B. cereus* food poisoning has been associated with a wide range of proteinaceous foods, most commonly meat or vegetable dishes, soups, sauces and puddings. In general, this type of food poisoning occurs when spores survive the cooking process, and unsatisfactory post-cooking storage conditions permit the spores to germinate and growth to occur (2).

Incidence of *B. cereus* Food Poisoning

The worldwide reported incidence of *B. cereus* food poisoning ranges from <1 - 22% of total food-poisoning outbreaks (<1 - 18% of cases) of known bacterial origin, according to country (2).

In the US between 1988 and 1992, 21% of food poisoning outbreaks were due to *B. cereus*. On average, about 14% of food poisoning outbreaks in Canada are associated with *B. cereus* per year. In the UK, between 1989 and 1993, 68% of food poisoning outbreaks were due to *B. cereus* food poisoning (8). Between 2000 and 2006 recorded outbreaks per year were between 0 and 4 in England and Wales with a maximum number of cases in any one year 41 (2003) (source: Health Protection Agency). Because of the relatively mild and short-lived nature of the illness caused by *B. cereus*, a large proportion of food-poisoning incidents caused by this organism may go unreported.

Information from the surveillance for foodborne outbreaks in the USA indicates that *B. cereus* is not a major concern. *B. cereus* is the cause of 2.7% of foodborne outbreaks and 0.83% of outbreak cases (9).

Sources

Humans

Bacillus species are often present in low numbers in normal stools as transient flora, which reflects dietary intake (low numbers of *B. cereus* can be ingested with no ill effect). In cases of food poisoning, the organism can be excreted in fairly high numbers for up to 48 hours after onset (7,10).

Animals and environment

B. cereus is widely distributed in nature. It is common in a wide range of environments, including soils (especially rice paddy soil), sediments, dust, natural water, animal hair and vegetation.

Foods

Because the *B. cereus* group is ubiquitously distributed in the environment, it can easily contaminate various types of foods, especially products of plant origin. It is common in many foods, notably cereals and cereal derivatives, as well as spices, meat and meat products, pasteurised liquid egg, rice, ready-to-eat vegetables (RTE), milk and dairy products, and dried foods. Its association with milk, where it can be a significant cause of spoilage in raw and heat-treated products (especially 'bitty cream'), as well as with rice, may be a particular cause for concern. However, proper cold storage appears to be effective in preventing spore outgrowth and toxin production.

Growth/Survival Characteristics of the Organism in Foods

Temperature

Typically, the vegetative cells of *B. cereus* have an optimum growth temperature of 30 - 35 °C, and a maximum ranging from 48 - 55 °C (4, 11, 12). However, psychrotrophic strains have been identified - especially in milk and dairy products - capable of growing within the temperature range 4 - 37 °C (13). In addition, most of the psychrotrophic strains tested have been shown to produce enterotoxin (14) and production at temperatures as low as 4 °C has been reported, although concerns have been raised about the

methodology used in these studies (12). The emetic toxin has been shown to be produced optimally at 25 - 30 °C (12).

Heat resistance

Vegetative cells of *B. cereus* are readily destroyed by pasteurisation or equivalent heat treatments. However, spores can survive quite severe heat processes. *B. cereus* spores from different strains show varying heat resistance; $D_{95\ °C}$ values of between 1.2 and 36 minutes have been reported (2). It has been shown that strains commonly implicated in food poisoning are more heat resistant than other strains, and are therefore more likely to survive cooking. The heat resistance of spores is also enhanced in low water activity (a_w) or high fat foods.

The diarrhoeal enterotoxin is heat-sensitive, being destroyed at 56 °C for 5 minutes (2, 4). However, the emetic toxin is extremely heat-resistant, and can withstand heating at 126 °C for 90 minutes (2, 12). This heat resistance is very significant in the context of food safety, where food, especially rice, is prepared in advance and left at room temperature before reheating. This practice can allow the growth of *B. cereus* and formation of heat-stable toxin, which is not affected by reheating of the food (such as may occur in the preparation of fried rice in restaurants).

pH

$D_{100\ °C}$ has been reported to be capable of growth at pH values between 4.3 and 9.3, under otherwise ideal conditions (2,10).

The emetic toxin is stable in the pH range 2 - 11 (4), whereas the diarrhoeal enterotoxin is unstable at pH values of <4 or >11 (2).

Water activity/salt

In the presence of sodium chloride (NaCl) as a humectant, *B. cereus* will not grow at a_w of 0.93. However, growth was found at a_w of 0.93 (but not at a_w of 0.92) when glycerol was used as a humectant (8). As fried rice is known to support the growth of *B. cereus*, and the a_w of fried rice may be as low as 0.91, this may be a more reliable guide to its minimum (2).

B. cereus can tolerate salt concentrations up to 7.5% (4).

Atmosphere

Most studies have shown *B. cereus* to be sensitive to the antimicrobial effects of carbon dioxide (CO_2). It affects both spore germination and growth of the organism. Under 100% CO_2, *B. cereus* growth is only 17% of that observed under aerobic conditions, and 33% of that under anaerobic conditions (15).

Summary of Control of *B. cereus* in Foods

Because *B. cereus* is ubiquitous and has the ability to form spores, some degree of contamination of the food chain and survival through all stages of food processing short of retorting is likely. As already mentioned, rice, in particular, can harbour *B. cereus*.

The ingestion of low levels of the organism does not represent a hazard. However, efforts must be made to minimise the germination and outgrowth of spores that survive thermal treatment. Primary control of this type of intoxication consists of prevention of time and temperature abuse, particularly in cooked products. Cooked foods should be kept hot before consumption (minimum 63 °C), or, if such foods are to be stored, they must be cooled rapidly and kept at refrigeration temperatures for a limited time, unless the pH and/or a_w of the food are such that growth is prevented.

OTHER *BACILLUS* SPECIES

Species of *Bacillus* other than *B. cereus* have been shown to be responsible for incidents of food poisoning. These species include *Bacillus licheniformis* and *Bacillus subtilis*. In comparison with *B. cereus*, the accumulation of knowledge about these organisms in relation to food safety is in its infancy. Foodborne incidents involving these bacteria, however, have occasionally been reported.

B. subtilis has been implicated in food poisoning caused by 'ropy' bakery products, as well as a number of other foods, such as custard powder, synthetic fruit drinks, mayonnaise, meat, seafood with rice and, pastry. *B. subtilis* food poisoning has a rapid onset (median 2.5 hours) and the main symptom is acute vomiting, with diarrhoea commonly following.

B. licheniformis food poisoning is also associated with cooked meat and vegetable dishes, as well as meat or vegetable stews, or curried meat or

poultry with rice. *B. licheniformis* has a slightly longer onset (median 8 hours), and the main feature is diarrhoea, although vomiting occurs in about half of cases. This type of food poisoning closely resembles that caused by *Clostridium perfringens*.

B. subtilis has been implicated in food poisoning more often than *B. licheniformis*. The Health Protection Agency (HPA) stated that *B. subtilis* was implicated in a total of 70 outbreaks reported to the Food Hygiene Laboratory during the period 1974 - 89. In the same period, *B. licheniformis* was implicated in a total of 32 outbreaks (2, 7). In general, where either of these bacteria has been implicated in food poisoning incidents, the contamination level in food is high (105 /g or more) (2,7).

It is currently very difficult to differentiate *B. cereus* and *Bacillus thuringiensis* by conventional methodology. However, since *B. thuringiensis* is now widely used in agriculture for its insecticidal properties, it is likely to occur more frequently in some food materials in future. Therefore there is a need for better identification methods to be developed. Furthermore, there have been reports of enterotoxin production by *B. thuringiensis* strains isolated from food (16).

It can be concluded that the presence of large numbers of *Bacillus* in any food should be viewed with suspicion.

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CAMPYLOBACTER

Campylobacter fetus was first isolated from aborted sheep foetuses, and it was initially called *Vibrio fetusovid* because of the curved shape. It was later known by various names, for example, *Vibrio fetus* - isolated from aborted cattle, and *Vibrio jejuni*, which is a cause of winter dysentery outbreaks in cattle. Finally, the species was given the new genus name *Campylobacter*, meaning curved rod in Greek (1). Its role as a human pathogen was not appreciated until 1977, when suitable methods were developed for its isolation. Since then, it has become increasingly recognised as an important cause of gastroenteritis.

The Organism *Campylobacter*

Campylobacter are Gram-negative, oxidase and catalase-positive, non-spore-forming rods with a characteristic spiral (s-shape) morphology belonging to the family Campylobacteriaceae. Most *Campylobacter* species are motile (corkscrew-like darting motility) and produce a single polar flagellum. They are microaerophilic, requiring special atmospheric conditions for growth. For these reasons they are not normally capable of growing in foods. This distinguishes them from other food poisoning bacteria, but despite this fact, *Campylobacter* is the leading cause of bacterial gastrointestinal illness in the UK (2). Because of their small size, they can be separated from most other Gram-negative bacteria by use of a 0.65 μm filter (3).

The genus *Campylobacter* currently consists of 16 species and 6 sub-species (1,4). Some former species of *Campylobacter* have been placed in the genus *Arcobacter* and some are now classified as species of *Helicobacter*.

Campylobacter Enteritis

Campylobacter enteritis/campylobacteriosis can be caused by a number of species, namely *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter fetus* and *Campylobacter upsaliensis* (1). *C. jejuni* and *C. coli* are the most common species associated with diarrhoeal illness and produce indistinguishable clinical symptoms. *C. jejuni* is the most prevalent in the UK, accounting for 92% of all cases (2).

The illness may start with cramping abdominal pain and diarrhoea. Typical symptoms include fever, chills, headache, myalgia and occasionally delirium (1). *C. jejuni* poisoning gives rise to a more intense and long-lasting abdominal pain than other foodborne illnesses, and the occasional presence of blood or mucus in the stools may be cause for alarm for sufferers. This may explain the large number of cases that are reported to doctors, in contrast to the milder, shorter-lived symptoms caused by other food-poisoning organisms such as *Clostridium perfringens* or *Bacillus cereus*.

For patients who require antimicrobial therapy, increasing resistance to fluoroquinolones has become a problem in most parts of the world (4).

Incubation time

The onset of illness caused by *Campylobacter* occurs between 1 and 11 days after exposure, but is usually 2.5 days (5).

Symptoms

The illness caused by *Campylobacter* is not easily distinguished from other types of gastrointestinal disease. Symptoms vary widely; they can be mild, with little obvious sign of illness, or quite severe, and usually last for between 1 and 7 days, but sometimes last for several weeks. Almost all cases experience diarrhoea, which may be profuse, lasting for 1 - 3 days, and sometimes involve the excretion of blood and mucus in the stools. Abdominal pain can persist for up to 7 days and recurrence of symptoms can occur. Nausea is common, but vomiting is uncommon (1,6,7).

The disease is usually self-limiting, but complications such as appendicitis, meningitis and reactive arthritis occur in about 1% of patients, 1 - 2 weeks after onset of the illness. Bacteraemia and Guillain-Barré syndrome are also possible complications (2,7).

Mortality

Almost all cases of campylobacteriosis recover from illness with a week; although there may be complications, death from campylobacteriosis is very rare. Mortality associated with infection maybe underestimated, however, and in the US, it has been estimated that the case fatality rate is about 124 deaths per annum (1, 7).

Infective dose

The infective dose for *Campylobacter* enteritis is thought to be low; infection has been established with as few as 50 - 500 organisms. The fact that *Campylobacter* cannot normally grow in foods does not necessarily limit its ability to cause infection (1).

Foods involved

Because the vast majority of reported cases of *Campylobacter* enteritis are sporadic rather than associated with outbreaks, food vehicles are difficult to identify. Furthermore, *Campylobacter* is not often fully identified, and only rarely serotyped (about 15% of isolates from reports to Communicable Disease Surveillance Centre (CDSC) in 1989 - 1991 were speciated). Consequently, in contrast to salmonellosis, where specific serotypes of *Salmonella* can be traced to specific foods, *Campylobacter* infections are more difficult to trace to a specific food vehicle (8,9,10).

Where a food vehicle has been implicated, *Campylobacter* enteritis has been most commonly associated with undercooked poultry, with cross contamination from raw poultry to other foods that are not subsequently cooked, and with raw or bird-pecked milk (2). Large outbreaks have been associated with raw and inadequately pasteurised milk and contaminated water supplies (5).

Incidence of *Campylobacter* Food Poisoning

Campylobacter enteritis is prevalent worldwide, and is recognised as an important source of diarrhoeal illness in the public health domain. In Europe and North America it is the number one bacterial cause of gastrointestinal disease (11,12). The increase in the number of reports of *Campylobacter*

infections may be due to a combination of factors, including increased surveillance, changes or improvements in culture and molecular methods, and the recognition that they cause primarily sporadic rather than outbreak disease (11).

A study of the levels of *Campylobacter* incidence in England and Wales between 1990 and 1999 revealed an average annual rate of 78.4 +/- 15 cases (11). Between 1997 and 2008, there were approximately 50,000 laboratory-reported infections annually in England and Wales (source: Health Protection Agency). This represents an annual reporting rate of about 900/million of the population. However, in 1997 only 10 general outbreaks were recorded, emphasising the sporadic nature of disease caused by *Campylobacter*.

Based on active surveillance data collected through the Centre for Disease Control's (CDC) FoodNet, it is estimated that 1% of the United States of America (USA) population develops *Campylobacter* infection each year. In the USA, gastroenteritis caused by *Campylobacter* peaked in 1997, declining to an infection rate of 13.8/100,000 of the population by 2001. Likewise, the incidence of *Campylobacter* infections in the USA appears to be decreasing – only 12.6 incidences per 100,000 persons were reported in 2003 as compared to 21.7 between 1996 and 1998 (4).

The greatest incidence of *Campylobacter* infection is seen in infants and young adults (peak age group is 10 - 29 years), especially young males (1). There is also a seasonal trend in incidence - the highest incidence occurs in spring/early summer (generally between mid-June and mid-July). This peak could be due to informal eating outside, such as barbecues, coupled with an increase in temperature. There is some evidence linking it to agricultural activities (11). There is also more infection amongst the rural community than in urban populations.

As in the case of other forms of foodborne infection, these reported isolations probably underestimate the true incidence of *Campylobacter* infection. One small study in the early 1980s indicated that the true incidence might be over half a million cases each year in England and Wales. Another study suggested that cases might exceed one million annually (13).

Sources

Humans

Faecal-oral, person-to-person transmission of infection has been reported for *C. jejuni*, but it is uncommon. This type of transmission can occur if personal hygiene is poor. Asymptomatic carriers are also reported to occur, but are rare in developed countries (14). However, humans may act as vectors and transfer the organism into poultry production areas on contaminated clothing and footwear.

Animals and environment

Campylobacter is not an environmental organism but rather a zoonotic organism; it can be found in the intestinal tract of a wide range of warm-blooded animals. It is especially common in birds, which relates to the organism's high optimum growth temperature. Wild birds are considered to be an important reservoir of infection for domestic and food animals. Poultry is also a natural host for *Campylobacter* (2).

In addition to birds, *Campylobacter* spp. may be found in the intestinal tract of cattle, sheep, pigs, goats and domestic pets, especially puppies and kittens. *C. jejuni* has also been isolated from houseflies (15).

Water can become contaminated with *Campylobacter*, and can act as a vehicle for *Campylobacter* infections - either directly or indirectly. There have been several small waterborne outbreaks in the UK, and large waterborne outbreaks have been reported in other countries. These have mainly been associated with defective chlorination systems. *Campylobacter* may enter a viable but non-culturable state in water (although this is disputed by some researchers) (7,16).

Isolation of *Campylobacter* species has also been achieved from mud and sewage.

Foods

Poultry - either undercooked, or as a source of contamination of other foods, such as salads in the kitchen - is commonly considered to be the major cause of *Campylobacter* infections in man (2,6,16).

Cross contamination during processing of poultry can lead to a contamination rate of *Campylobacter* in processed poultry of 60% or more,

with numbers per carcass ranging from 10^2 - 10^4 cfu/g (3). The contamination rate of fresh poultry is higher than in frozen poultry, and it has been suggested that the increase in the reported incidence of *Campylobacter* infection over recent years may be linked to increased consumption in the UK of fresh (as opposed to frozen) poultry. In a Food Standards Agency (FSA) study carried out in 2001, the prevalence of *Campylobacter* in UK poultry was found to be 50%, but the contamination rate in fresh poultry samples was 63%, with that in frozen poultry being only 33%. Studies in Spain, Japan and Germany have produced similar results, reporting contamination rates of 49.5%, 45.8%, and 41.1% respectively (17,18,19).

Although *Campylobacter* can be isolated readily from fresh red meat carcasses, air and drying of the surface lead to poor survival, such that red meat is less prone to being contaminated than poultry. Reported incidence of *Campylobacter* spp. ranges from 0 - 23.6% in beef, from 0 - 15.5% in lamb, and from 1 - 23.5% in pork (20). *C. coli* tend to be isolated from pork more frequently than *C. jejuni*.

Milkborne *Campylobacter* infections are still a problem in England and Wales, where the sale of raw milk is still permitted, although declining in importance. This is in contrast to Scotland, where the incidence of outbreaks from *Campylobacter* infections associated with milk has seen a major decline since the pasteurisation of milk was made compulsory in 1983. Bird-pecked milk bottles are also becoming recognised as a major source of *Campylobacter* infections, especially in the late spring. Recent studies have shown that in some parts of the UK, a high percentage of cases in the early summer peak of *Campylobacter* infections is associated with consumption of milk from bottles pecked by magpies or jackdaws (1,2,16). A recent *Campylobacter* outbreak in 1997 was thought to be associated with bird-pecked milk (21).

In addition to animal sources, vegetables (e.g. lettuce) and shellfish may also be contaminated with *Campylobacter*, and prevalence in oysters and mussels has been reported as 47 - 69% and 6 - 27% respectively (18). More recently, on the basis of epidemiological data, bottled mineral water has been proposed as a significant vehicle for *Campylobacter* infection. More unusual vehicles for foodborne campylobacteriosis include garlic butter, sweet potato, stir-fried food and tuna salad (2).

Growth/Survival Characteristics of the Organism in Foods

As already indicated, *Campylobacter* is a fragile and fastidious organism. It cannot normally grow in foods (requiring a mammalian or avian host for growth), although it may survive in food. *Campylobacter* spp. have specific temperature atmosphere requirements, as well as being particularly sensitive to oxygen breakdown products and to drying. Viable, but non-culturable *Campylobacter* spp. have been reported (22).

Temperatures

C. jejuni and *C. coli* can only grow at temperatures above about 30 °C; they (and *C. lari*) are consequently referred to as the thermophilic group of *Campylobacter* spp. Their optimum temperature for growth is between 42 - 43 °C, with a maximum of 45 °C (6). *Campylobacter* survives poorly at room temperatures (RT) (around 20 - 23 °C) consequently they will not multiply during food processing or food storage.

Chilling is known to promote survival of *C. jejuni*. Conversely, it is generally more sensitive to freezing, although there may be some survival for long periods (1, 2, 23). Despite its inability to grow at low temperatures *C. jejuni* is still metabolically active at temperatures far below its minimal growth temperature and also remains motile. As a consequence of this, the organism is still able to move to favourable environments at temperatures as low as 4 °C (2).

Heat resistance

C. jejuni is very heat-sensitive. Heat injury can occur at 46 °C or higher; D-values at this temperature (in skimmed milk) of 7.2 - 12.8 minutes have been reported (24). At 55 °C, the range was 0.74 - 1.0 minutes (6). The organism cannot survive normal milk pasteurisation. In meat and poultry, heat treatments sufficient to kill *Salmonella* will also readily kill *Campylobacter*. D-values ranging from a few seconds to less than 1 minute at temperatures between 57 - 60 °C have been reported for *Campylobacter* in meat. Low z-values (*ca* 3.5 °C) have also been demonstrated for *Campylobacter* (25).

pH

The optimum pH for growth is reported to be in the range 6.5 - 7.5 and no growth is observed below pH 4.9 (6). The response of *C. jejuni* to pH is influenced by temperature and the type of acid used to adjust the pH. At similar pH values, the organism was most rapidly inactivated at 42 °C, at an intermediate rate at 25 °C, and slowly at 4 °C (25). Lactic acid is more inhibitory than hydrochloric acid at the same pH level (23).

Water activity/salt

Campylobacter is quite sensitive to sodium chloride (NaCl); levels of 2% or more NaCl can be bactericidal to the organism. The effect is temperature-dependent, and decreases with decreasing temperature (24).

Campylobacter is particularly sensitive to drying; it does not survive well in dry environments. Minimum water activity (a_w) for growth is 0.98. However, a study in 1998 suggested that *Campylobacter* may be able to survive for some time on wooden cutting boards. It may even be able to grow under certain circumstances if the ambient temperature is high. There is always a potential cross contamination risk (26).

Atmosphere

Campylobacter is both microaerophilic and 'capnophilic' (liking carbon dioxide). It will grow best in an atmosphere containing 10% carbon dioxide (CO_2) and 5-6% oxygen (O_2). Growth is also enhanced by hydrogen. The organism will normally die quickly in the presence of air; it is particularly sensitive to oxygen breakdown products. Vacuum- or gas-packing appears to have little major impact on the survival of *Campylobacter* in chilled meat or poultry (23, 24).

Irradiation

Campylobacter spp. are more sensitive to ionising radiation than salmonellae or *Listeria*, and therefore treatments applied to poultry that are designed to eliminate these pathogens are effective against *Campylobacter* (27). D-values of 0.08 - 0.32 kGy have been reported, but the rate of inactivation is dependent on food type and temperature, with frozen foods

requiring more severe treatments than chilled foods (6). Rate of killing is also dependent on the growth phase of the organisms.

Summary of Control of *Campylobacter* in Foods

Since poultry is considered to be one of the most important sources of human infection, prevention of foodborne *Campylobacter enteritis* rests heavily on the control of the organism in poultry. Measures should be taken to minimise the contamination of chickens during production and to minimise the spread of contamination amongst carcases during processing. Several European countries, notably Denmark and Norway, have significantly reduced the prevalence of *Campylobacter* in poultry flocks by improving hygiene in production units and introducing new measures to prevent infection in disease-free flocks. Further control measures involve practicing good food hygiene, e.g. prevention of cross contamination of prepared foods with *Campylobacter* spp. from raw meat (particularly poultry) and adequate refrigeration. Poultry must be thoroughly cooked. The consumption of raw milk and 'bird-pecked' milk also involves a risk of *Campylobacter* infection.

There is a lack of information on the effects of preservatives on *C. jejuni*, but it is likely that preservatives will inhibit the growth of organisms rather than kill them (2).

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CLOSTRIDIUM BOTULINUM

The organism *Clostridium botulinum* was named after early investigations that linked outbreaks in central Europe with sausages. Hence the name 'botulism' from the Latin word 'botulus' meaning 'sausage'. In 1897, a Belgian physician found that an outbreak of alimentary intoxication was related to the 'ham bacillus' and called it *Bacillus botulinus*. However it was redesignated as *C. botulinum* due to the phylogenetic difference between *Clostridium* and *Bacillus*.

The Organism *C. botulinum*

Clostridia belong to the family Bacillaceae. *C. botulinum* is a Gram-positive, catalase and oxidase-negative, spore-forming, motile rod with subterminal oval endospores and peritrichous flagellae. It can form toxins, and can grow only under anaerobic conditions.

There are four forms of botulism: 'classic' botulism, caused by ingestion of toxin in food; infant botulism (or 'floppy baby syndrome'); the rare wound botulism; and unclassified - this includes cases of unknown origin and adult cases that resemble infant botulism (1, 2). This chapter considers foodborne and infant botulism.

Seven different types of *C. botulinum* are known, forming at least seven different toxins, A to G. Types A, B, E and, to a lesser extent, F are the types that are responsible for most cases of human botulism (2, 3).

Botulism - the illness caused by the consumption of preformed botulinum toxin of *C. botulinum* - is extremely serious and, unless recognised and treated promptly, carries a high risk of mortality. It is, therefore, the most severe form of food poisoning.

The *C. botulinum* types can be divided into four groups based on their physiological differences (4).

Group	Types	Physiology
I	A,B,F	Proteolytic, mesophiles
II	B,E,F	Non-proteolytic, psychrotrophs
III*	C,D	Non-proteolytic
IV†	G	Weakly proteolytic

* these strains are not involved in human botulism
† no outbreaks of type G strains have been reported

Until recently *C. botulinum* was defined by the production of neurotoxin causing botulism, and this resulted in a very diverse species. Strains of other species have also been found to produce botulism neurotoxins (*Clostridium butyricum* and *Clostridium baratii*) and there have been reported outbreaks of foodborne illness associated with these organisms (5, 6, 28). It has been suggested that the four groups above should each be considered to be a separate species (7).

C. botulinum Food Poisoning

C. botulinum was first linked with an outbreak of botulism in Belgium in 1895. Since then, a number of outbreaks have occurred all over the world, associated mainly with home-preserved vegetables, fish or meat products. The illness is most commonly associated with home-produced foods, or small catering units, rather than commercially prepared foods (3). Nevertheless, the impact of botulism associated with commercially manufactured foods, although rare, can be devastating.

'Classic botulism' is caused by ingestion of toxin that is pre-formed in food. *C. botulinum* spores present in food must be given favourable conditions to germinate and grow to sufficient numbers to form toxin in the food, and the contaminated food must then be eaten raw, or without adequate heat treatment.

Incubation time

The incubation period is usually 12 - 36 h, but can be from a few hours up to 14 days, depending on the amount of toxin consumed. The earlier the symptoms occur, the more serious they are likely to be (2).

Symptoms

The severity of symptoms relates to the amount of toxin ingested, but the first symptoms are generally nausea and vomiting, followed by neurological symptoms, including blurred and/or double vision, fixed and dilated pupils, loss of normal mouth and throat functions (dryness of the mouth, followed by difficulty in talking, swallowing, a sore throat), general fatigue and lack of muscle coordination, and respiratory impairment. Other gastrointestinal symptoms may include abdominal pain, diarrhoea, or constipation. Nausea and vomiting appear more often in cases of botulism types B and E; dysphagia and muscle weakness are more common in outbreaks of types A and B (2). Very early vomiting often occurs with type E botulism, and this may be linked to lower mortality rates.

Mortality

Previously, the mortality rate for botulism was high, between 30 and 65%, with the rate being generally lower in European countries than in the United States of America (USA). Nowadays, the mortality rate is lower, below 10%, as a result of prompt treatment, administration of antitoxin and respiratory support. For example, the first outbreak to occur in the United Kingdom (UK) in 1922, which was caused by a commercially prepared duck paste, containing type A toxin, reported 8 cases and 8 deaths. In another UK outbreak in 1989 involving hazelnut yoghurt, 26 out of 27 people affected were admitted to hospital and only 1 person (an elderly woman) died (8).

In 1978, a single can of salmon imported from the USA, containing type E toxin, caused a family outbreak resulting in 4 cases and 2 deaths.

Infective dose

No live organisms of *C. botulinum* need to be consumed for foodborne botulism to occur. *C. botulinum* toxin is lethal - in fact, probably the most potent natural toxin known to man. It is believed that the oral lethal dose is between 0.005 and 0.1 µg for proteolytic types and between 0.1 and 0.5 µg for non-proteolytic types and this may be as low as 30 ng (28). As little as 0.1 g of food in which *C. botulinum* has grown and produced toxin can cause botulism (3). In effect, the presence of toxin at any concentration cannot be tolerated, and neither can conditions in foods in which toxin could be formed.

Foods involved

Foods involved in most cases and outbreaks of botulism have been associated with meat, fish and vegetables. In the USA (apart from Alaska), home-preserved (particularly canned) vegetables are the most frequent cause of classic botulism (commonly involving type A). In central Europe, meats, such as home-cured hams, are the foods most commonly responsible (particularly involving type B). In Italy and Spain, vegetables have been most commonly implicated (again involving type B). In northern Europe, Alaska, Canada, Scandinavia and Japan, fish products (containing type E) have been involved in the highest number of outbreaks (1, 3, 9).

The different patterns of botulism throughout the world reflect different eating habits and preservation methods. In the UK, where botulism is very rare, 'high risk' (including home-bottled/canned) foods are not normally eaten, and commercial canning and bottling processes are designed to ensure the destruction of spores of the organism.

The largest outbreak to occur in the UK was caused in 1989 by hazelnut conserve, which contained type B toxin, used in flavouring yoghurt. The conserve was inappropriately processed, following a change in formulation, allowing the survival and subsequent growth and toxin formation by *C. botulinum* in the canned product, which was later added to yoghurt (3, 8).

Although most incidents of botulism have involved under-preserved meats, vegetables, or fish, more recent incidents worldwide have involved more unusual food types, including foil-wrapped baked potatoes used in potato salad, sautéed onions, cheese containing onion, Brie cheese, temperature-abused garlic stored in oil and commercially canned chilli sauce (1, 3, 4, 10, 11, 28).

Incidence of *C. botulinum* Food Poisoning

In the USA, an average of 9.4 outbreaks of botulism occur per year. Between 1950 and 1996 there were 44 foodborne outbreaks reported. The number of outbreaks per year increased up to 15 - 16 between 1978 and 1983, with an average of *ca* 3 cases per outbreak, and 17 deaths. Between 1983 and 1990 there were 107 outbreaks with 185 cases and 11 deaths. Therefore, the number of outbreaks, cases and deaths is decreasing with time (4). Currently, there are, on average, 110 cases of botulism reported in the US per year, of which 25% are foodborne and 72% are infant botulism. These figures have not changed significantly in recent years, but there has been an increase in

wound botulism mainly associated with injecting drug users [source: Centre for Disease Control (CDC)].

In the European Union, during the period 1988 - 1998, those countries reporting the highest numbers of cases of botulism were France (88 outbreaks of one or more cases), Germany (177), Italy (412) and Spain (92). Belgium, Denmark, England and Wales and Sweden generally reported less than one outbreak a year during the period 1988 - 1998 and outbreaks in these countries were usually small (less than three cases), with the exception of the hazelnut yoghurt outbreak in the UK in 1989 which resulted in 27 cases (12). A total of 6 cases were reported in the UK between 1990 and 2005, all from eating food produced from outside the UK (source: Health Protection Agency).

Poland has the highest incidence of botulism in Europe. Between 1988 and 1998 there were 1,995 outbreaks linked to the consumption of home-preserved meats (1, 2). A recent paper reported nearly 2,000 cases with at least 28 deaths from 1988 to 1998 (13). In 1990, 74% of the 285 cases registered were linked to the consumption of home-processed foods, the other 26% were associated with commercially processed products, mainly canned meat and fish (13). The numbers of cases of botulism associated with red meat and fish are however in decline in Poland. In 2004 17 cases of botulism were reported, 8 of which were associated with commercially processed meat and fish (28).

In Japan, from 1988 to 1998, there were 51 cases of foodborne botulism and many of those cases were associated with izushi (fermented raw fish and cooked rice) (source: National Institute of Health Sciences (NIHS), Japan).

Sources

Humans

Carriage of *C. botulinum* by humans does not play a significant role in the transmission of the organism.

Animals and environment

C. botulinum is ubiquitous - it is widely distributed in soil and marine sediments throughout the world. It is also found in the intestinal tract of animals, including fish. Surveys have shown geographical variations in the distribution of the different types of *C. botulinum* in the environment,

particularly in soils. Type A predominates in the Western US, South America and China, whereas type B is more common in the Eastern US, the UK and Europe. Type E is more often found in Northern regions and in temperate aquatic environments. A recent survey of marine sediments in the Baltic sea showed that type E was present in 81% of samples (14).

Types A or B have occasionally been isolated from cattle, sheep and pigs, and have been isolated in very low numbers (generally 0.04 to 2.2 spore/kg) from meats including pork, bacon and liver sausage (15).

Foods

Because of the widespread occurrence of *C. botulinum* in the environment, the organism can be isolated occasionally from many foods. Most studies into the occurrence of the organism have concentrated on fish, meats and infant foods, especially honey. The highest incidence is in fish, such as farmed trout, Pacific salmon, and Baltic herring, which commonly contain low numbers of *C. botulinum*. Type E is the most commonly isolated type in fish, but other types may also be encountered (15).

Meat and meat products have been studied less intensively because there is considerably less contamination of the farm environment (1,2).

Types A and B of Group I are commonly found in soil, and these organisms (especially type A) have been isolated from certain vegetables and fruit, including mushrooms. Other products in which contamination has often been detected include asparagus, beans, cabbage, carrots, celery, corn, onions, potatoes, turnips, olives, apricots, cherries, peaches and tomatoes (2). The organism has also been isolated from honey (55 - 60 spores/g) and other infant foods, namely corn syrup and rice cereal (but not in the UK) (2, 15).

Growth/Survival Characteristics of the Organism in Foods

The prevention of growth and toxin production by *C. botulinum* - particularly by ensuring the destruction of the most heat-resistant spores of *C. botulinum* - is the basis of all commercial canning and many other food preservation processes.

It is important to note that toxin production by *C. botulinum* does not necessarily coincide with obvious food spoilage. A food that contains toxin can be free of any odour, flavour or appearance that might warn the consumer that it is hazardous.

Temperature

All strains of *C. botulinum* grow reasonably well in the temperature range 20 - 45 °C, but the low temperatures required to inhibit Groups I and II are different. Group I has an optimal temperature for growth at 37 °C with growth occurring between 10 – 48 °C (they will not grow at temperatures less than 10 °C), but Group II optimum growth temperature is between 25 - 30 °C. These strains are psychrotrophic, being capable of slow growth and toxin production (within 36 days) at low temperatures with an accepted minimum growth temperature of 3 °C. This ability to grow at chill temperatures is of particular concern in relation to products that are given a mild heat treatment under, or followed by, modified-atmosphere/vacuum packaging (MAP/VP), and are expected to have a relatively long shelf-life at chill temperatures (16).

Continuous storage of products at <3.3 °C could be used as a method for the control of growth and toxin production. However, in practice, the temperatures used for chill food storage, by retailers and consumers, may allow growth and toxin production, and thus this is one of the major contributory factors in most foodborne botulism outbreaks in the US. It should be noted that, in a study into the incidence of psychrotrophic *C. botulinum* in refrigerated, packaged (vacuum, overwrap or modified atmosphere) foods in the UK, the organism was not detected (17).

Heat resistance

While the vegetative cells of *C. botulinum* are not particularly heat resistant, the spores of this organism are more so. Spores of Group I (proteolytic) strains are those with the greatest heat resistance. Thermal inactivation times (D-values) vary between the *C. botulinum* strains, and are dependent on the way in which the spores were produced and treated, the heating environment, and the recovery system. The most heat-resistant spores of Group I *C. botulinum* are produced by type A and proteolytic B strains ($D_{121\,°C}$ = *ca* 0.21 min.) (18).

The heat resistance of these Group I spores of *C. botulinum* has been studied extensively, as it is the basis of safe canning processes for foods that have a pH value above 4.5 (‘low-acid’). A ‘Botulinum Cook’ is required for foods destined to be stored at ambient temperatures (principally canned foods) that are capable of supporting the growth of *C. botulinum*. These foods are those with a pH value >4.5 and water activity (a_w) that is high

enough to allow growth. In theory this is >0.93, but a margin for safety is recommended; in the USA, low-acid foods are defined as those with a pH greater than pH 4.6.

A Botulinum Cook is defined as that giving a 12 log cycle kill, i.e. destruction of 10^{12} spores of the most resistant proteolytic strain of *C. botulinum*. In commercial terms, this is a cook of equivalent to at least 3 minutes at 121 °C at the slowest heating point in the container, which then allows the safe storage of foods at ambient temperatures (4).

The spores of Group II (non-proteolytic/psychrotrophic) strains are less heat-resistant than those of Group I strains. However, they may survive processing at temperatures of 70 - 85 °C and their ability to grow at refrigeration temperatures necessitates their control in foods capable of supporting their growth (e.g. VP, par-cooked meals with pH value >5.0 and a_w >0.97) (19, 20).

All *C. botulinum* types produce heat labile toxins, which may be inactivated by heating at 80 °C for 20 - 30 minutes, at 85 °C for 5 minutes, or at 90 °C for a few seconds.

The potential for growth of psychrotrophic *C. botulinum* in REPFEDS (Refrigerated Processed Foods With Extended Durability) or 'sous vide' products has led to a number of authors and associations recommending appropriate processing conditions. In general, it is recommended that the product is heated at 90 °C for 10 minutes to achieve a 6 log cycle kill. For some products, it may not be appropriate to follow these process conditions. In these cases, a combination of heat and other factors should be used to prevent the growth of *C. botulinum*, e.g. a_w <0.97, pH <5.0, and salt >3.5%.

The control of non-proteolytic, psychrotrophic strains of *C. botulinum* in chilled, modified atmosphere and vacuum-packed foods is considered in detail in the 'Food Standards Agency Guidance on the Safety and Shelf-life of Vacuum and Modified Atmosphere Packed Chilled Food with Respect to Non-proteolytic *Clostridium botulinum*' (21). Food producers are strongly advised to follow the advice given in this guidance in establishing appropriate controlling factors such as heat treatments and shelf lives for any VP or MAP products that might have the potential to support the growth of *C. botulinum*.

pH

Generally, it is accepted that the minimum pH for the growth of *C. botulinum* Group I is pH 4.6, and many regulations worldwide use this

limit. The limit for Group II organisms is pH 5.0 (1, 18). However, there have been examples of growth and toxin production at pH levels below 4.6. These data were obtained in laboratory media, or under laboratory conditions, and the presence of high concentrations of proteins protected the organism (16). This does not occur in foods preserved by acidity (18).

As pH has a major bearing on the ability of foods to support the growth of *C. botulinum*, foods are traditionally categorised into 'high-acid' and 'low-acid' products. In the UK, low-acid products are defined as those having a pH value equal to, or greater than, 4.5. These products must be given a Botulinum Cook or be controlled by some other factor such as a_w. (Note that in the Department of Health guidelines for the canning of low-acid foods, if a heat process less than the Botulinum Cook is given, the onus is on the canner to show that the process provides equivalent protection against *C. botulinum*) (22).

The growth of acid-tolerant micro-organisms, such as yeasts, moulds and bacilli can increase the pH in their immediate area to a level that allows growth of *C. botulinum*. All of the 35 outbreaks of botulism attributed to normally 'high-acid' foods had been spoiled by yeasts or moulds (23).

Water activity/salt

In foods, several different solutes can alter the a_w. The most common humectant used in foods is sodium chloride (NaCl) or salt. It is generally accepted that under optimal growth conditions 10% (w/w) NaCl is required to prevent growth of Group I organisms and 5% (w/w) NaCl is necessary to prevent growth of Group II organisms. These concentrations correspond to limiting a_w of 0.94 for Group I and 0.97 for Group II (2). The type of solute used to control a_w may influence these minimum values. Generally, sodium chloride, potassium chloride, glucose and sucrose result in similar limits; however, glycerol will allow growth at lower a_w values (by up to 0.03 units) (16,18).

These values have been established under carefully controlled laboratory conditions. In commercial situations, safety margins must be introduced to allow for process variability.

Atmosphere

Although *C. botulinum* is a strict anaerobe, many foods that are not obviously 'anaerobic' can provide adequate conditions for growth. Thus, an aerobically packed product may not support the growth of the organism on the surface, but the interior of the food may do so. It is also important to note that the inclusion of oxygen (O_2) as a packaging gas cannot ensure that growth of *C. botulinum* is prevented; the Eh (reduction-oxidation potential or redox) of most foods of this type is usually low enough to permit its growth (2,16). In an investigation of *C. botulinum* toxin production in inoculated pork, it was shown that initial atmospheres with 20% O_2 did not delay toxin production, compared with samples packaged in 100% nitrogen (N_2) (1,2).

Increasingly, MAP is being used to extend the shelf-life, and improve the quality of foods. However, depending on the particular atmosphere, it may allow the growth of *C. botulinum*. Many MAP products utilise carbon dioxide (CO_2) to control spoilage and pathogenic bacteria, but CO_2 may allow the growth of *C. botulinum* dependent upon the gas concentration. Studies with inoculated pork showed that CO_2 levels of 15-30% did not inhibit *C. botulinum*; only 75% CO_2 showed significant inhibition (1,16).

Summary of factors controlling the growth of* C. botulinum *in foods (18)

	Group I (proteolytic)	**Group II (non-proteolytic)**
	A,B,F	B,E,F
pH	<4.6	<5.0
a_w	<0.94	<0.97
Inhibitory (NaCl)	10%	5%
Temperature	<10 °C	<3 °C
$D_{100\ °C}$ of spores	25 min	0.1 min
$D_{121\ °C}$ of spores	0.1-0.2 min	<0.001 min

Summary of Control of *C. botulinum* in Foods

It must be assumed that spores of *C. botulinum* may be present in raw foods. Processing or preservation conditions must, therefore, be adequate to

destroy spores or to prevent germination, subsequent growth and toxin production. This relies on the appropriate use of heat, manipulation of pH, a_w, addition of nitrite or other preservatives, temperature control, etc. Appropriate codes of practice should be followed where available. Changes in established, validated formulations or processes, whether intentional or inadvertent, must be evaluated by a hazard analysis, and appropriate controls instituted for those parameters responsible for destroying or controlling the growth of the organism.

A variety of chemical preservatives have been shown to inhibit *C. botulinum*, either alone or in combination. These include: nitrites, sorbates, parabens, nisin, phenolic antioxidants, polyphosphates, ascorbates, ethylenediaminetetraacetic acid (EDTA), metabisulfite, n-monoakyl maleates and fumarates, and lactate salts. In fish, the addition of liquid smoke has been found to reduce the amount of salt needed to inhibit growth. Comprehensive reviews on the effects of these preservatives have been published (1, 7, 16, 18, 24).

Note: Infant Botulism

Infant botulism was first reported in 1976, and since that time over 1,000 cases have been confirmed worldwide. In the USA, infant botulism is now the most common form of botulism (1, 2). However, it appears to be very rare in Europe, where there have only been 49 recorded cases, of which 6 were in the UK (25).

Toxin-producing *C. butyricum* and *C. baratii* have also been implicated in 7 cases of 'infant botulism' (25).

It is thought that, in infants under a year old, ingested spores of *C. botulinum* (which do not pose a direct hazard to adults because of the presence of an inhibitory normal gut flora) can germinate and multiply in the intestine, forming toxin *in situ*. Symptoms include generalised weakness and weak cry. They may also include feeding difficulty and poor sucking, lethargy, lack of facial expression, irritability and progressive 'floppiness'.

Honey (and possibly glucose syrup), that is sometimes used on 'pacifiers' for infants, may attract spore-contaminated dust. Contamination of the honey may result from growth of the organism in dead larvae present in bee hives. The prevalence of *C. botulinum* spores in honey is usually low (typically <1 cfu/g), but they are occasionally found at levels as high as 60 cfu/g (26). 80 spores/g of *C. botulinum* types A and B were reported in one sample of honey incriminated in an incident of infant botulism (27), and the

minimum infective dose of *C. botulinum* spores for infants has been estimated to be as low as 10 - 100 spores (25). Although many studies have failed to detect *C. botulinum* spores in honey, 5.1 % samples, out of 2,033 tested, from various parts of the world contained detectable levels of *C. botulinum* spores (26).

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CLOSTRIDIUM PERFRINGENS

Clostridium perfringens was first described as *Bacillus aerogenes* in 1892 and later called *Clostridium welchii*. The main species of *Clostridium* that is of concern in the context of food safety is *Clostridium botulinum*. However, *C. perfringens* is the cause of a much less severe but more common type of food poisoning.

The Organism *C. perfringens*

Like other species of clostridia, *C. perfringens* is a Gram-positive, catalase and oxidase negative, non-motile, encapsulated bacillus belonging to the family Bacillaceae. Also, like other clostridia, it forms spores (endospores), that are heat-resistant, surviving normal cooking conditions. It is also anaerobic, but can tolerate moderate exposure to air.

Strains of *C. perfringens* are classified into five types, A-E, according to the toxin produced, and it is *C. perfringens* type A and, to a lesser extent, C, that is of primary concern in the context of food safety (1, 2).

C. perfringens Food Poisoning

This organism first earned its reputation as a pathogen as the cause of gas gangrene, but its association with food poisoning has been recognised since the 1940s. It has since become recognised as a significant cause of food poisoning worldwide (3).

C. perfringens is capable of producing at least 13 different toxins, and it is primarily the formation of enterotoxin that is responsible for the symptoms of food poisoning by this organism. This toxin is not normally formed by *C. perfringens* until the organism enters the human intestine and begins to multiply. It is commonly thought that the organism then sporulates, at the same time releasing the enterotoxin responsible for the symptoms (1, 2). Symptoms are therefore the result of an infection rather than intoxication.

Incidents of rapid onset symptoms have indicated that a toxin may be preformed in foods. However, this would appear to be rare; the enterotoxin is not normally formed in sufficient quantities in food to cause illness, but preformed toxin may contribute to the rapid development of symptoms (3, 4).

Incubation time

Symptoms of *C. perfringens* food poisoning occur between 8 and 22 hours (usually 12 - 18 hours) after the ingestion of contaminated food.

Symptoms

The symptoms of food poisoning caused by *C. perfringens* normally comprise diarrhoea and severe abdominal pain. Nausea occurs occasionally, but fever and vomiting are unusual. The illness is a relatively mild one (5).

Mortality

Recovery from *C. perfringens* is normally complete within 24 h. However, complications and death from *C. perfringens* have been recorded on very rare occasions, amongst elderly debilitated people (5).

Infective dose

Large numbers ($>10^5$/g - typically 10^6 - 10^8/g) of viable vegetative cells of *C. perfringens* are needed in foods to cause food poisoning by this organism (5, 6).

Foods involved

Most outbreaks of food poisoning caused by *C. perfringens* are associated with meat dishes; especially beef, as well as poultry. Such food poisoning is most commonly reported as a consequence of institutional and restaurant or reception catering. Virtually no outbreaks have been linked with commercially prepared processed foods (1, 4).

Spores of *C. perfringens* on meat or poultry can survive cooking (especially if protected within the cavity of poultry carcasses, or in the centre

of rolled roasts or casseroles, stews, pies, etc.), and may in fact be stimulated to germinate by the heat applied (6). Inadequate cooling facilities for cooked foods, leading to slow cooling, can allow germination and rapid multiplication of the organisms to numbers that constitute an infective dose. This happens most commonly during mass catering; if large quantities of food need to be cooked well in advance of being consumed, and facilities are not adequate for rapid chilling and refrigerated storage. Dishes containing high numbers of *C. perfringens* may remain palatable, giving no warning to the consumer of any hazard. Spores are not usually present (4, 6, 7).

Incidence of *C. perfringens* Food Poisoning

As is the case with other minor forms of food poisoning, it is thought that the vast majority of incidents of food poisoning caused by *C. perfringens* (perhaps 90-95%) go unreported. Nevertheless, *C. perfringens* features quite significantly amongst the food poisoning statistics both in the United Kingdom (UK) and in the United States (US) (6).

In England and Wales, the reported incidence of food poisoning attributed to *C. perfringens*, although low in comparison with that caused by *Salmonella*, is greater than that of *Bacillus cereus* and *Staphylococcus aureus*; after *Salmonella* and *Campylobacter*, it is the most commonly reported agent. There was an average of 50 outbreaks of food poisoning per year reported to Communicable Disease Surveillance Centre (CDSC) between 1989 and 1991 attributed to *C. perfringens*, totalling 3,064 cases (8).

During 1992-93, there were only 68 reported outbreaks due to *C. perfringens* - an average of 34 per year (9). Therefore, this type of food poisoning appears to be decreasing in incidence in the UK. Outbreaks reported between 1992-1998 totalled 29, with 540 individual cases. Between 1999-2006 reported outbreaks were 9 with a total of 299 cases. Thus incidence of *C. perfringens* is certainly in decline but there appears to have been a shift from the disease occurring in outbreaks to being individual point cases of food poisoning (source: Health Protection Agency).

Similar trends have been observed in the US; during 1983-1987, there were 24 confirmed *C. perfringens* outbreaks, totalling 2,743 cases and 2 deaths (10). During 1993-97, 57 outbreaks and 2,772 cases of *C. perfringens* food poisoning were reported by the CDC (11). In 1999, it was estimated that there were approximately 250,000 annual cases of foodborne illness in

the US caused by *C. perfringens*, resulting in 41 patients requiring hospitalisation and 7 deaths (12).

Sources

Humans

C. perfringens spores are present in numbers between 104 and 106 as part of the normal faecal flora of most individuals.

The high incidence of *C. perfringens* in faecal flora makes it necessary to detect enterotoxin from isolates for outbreak confirmation (1).

Animals and environment

The spores of *C. perfringens* type A are widely distributed in the environment, and are usually present in soil - especially well-manured soil - in high numbers (10^3 - 10^4/g).

Spores of *C. perfringens* are commonly found in the faeces of many animals. The number of *C. perfringens* in the intestinal tract varies from animal to animal, and among individuals of the same species.

Foods

C. perfringens is commonly found in low numbers in many foods, especially in meat and poultry and their products, and in Mexican foods (3). Because of the common occurrence of *C. perfringens*, the interpretation of the significance of finding low numbers of *C. perfringens* in foods - especially meat and poultry and their products - should be made with caution; many wholesome foods may contain low numbers of the organism (13). It has been suggested that only strains of *C. perfringens* that have been subjected to repeated heating are able to cause food poisoning, and that strains freshly isolated from the environment do not (14).

Growth/Survival Characteristics of the Organism in Foods

Temperature

The most significant characteristic of *C. perfringens* in relation to food safety is the organism's ability to grow extremely rapidly at high temperatures. Its optimum temperature for growth is 43 - 45 °C. At these temperatures, *C. perfringens* has one of the fastest rates of growth (shortest generation times) of any bacterium; a generation time of approximately 7 minutes has been recorded at 41 °C for one strain of *C. perfringens*, although 10 minutes is more typical (3,6).

However, *C. perfringens* has the potential ability to grow within the temperature range 15 - 50 °C, depending on strain and other conditions. Although some growth can occur at 50 °C, death of the vegetative cells of this organism usually occurs rapidly above this temperature (1, 3). At cold temperatures, 0 - 10 °C, vegetative cells die rapidly (1).

Heat resistance

Exposure to a temperature of 60 °C or more will result in the death of vegetative cells of *C. perfringens*, although prior growth at high temperatures or the presence of fat in a food will result in increased heat resistance.

The spores of *C. perfringens* can vary quite considerably in their heat resistance, and their heat resistance is also affected by the heating substrate. Recorded heat resistance values at 95 °C (D-values) range from 17.6-63 minutes for heat-resistant spores to 1.3 - 2.8 minutes for heat-sensitive spores (1). Recent studies suggest that at 55 °C, vegetative cells of food poisoning isolates are about twice as heat resistant as vegetative cells of other *C. perfringens* isolates (5).

In addition, the enterotoxin is not heat-resistant - it is destroyed by heating at 60 °C for 10 minutes (4, 6, 15).

pH

C. perfringens is not a tolerant organism with respect to pH. It grows best at pH values between 6 and 7 (the same pH as most meats). Under otherwise ideal conditions, very limited growth may occur at pH values over the range

≤5 and ≥8.3. Spores, however, will survive greater extremes of pH [and water activity (a_w)] (1,3).

Water activity/salt

C. perfringens is not tolerant of low a_w. As in the case of other factors limiting the growth/survival of this organism, the limits for a_w are affected by temperature, pH, type of solute, etc. The lowest a_w recorded to support the growth of *C. perfringens* is 0.93 to 0.97 depending on the solute used to control the a_w of the medium (3,5).

Salt concentrations of 6 - 8% inhibit growth of most *C. perfringens* strains, but lower concentrations may be effective in combination with other factors. Some studies have indicated that the presence of 3% sodium chloride (NaCl) delays growth of *C. perfringens* in vacuum packed beef (5).

Atmosphere

C. perfringens - like other clostridia - is an anaerobe. It will not, therefore, grow on the surface of foods unless they are vacuum or gas-packed. The organism will grow well in the centre of meat or poultry dishes, where oxygen levels are reduced, particularly by cooking (15).

The oxidation-reduction potential (Redox or Eh) for growth is reported to be between -125mV and +300mV (1,3). Once growth is initiated, the cells are able to modify the local Eh to favour more rapid growth, probably by production of substances such as ferredoxin (15).

Summary of Control of *C. perfringens* in Foods

Food poisoning from *C. perfringens* occurs most commonly where there is inadequate temperature control after cooking. Improper cooking of foods and contaminated equipment also contribute to infection. The prevention of food poisoning by *C. perfringens* can be assured by thoroughly cooking the food. In addition, prevention can be guaranteed by the rapid cooling of any large meat or poultry portions or dishes, after cooking, to temperatures below those allowing the growth of the organism (below 15 °C, or preferably below 10 °C, within 2 - 3 hours). Alternatively, such cooked foods should be kept until serving at temperatures too high for the growth of *C. perfringens* (>60 °C).

Pre-cooked foods should be reheated before serving, to an internal temperature of at least 75 °C, to ensure the destruction of vegetative cells of *C. perfringens*.

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CRONOBACTER SAKAZAKII

The organism, until 1980, was classified as the 'yellow-pigmented' *Enterobacter cloacae*. It was then renamed *Enterobacter sakazakii*, after the Japanese bacteriologist Riichi Sakazaki and was renamed again in 2008 to become *Cronobacter sakazakii* in order to clarify the taxonomic relationship of several strains. The change in classification is based on improved identification methods. *C. sakazakii* is considered an opportunistic pathogen and some strains are known to produce an entero-toxin (1, 2).

The Organism *C. sakazakii*

C. sakazakii is a Gram-negative, catalase-positive, oxidase-negative, motile, non-sporing rod, with a facultative anaerobic metabolism, belonging to the family Enterobacteriaceae. It has a biochemical profile similar to *E. cloacae*, but is usually D-sorbitol negative. Other distinguishing characteristics include greater pigment production at temperatures less than 36 °C and utilisation of citrate as the sole source of carbon.

Two morphologically different colony types have been observed, broadly as 'matt' or 'glossy'.

Strains currently classified as *C. sakazakii* fall into two distinct groups; these are further subdivided into 57 strains based on DNA hybridisation, antibiotic susceptibility and biochemical reactions. (3, 4, 5).

C. sakazakii Food Poisoning

Despite its ubiquity in nature, *C. sakazakii* has not equalled *Salmonella'*s reputation as a pathogen, because the number of outbreaks reported is relatively low. *C. sakazakii* is a frequent cause of nosocomial disease. It has been implicated in sporadic outbreaks of neonatal diseases due to the consumption of contaminated infant formula. Although fresh produce has

not been implicated in *C. sakazakii* food poisoning, its ability to survive and grow on fresh produce raises some concern (6).

Incubation time

Challenge studies involving intra-peritoneal injections of suckling mice at a dose of 10^8 cfu/mouse showed that death occurred within 3 days after dosing, typically after 24 to 48 hours (7).

According to published studies, infections are reported within the first day, and up to 18-30 days after birth in premature babies (8).

Symptoms

C. sakazakii-induced neonatal meningitis results in ventriculitis, brain abscess or cyst formation and the development of hydrocephalus. Another clinical manifestation is the development of neonatal necrotising enterocolitis (NEC) characterised by intestinal necrosis and pneumatosis intestinalis.

There are only a few reports of the infection among adults and almost all cases were associated with immunocompromised adults. *C. sakazakii* has been obtained from patients suffering from bacteraemia and osteomyelitis, but no reported cases of meningitis (3, 4, 5).

Mortality

Neonatal meningitis and necrotising enterocolitis are the most common cause of mortality in neonates. Mortality rates are between 40 and 80% (1, 8).

Infective dose

Since no quantitative determinations of the ingested *C. sakazakii* have been performed the dose-response curve is not known. The effect is thought to be linear to the dose.

Very low initial counts of 1 cfu/ml in bottled infant formula have led to counts of 10^7 per serving of 100 ml in bottles stored at room temperature for 10 hours, even sooner in formula held at 35-37 °C (3, 5).

Infection

Species of *Cronobacter* are considered opportunistic pathogens and rarely cause disease in otherwise healthy individuals. The bacterium has been implicated most frequently in causing illness in neonates and children from 3 days to 4 years of age, with low birth weight, premature and immunosupressed babies being most at risk of infection. At least 111 cases of *C. sakazakii* infection and 26 deaths in infants have been reported (source: Health Protection Agency). Nine cases of *C. sakazakii* infection in adults have been reported (4).

C. sakazakii has been documented to cause meningitis, sepsis and necrotizing enterocolitis (9). Pathogenesis in neonates frequently involves bacteraemia and/or sepsis, cerebrospinal fluid (CSF) infection and meningitis with other neurological complications, and necrotizing enterocolitis.

Incidence of *C. sakazakii* Food Poisoning

The number of documented cases and outbreaks of *C. sakazakii* infection are few. The first cases of *C. sakazakii* were in 1958 in St Albans, England where two cases of terminal neonatal meningitis were reported. The next distinctive report of *C. sakazakii*-induced meningitis infection was in Denmark. The child survived meningitis but experienced severe mental and neurological impairment (4). Additional cases have since been reported and described in America, Netherlands, Greece, Iceland, Belgium, Israel and Germany where *C. sakazakii* was responsible for non-meningital bacteraemia, necrotizing enterocolitis, neonatal septicaemia and *C. sakazakii*-induced meningitis (4, 10). In some cases, even though the child survived infection, they were severely mentally retarded or suffered from seizure disorders.

Sources

C. sakazakii sources have been investigated for only a few cases of reported illness, thus the exact reservoir for the organism is unknown. The organism is considered to be ubiquitous.

Clinical

C. sakazakii has been found in the human and animal gut (9). Most isolates are rare and have originated from clinical sources such as blood, CSF, sputum, lower and upper respiratory tract, digestive tract, superficial wounds and urine (3, 4, 5).

Environment

Little is known about the presence of the organism in the environment but it is suspected that water, soil and vegetables are the primary sources of contamination, with flies and rodents secondary means of contamination (4, 11).

C. sakazakii has also been isolated from production environments of food factories (milk powder, chocolate, cereal, potato and pasta), and households (3) and hospitals.

Foods

The foods most commonly associated with *C. sakazakii* contamination are dried milk, dried infant foods and powdered infant formula milk. However, *C. sakazakii* has been isolated from a wide range of foods including cheese products, khamir (a fermented bread), sobia (a fermented beverage), minced beef, sausage meat, poultry, grain, various dry food ingredients (e.g. herbs and spices), raw lettuce and other vegetables, (3, 9) and drinking water (4).

Growth Survival Characteristics of the Organism in Foods

Temperature

The minimum growth temperature is between 5.5 - 8 °C. The lowest recorded temperature that allowed growth of *C. sakazakii* was 3.4 °C, suggesting that the organism is able to grow during refrigeration. The maximum growth temperature ranges from 41 - 45 °C, in general. However, under laboratory conditions, 22 strains examined were all capable of growing in brain heart infusion broth at 47 °C (1, 3, 4, 9, 12).

pH

Like other members of the Enterobacteriaceae, *C. sakazakii* is presumed to have good resistance to low pH. Survival of the organism in acid environments depends on a number of factors such as pH, acidulant identity, acidulant concentrations, temperature, water activity (a_w), atmosphere and the presence of other inhibitory compounds (6).

Survival characteristics of 12 strains of *C. sakazakii* in tryptic soy broth, adjusted to pH 3.0 and 3.5 with hydrochloric acid (HCl), revealed that 10 of the 12 strains showed less than a 1 log decline over a 5 hour period at 37 °C (4).

Heat resistance

C. sakazakii is considered to be one of the most thermo-tolerant among the Enterobacteriaceae. As *C. sakazakii* can survive at elevated temperatures (45 °C), and has the ability to grow at temperatures of up to 47 °C in warm and dry environments such as in the vicinity of drying equipment in factories, it has a competitive advantage when compared to other members of the Enterobacteriaceae. This also favours its growth even when present at low levels. However, *C. sakazakii* does not survive a standard pasteurisation (>60 °C) process (2, 11, 12, 13).

In a study of 10 strains tested, 5 clinical and 5 food isolates, in reconstituted dried infant formula, a mean $D_{60\ °C}$ of 2.5 minutes and z of 5.82 °C was found. The mean $D_{60\ °C}$ for the 5 clinical isolates was 2.15 minutes and 3.06 minutes for the 5 food isolates. In another study the $D_{58\ °C}$ of 12 strains in rehydrated infant formula ranged from 30.5 - 591.9 seconds (0.51 - 9.87 minutes). When the most heat-resistant strain (z = 5.6 °C) was added to a dry infant formula rehydrated at 70 °C, greater than a 4 log reduction was achieved. If 1 cfu/100g of *C. sakazakii* is present in infant formula, a 4-D treatment should ensure absence of the bacterium after cooling (1, 12).

Water activity/salt

C. sakazakii can survive in dried infant formula having a_w of *ca* 0.2. Like many other organisms, *C. sakazakii* is relatively resistant in the stationery phase to osmotic and dry stress.

The bacteria protect themselves to increasing osmolarity by the rapid intracellular accumulation of ions, mainly potassium (12).

Control of *C. sakazakii* in Foods

The occurrence of *C. sakazakii* can be reduced through a combination of control measures, for example, the implementation of more stringent hygiene during manufacture, including prevention of recontamination after processing from the environment. Other measures include filtering the air that enters the processing area, keeping overpressure in rooms around the dryer and minimising the use of water for cleaning.

Adherence to hygienic rules during preparation and handling; up to consumption is equally important. When preparing and handling infant formula, care should be taken to prevent recontamination through the environment or by improperly sanitised utensils. In addition, holding time (i.e. the time between preparation and consumption) and 'hang time' (i.e. the amount of time the formula is held at room temperature or in the bottle warmer) should be minimised (5, 8, 14).

In hospitals and maternity units, where central milk kitchens supply prepared bottled feeds for distribution, milk should be sterilised in bottles with the teat already in place. Sterile water should be used for the distribution of feeds (15).

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LISTERIA MONOCYTOGENES

Until 1927, the organism was called *Bacterium monocytogenes* and then renamed *Listeria monocytogenes*.

In England and Wales, listeriosis is a comparatively rare disease, and the reported incidence is now about 2-3 cases per million of the population, following peaks in 1988 and 2003. Until 2000 an average total of approximately 100 cases has been reported annually (1). The figure for 2001 was 136, the highest total since 1989, and since 2001 the numbers of reported cases have been increasing with an average total of approximately 200 cases reported within the period 2003-2008 (source: Health Protection Agency). In the United States of America (USA), preliminary data from the Foodborne Diseases Active Surveillance Network (FoodNet) indicated an overall incidence of 3 cases per million of the population for 2001. However, because of the potential severity of the disease, food microbiologists need to be well aware of the characteristics of the causative organism and measures for its control in foods. It is generally believed that the majority of cases are foodborne and may be preventable.

The Organism *L. monocytogenes*

Listeria are catalase positive, oxidase negative, short Gram-positive, rods with rounded ends, which are non-sporing and have a facultative anaerobic metabolism. They are motile with motility manifesting itself at 20 - 25 °C; motility is absent at 37 °C. *L. monocytogenes* is a species belonging to the Listeriaceae family and a genus of bacteria that also includes *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, *Listeria ivanovii* and *Listeria grayi*. *L. innocua* and *L. grayi* are considered to be non-pathogenic, and although *L. ivanovii*, *L. seeligeri* and *L. welshimeri* have very rarely caused human infection (2), almost all cases have been recognised as being due to *L. monocytogenes*.

L. monocytogenes can be divided by serotyping into 13 different serotypes, all of which can cause human listeriosis. However, this is of

limited epidemiological value because the majority of cases are caused by serotypes 1/2a, 1/2b, and 4b, and the majority of significant recorded outbreaks have been caused by serotype 4b.

L. monocytogenes Food Poisoning

L. monocytogenes was discovered almost 100 years ago, but it has only been recognised as an important foodborne pathogen since the early 1980s.

Listeriosis is a generic term for a variety of syndromes caused by *L. monocytogenes*. Many factors have been shown to affect the pathogenicity of *L. monocytogenes*, including the production of a haemolysin and two phospholipases. These virulence mechanisms have been extensively reviewed (2).

Incubation time

The incubation period can vary from 3 - 70 days (for reasons that are not understood). Symptoms of gastroenteritic illness occur within hours - usually 18 - 27 hours (3).

Symptoms

Listeriosis most typically takes the form of meningitis, septicaemia and meningoencephalitis, which occurs most commonly in immunosuppressed middle-aged or elderly people. In addition, a flu-like illness can occur in pregnant women, which leads to infection of the foetus and, in turn, can result in miscarriage, stillbirth or birth of a severely ill infant. Currently, about 25% of the cases of listeriosis in England and Wales are pregnancy-associated, but this figure can vary significantly from year to year. Only a small proportion (about 15% in England and Wales) of cases of listeriosis occur amongst people with no known risk factors. Outbreaks have been reported where the principal symptoms were those of gastroenteritis, rather than classical listeriosis (4, 5).

Mortality

The overall mortality rate amongst cases of listeriosis is about 30%, but may be as high as 40% in susceptible individuals (6).

Infective dose

The infective dose for listeriosis is uncertain. Food recovered from the homes of a small number of patients who developed listeriosis has been generally heavily contaminated (>10^3 cfu/g), and the typical infective dose is likely to be high (7). However, frankfurters implicated in an outbreak in the US in 1998 were reported to contain less than 0.3 cfu/g (8), and it has been suggested that this may have been an indication of a strain with enhanced virulence (8). The potentially long incubation period for listeriosis causes difficulties in the determination of an infective dose.

The infective dose for gastroenteritic illness is higher (1.9×10^5 to 10^9 cfu/g) (3).

Foods involved

Many cases of listeriosis have not been directly related to a known food source, and it is often difficult to differentiate between cases that are food-linked and those that are not, again because of the long incubation period involved.

The first outbreak of human listeriosis where food was convincingly implicated occurred in 1981, involving coleslaw salad in an outbreak in Canada, with at least 41 cases and 7 deaths. In this outbreak, cabbage had become contaminated with *Listeria* from infected sheep manure, which was used as fertiliser in the soil in which the cabbages were grown. The manure used was from sheep suspected of suffering from listeriosis (9).

At least 11 further major outbreaks of human foodborne listeriosis have occurred: a possibly milkborne outbreak in Boston, USA; 'Mexican-style' cheese in Los Angeles, USA; 'Vacherin Mont d'Or' cheese in Switzerland; pâté in the UK; and pork tongue in aspic in France (7). Also 'rillettes' (potted pork) were implicated in a further outbreak involving at least 25 cases in mid-1993 in France (7). Chocolate milk caused an outbreak of 45 cases in the USA in 1994 (5), and a raw milk soft cheese was implicated in a 1995 outbreak in France (7). More recently, contaminated frankfurters were associated with a multi-state outbreak of over 100 cases in the US that took place between August 1998 and March 1999, during which 15 adult deaths and 6 miscarriages were recorded (8). An outbreak in Finland between 1998 and 1999 was linked to butter (10), pork tongue in jelly was implicated in an outbreak in France in 1999-2000 (11), and an outbreak in the US in 2000 was associated with ready-to-eat turkey deli meat (12).

Incidence of *L. monocytogenes* Food Poisoning

The incidence of listeriosis worldwide increased dramatically throughout the early to mid-1980s. In 1988, numbers in England and Wales peaked at nearly 300, possibly as a result of a single foodborne outbreak (13). However, despite rigorous surveillance, numbers (until 2003) have since seen a decline to a level similar to that in the early 1980s. The decline in cases coincided with the issue of United Kingdom (UK) Government health warnings, concerned with the consumption of pâté. The average number of cases has risen in the last 5 years however.

Similar trends were observed in France and the USA. Before 1974, there were about 15 cases per year in France compared with 687 cases in 1987. In the US, during 1967-69 there were 255 cases compared with about 1,700 cases in 1987 (14). In Canada, between 1989 and 1990, 24% of food poisoning incidents were caused by *L. monocytogenes*, but this declined to ≤3% during 1991-92 (3).

In 2007 the Health Protection Agency (HPA) provided two briefings on the continued high level of reporting of listeriosis in England and Wales, especially in patients over 60 years of age. The possible factors identified by The Advisory Committee on the Microbiological Safety of Foods (ACMSF) were improper refrigerator temperatures, dirty dish-cloths, and non-adherence to 'use by dates' (15).

In 2008 at least 20 people died following consumption of *Listeria* contaminated cured meats manufactured by a Canadian company, Maple Leaf Foods Inc. In the same year, three deaths and a miscarriage, in Boston, USA were linked to contaminated dairy products.

In 2010 at least 7 people died in Austria and Germany and a further 14 cases of listeriosis were reported following the consumption of *Listeria* contaminated acid curd cheese made by Prolactal.

Sources

Humans

Person-to-person transmission of infection has been documented as person-to-person spread in hospitals, but only during the neonatal period.

Surveys into the carriage rate of *L. monocytogenes* in healthy humans have shown a wide range in percentage carriage rates; 5 - 10% is a figure that is commonly mentioned.

Animals and the environment

Infection from *Listeria* can originate from direct or indirect contact with animals (sheep, goats, and cows can excrete *L. monocytogenes*, in faeces or sometimes in milk). *L. monocytogenes* is also ubiquitous in the environment, and survives and grows in soil and water, so it can be transferred to foods from a wide range of sources. However, evidence for tracing the transmission of human listeriosis suggests that post-process contamination within the food manufacturing unit, usually from plant or machinery, is of particular importance (16).

Pasture grass and silage - especially silage of poor quality - are important sources of *Listeria* in ruminant livestock.

Foods

Foods can become contaminated with *Listeria* at any stage in the food chain, from the farm, through processing and distribution, to the consumer's kitchen, especially in moist/wet environments. Numerous studies have now indicated that *L. monocytogenes* can be found in a very wide range of foods, including milk, soft cheese, raw and pre-cooked chicken and meats, pâté, fermented sausage, vegetables, smoked and lightly processed fish products (17), and seafoods (2, 3, 18). In the UK, *L. monocytogenes* has been found in many foods, usually in very low numbers, but occasionally in numbers exceeding 10,000/g, particularly in pâté and soft cheese (19). Most foods where *L. monocytogenes* has been found have contained serotype 1/2a, b or c. However, pâté has frequently been found to contain serotype 4b - the serotype most commonly isolated from humans (19). Cooked, chilled, ready-to-eat meat, poultry, and sausage products are now of particular concern, and *L. monocytogenes* has been isolated from samples of these foods in many countries (17).

Although *Listeria* spp. other than *L. monocytogenes* are not a cause of human listeriosis, their presence in foods, particularly those that have undergone a listericidal process, may be indicative of poor hygiene practices.

There is evidence that the microbiological quality of some foods, with respect to contamination by *Listeria*, improved during the late 1980s, possibly as a result of changes in UK legislation and implementation of better hygiene standards by the food industry (13, 20).

Foods associated with transmission of infection are diverse; processed meat, dairy, vegetable or marine products have all been implicated. However, these generally have been able to support the growth of *L. monocytogenes*, and have a high degree of processing, and an extended shelf-life, often at chill temperatures.

Growth/Survival Characteristics of the Organism in Foods

Temperature

L. monocytogenes is unusual amongst foodborne pathogens in that it is psychrotrophic, being potentially capable of growing at refrigeration temperatures down to, or even below, 0 °C. Growth at low temperatures can be very slow, requiring days to double in number. As part of a mixed inoculum with other psychrotrophic organisms, *L. monocytogenes* was found to grow in vacuum packed (VP) sliced roast beef at a temperature of -1.5 °C, but with a lag time of 174 days and a generation time of 100 hours (20). However, -0.4 °C is probably a more likely minimum in foods (22). Its optimum growth temperature is between 30 °C and 37 °C.

The upper temperature limit for the growth of *L. monocytogenes* is reported to be 45 °C. *L. monocytogenes* is killed at temperatures of >50 °C (3).

The organism survives well for several weeks in frozen foods (23), but less well under acid conditions.

Heat resistance

L. monocytogenes is not a particularly heat resistant organism; it is not a spore-former, so can be destroyed by pasteurisation. It has, however, been reported to have slightly greater heat resistance than certain other foodborne pathogens such as *Salmonella*. It is generally agreed that commercial milk pasteurisation will destroy normal levels of *L. monocytogenes* in milk ($>10^5$/ml). In milk at 62.8 °C for 30 minutes 39D and at 71.7 °C for 15 seconds 5.2D, reductions have been obtained. The UK Department of Health advised that ready meals or similar products should receive a heat treatment of at least 2 minutes at 70 °C or equivalent to ensure the destruction of *Listeria*. In the US, standards for the heat treatment of frozen dessert mixes require a process of 68.3 °C for 30 minutes, or 79.4 °C for 25 seconds to ensure destruction of *L. monocytogenes*. The addition of stabilisers such as

guar gum and carrageenan to ice cream mixes is thought to increase the heat resistance of the organism in these products (24).

Typical D-values in foods range between approximately 5 - 8 minutes at 60 °C and 0.1 - 0.3 minutes at 70 °C, depending on strain and substrate. Heat shocked cultures have been reported to show enhanced heat resistance (25).

pH

The ability of *Listeria* to grow at different pH values (like other bacteria) is markedly affected by the type of acid used and temperature. Under otherwise ideal conditions, the organism is able to grow at pH values well below pH 5; pH 4.3 is the lowest value where growth has been recorded, using hydrochloric acid as an acidulant. In foods, however, the lowest limit for growth is likely to be considerably higher - especially at refrigeration temperatures, and where acetic acid is used as an acidulant; pH <5.2 has been indicated as inhibitory for growth in foods (17). Nevertheless, *L. monocytogenes* is relatively acid tolerant, and has been shown to survive for 21 days in orange serum adjusted to pH 3.6 and incubated at 4 °C (26).

Water activity/salt

L. monocytogenes is quite tolerant of high sodium chloride (NaCl)/low water activities (a_w). It is likely to survive, or even grow, at salt levels found in foods of 10 - 12% NaCl or more. It grows best at a_w of $\geq$0.97, but has been shown to be able to grow at a_w level of 0.90. The bacterium may survive for long periods at a_w values as low as 0.83 (3). Tolerance of a_w levels is influenced by temperature, pH and type of solute (a slightly lower a_w minimum - 0.90 - has been observed with glycerol rather than NaCl or sucrose at 30 °C) (17).

Atmosphere

L. monocytogenes grows well under aerobic and anaerobic conditions, and a study using broth culture showed that anaerobic incubation favoured growth (27). Also, several studies with modified atmospheres showed that *L. monocytogenes* grew at low temperatures (3 - 7 °C) on meat in reduced-oxygen or anaerobic conditions (28, 29). Similarly, *L. monocytogenes* grew at low temperatures (5 °C) on various vegetables stored under modified

atmospheres (3-10% CO_2) (30). High concentrations (80%) of CO_2 have been found to be necessary to inhibit growth (31). Studies have also shown that *L. monocytogenes* is not significantly affected by VP (3).

Irradiation

L. monocytogenes cells are more resistant to radiation than are the cells of Gram-negative pathogens such as *Salmonella*. Studies of the resistance of *L. monocytogenes* to gamma irradiation (0.38–1.00 kGy) in selected products gave a range of D-values (16). Resistance is generally greater in foods than in culture medium (32). Treatments of 1.7-4.0 kGy have been found sufficient to give a 6-7 log reduction in numbers of *Listeria* spp. (33).

It has been reported that irradiation treatments were significantly more lethal to *L. monocytogenes* in turkey meat packed in the presence of air than in modified atmosphere packed (MAP) or VP meat, suggesting increased sensitivity in the presence of oxygen (33).

Summary of Control of *L. monocytogenes* in Foods

The World Health Organisations (WHO) Informal Working Group on Foodborne Listeriosis in 1988 concluded that "the total elimination of the organism from all food is impractical and may be impossible" (34). This is still a generally accepted view. The common occurrence of *L. monocytogenes* in the environment and in foods indicates that exposure to *L. monocytogenes* does not usually lead to infection. The incidence of listeriosis, in comparison with other foodborne illness, such as salmonellosis, is very rare. Nevertheless, the potential severity of listeriosis demands that every effort should be made to reduce the incidence of the organism in foods to a minimum. In addition, the presence of *Listeria* in a food - especially a cooked food - may be an indication of poor hygiene during manufacture, distribution or retailing. The United States Department of Agriculture (USDA) has adopted a 'zero tolerance policy' for *L. monocytogenes* in ready-to-eat foods, but in Europe counts of up to 100 cfu/g at the end of shelf-life are generally considered acceptable.

The Hazard Analysis Critical Control Points (HACCP) approach to food quality assurance should be applied at all levels in the food industry, with particular attention being paid to temperature control, and the strict adherence to practices that prevent contamination, especially cross contamination between raw and processed foods, and wet

processing/manufacturing or packaging environments and the processed foods. Storage times for sensitive foods should also be carefully controlled.

The UK Department of Health advises vulnerable individuals, especially pregnant women and the immunosuppressed, to take appropriate precautions against *Listeria* infection, including avoiding eating pâté and soft (mould-ripened) cheeses, and to reheat cook-chill foods adequately (19). In the US, recent advice from the Food and Drug Administration (FDA) adds chilled ready-to-eat meats, hot dogs, and smoked seafood, unless properly reheated, to the list of foods that 'at risk' consumers should avoid.

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SALMONELLA

Salmonella is a common cause of food poisoning worldwide. There are many different types of *Salmonella* but, with the exception of the few which cause typhoid or paratyphoid fever, the illness they cause is similar. *Salmonella* is widespread in cows, poultry, eggs, pigs, pets and wild animals.

The Organism *Salmonella*

Salmonellae belong to the family Enterobacteriaceae. They are Gram-negative, non-sporing, oxidase-negative and catalase-positive rods. Most strains are motile and can grow both aerobically and anaerobically.

The nomenclature of *Salmonella* has been progressively revised on the basis of biochemical and serological characteristics. Recently, a classification based on that proposed by Le Minor and Popoff (1) has been increasingly adopted. On the basis of DNA/DNA hybridisation, it has been proposed that there are only two species of *Salmonella*; *Salmonella enterica*, and *Salmonella bongori*. However, *S. enterica* can be further divided into six sub species. The genus is further divided, serologically, into over 2,400 serovars (or serotypes), these being distinguished according to their possession of different antigens (somatic antigen O, flagella antigen H, or capsular antigen Vi). Most of the recognised serovars belong to the species *S. enterica*, and only 20 are included in *S. bongori*. Nearly 1,500 serovars, including almost all of those important in foodborne disease, belong to a single subspecies, *S. enterica* subsp. *enterica*. The serotype names have traditionally been used as they denote species (e.g. *Salmonella enteritidis*, *Salmonella typhimurium*), but an example of the correct nomenclature under the currently accepted classification would be *S. enterica* subsp. *enterica* serovar Enteritidis (2). For convenience this can be abbreviated to *S.* Enteritidis, and this form will be continued here. Further differentiation of *Salmonella* isolates can be done by biochemical characteristics and by 'phage-typing'.

There are three different syndromes in man caused by salmonellae. The most severe is enteric (typhoid) fever, caused by the host adapted organisms *Salmonella* Typhi, *Salmonella* Paratyphi A, B (*Salmonella* Schottmuelleri) and C (*Salmonella* Hirschfeldii), and this will not be considered further here. The second syndrome is by far the most frequently encountered, gastroenteritis or food poisoning caused by non-typhoid salmonellae. Finally, a variety of systemic infections (such as septicaemia) and chronic conditions may develop following infection of susceptible individuals by non-typhoid strains. Certain non-human adapted serotypes such as *Salmonella* Dublin, *S.* Enteritidis and *Salmonella* Virchow, can be invasive and give rise to such infections.

The two main serotypes of *Salmonella* that are of current concern in Europe and the United States (US) are *S.* Enteritidis and *S.* Typhimurium. Their importance is due to their frequency of isolation from incidents of food poisoning, and their involvement in the on-going issue of the safety of poultry and eggs (3). However, a large number of other serotypes, particularly *S.* Virchow, have been involved in food poisoning, and all serotypes of *Salmonella* can be considered to be equally significant in relation to food safety. Recently, there has been concern over increasing antibiotic resistance in some salmonellae, such as *S.* Typhimurium DT104. This strain has become a common human pathogen in England and Wales, and has also been isolated from livestock, domestic pets, and wildlife. It has shown an increasing spectrum of antibiotic resistance since 1990 (4), and has recently been documented as developing resistance to fluoroquinolones in England and Wales, and in Germany (5). The World Health Organisation (WHO) reports that multidrug-resistant strains of *Salmonella* are now frequently encountered, and that this characteristic is an integral part of the genetic material (6).

Salmonella Food Poisoning

Gastroenteritis is the usual manifestation of infection caused by *Salmonella*. After *Campylobacter*, *Salmonella* is the most commonly reported cause of gastroenteritis in the United Kingdom (UK), and it continues to be one of the main causes of foodborne illness the world over.

The severity of illness varies according to the strain of *Salmonella* involved and to the susceptibility of the host. The most susceptible are infants, the elderly, and individuals who are immunocompromised.

Individuals recovering from salmonellosis can continue to shed salmonellae in their stools for some time after illness; however, long-term carriage is uncommon.

Incubation time

The incubation period for *Salmonella* food poisoning is between 8 and 72 hours.

Symptoms

The symptoms of foodborne *Salmonella* infection include diarrhoea, abdominal pains, chills, fever, nausea and vomiting, as well as dehydration and headache. The symptoms usually last from 2-5 days.

Mortality

Death from salmonellosis is rare (less than 1% of cases), but is more likely amongst the susceptible group outlined above (3).

Infective dose

Until recently, it was considered that the ingestion of large numbers (minimum 106) of salmonellae was needed in order for salmonellosis to occur. However, more recent outbreaks have indicated that under certain conditions and in certain foods (e.g. chocolate, or cheese, where high fat content/low water activity (a_w) appears to protect *Salmonella* from stomach acidity), only small numbers need to be ingested to induce illness in the very young or elderly, possibly as low as 10-100 cells (7,8). The infective dose for a *S.* Enteritidis outbreak, associated with ice cream that occurred in the United States (US) in 1994, has been estimated at no more than 25 cells (9).

Foods involved

Types of food involved in foodborne salmonellosis have been wide-ranging, including poultry, meat products, dairy products, eggs, fruit juice, tomatoes, lettuce, canteloupe melons, seed sprouts, toasted oat cereals, paprika

flavoured potato crisps, coconut, almonds, peanut butter and chocolate. Fermented meats such as salami have been implicated in *Salmonella* outbreaks. In the UK, during 1988, there was an outbreak due to *S.* Typhimurium DT124 and 71 cases were confirmed, of which 55 concerned children (10). A very large outbreak of *Salmonella* occurred in the US in 1985; there were over 16,000 cases associated with contaminated pasteurised milk (11). In the same year, in the UK, an outbreak of *Salmonella* Ealing was associated with contaminated milk powder (12). The 1994 *S.* Enteritidis outbreak, associated with ice cream in the US, is estimated to have infected up to 224,000 people, and is probably the largest recorded (13).

There have been several outbreaks involving chocolate. If chocolate becomes contaminated with *Salmonella* during the production process, or is made using contaminated ingredients; the finished product will also be contaminated because the process of chocolate manufacture cannot include a sufficiently severe heat process to kill *Salmonella*.

Incidence of *Salmonella* Food Poisoning

In England and Wales, there was a marked increase in the incidence of foodborne salmonellosis, especially after 1985, reaching a peak of 32,000 reported cases in 1997. This was attributed mainly to the rise in *S.* Enteritidis PT4 infections. In 1993, the UK Advisory Committee on the Microbiological Safety of Food (ACMSF) concluded that, between 1981 and 1991, there was a rise of over 170% in reported cases of salmonellosis, primarily because of the increase in *S.* Enteritidis (particularly *S.* Enteritidis PT4). It is probable that this increase was due to its presence in certain foods - in particular, eggs and poultry. However, since 1997, there has been a dramatic decrease in the incidence of infection, and only about 12,500 cases were recorded in 2006 compared to approximately 31,000 in 1997 (source: CDSC). This is thought to be largely attributable to the reduced prevalence of *S.* Enteritidis in eggs and poultry, as a result of action taken by producers.

In November 2003, a large outbreak in the UK (324 cases) was traced to a single source. 19% of the cases were admitted to hospital, and *S.* Enteritidis PT56 was found to be causative agent.

A shipment of raw beef was implicated in an outbreak of *S.* Typhimurium DT104 identified in the Netherlands during October-December 2005 (14). Forty two cases of *Salmonella* Infection occurred in the UK in 2006 from the consumption of *Salmonella* Montevideo-contaminated chocolate. An

international outbreak, in 2008, involved seven European Union (EU) countries. Meat contaminated with *Salmonella* Agona was identified as the cause of the outbreak which involved 163 cases and 1 death (15, 16).

Since 1989, there have been about 30,000 *Salmonella* infections reported each year in England and Wales, and *S.* Enteritidis PT4 accounted for about 40% of these. In 2006, there were fewer than 2,000 reports of *S.* Enteritidis PT4 infections. In 2001, for the first time, *S.* Enteritidis PT4 accounted for less than 50% of all *S.* Enteritidis isolates from humans, as other phage types, such as PT1 and PT6 have become more common (17). Reported numbers of infections in England and Wales with *S.* Enteritidis have been in steady decline between 2001 and 2008 (source: Health Protection Agency).

Information worldwide suggests that the incidence of salmonellosis has increased in many countries, and infection caused by *S.* Enteritidis, in particular, has increased in several developed countries.

In European countries, the increase in incidence of *S.* Enteritidis is also due primarily to phage type 4.

There was a considerable increase in salmonellosis in the US in the early 1990s. This was also due largely to *S.* Enteritidis, but in North American countries the predominant phage types are PT8, PT13 and PT13a. However, during the period from 1996-2001, the incidence of salmonellosis decreased by 15% to an incident rate of 15.1 per 100,000 people. During the same period, the incidence of infection by *S.* Enteritidis, and *S.* Typhimurium fell by 22% and 24% respectively, but the incidence of some other serovars, such as *Salmonella* Newport, and *Salmonella* Heidelberg, increased significantly (18). There have been several outbreaks, in the US, involving *Salmonella* over the last couple of years. The major ones involved *Salmonella* Saintpaul involving alfalfa sprouts in which 228 cases were reported across 13 states during 2009 (19); *S.* Saintpaul was also implicated in an outbreak during 2008 involving 1,442 people and possibly 2 deaths where jalapeño and serrano peppers were identified as the major vehicles by which the pathogen was transmitted (20). Another outbreak during 2008-2009 involving *S.* Typhimurium-contaminated peanut butter was responsible for five deaths and infection of more than 400 people in 43 states (21). Other outbreaks involved a recall of 825,769 pounds of ground beef and tomatoes in which 40 and 150 people were ill, respectively.

In the US, it has been estimated that the true annual incidence of food poisoning due to *Salmonella* may be as high as 4.8 million (22). In the UK, a true annual incidence of more than 2 million has been suggested (3), but more recent estimates using the 'Delphi method' produced an annual figure of 537,000 cases (23).

Sources

Humans

Contamination of food by infected food handlers is unusual; it is only likely to occur when a handler has diarrhoea and contaminated hands come in direct contact with food that is not subsequently cooked.

Human transmission via the faecal-oral route can occur, especially among patients in hospitals and institutions. In these cases, poor personal hygiene has a vital role in the transmission.

Animals and environment

Food animals can become infected via direct contact with other infected or symptomless animals, via contaminated feed or water, via their environment, or via wild birds or rodent pests.

Salmonella may also infect common pets such as cats and dogs, and thus these animals may act as a source of the food contamination. Reptiles, such as terrapins, are also common carriers of *Salmonella*.

In addition, manufacturing, catering, or domestic environments can become contaminated with *Salmonella* and act as a source of the organism, for example, mops and cloths, refrigerators, slicing machines and other inadequately cleaned and sanitised equipment can harbour *Salmonella*.

Foods

The main source of *Salmonella* for man is food from infected food animals. Thus, meat, poultry, eggs, or raw milk can become contaminated with intestinal/faecal material from infected livestock. Eggs can be contaminated on the shell with *S.* Enteritidis, as well as other salmonellas, as a result of either faecal carriage of the chicken or contamination from the environment. Under certain conditions, shell organisms are able to penetrate into egg contents. More importantly, however, egg contents can be contaminated with *S.* Enteritidis as a result of systemic infection of the laying hen, which results in infection of the reproductive tissues, particularly the oviduct. In 1993, it was estimated that in eggs sampled from retail outlets, 1 in 880 were positive for *Salmonella* (24). In the light of continued problems with the contamination of chickens and eggs by *Salmonella*, the UK government instigated a vaccination policy for UK breeding flocks in 1993; vaccination

of breeding chickens began in 1994, and vaccination of commercial laying hens in 1997. This lead to a dramatic reduction in the number of *Salmonella* cases in humans from a peak of over 35,000 cases in 1997, down to 11,529 in 2005 (25). Large outbreaks of *Salmonella* have occurred in the UK between 2000 and 2002; the sources of which have been traced to products made with unpasteurised imported Spanish eggs where there was no requirement for vaccination (source: FSA).

Consumption of contaminated foods that are consumed raw or not properly cooked can lead to food poisoning. Cross contamination of other food materials that are not subsequently cooked - during processing, via chopping boards and other equipment used during food preparation - can lead to further incidents of food poisoning.

The prevalence of *Salmonella* in foods is variable. In 2001, a UK study reported that an average of 5.8% of poultry carcases were contaminated with *Salmonella* (26). This compares to a figure of about 40% reported in 1996 (27). In Spain, a study of the prevalence in chicken legs carried out in 1999 showed that 35.8% of samples were contaminated (28), and in the same year, a survey of *Salmonella* in processed broilers in the US gave a contamination rate of 11% (29). The prevalence in beef and lamb carcasses is reported to be less than 1% (27). Vegetables, fruit and herbs/spices can be contaminated with *Salmonella*, and levels reported are 1.9-8%, ≤5.4% and 6.7-13.8%, respectively (7). Data collected for seafood in the US over an eight-year period during the 1990s showed that the overall prevalence of *Salmonella* was 7.2% for imported products, and 1.3% for domestically produced seafood (30). A recent increase in the consumption of snacking nuts such as Brazil nuts, almonds and peanuts and seeds has resulted in them having been identified as a source of *Salmonella* infection. This was following the 'Assessment on the Microbiological Safety of Edible Nut Kernels on Retail Sale in the UK' between 2008 and 2009 by Local Authorities Co-ordinators Regulatory Services (LACORS) and the Health Protection Agency (HPA).

Growth/Survival Characteristics of the Organism in Foods

Temperature

Most *Salmonella* serotypes can grow in the temperature range of 7 - 48 °C. However, some strains are able to grow at temperatures as low as 4 °C (31). Growth is slow at temperatures below about 10 °C, the optimum being 35 -

37 °C. Although most salmonellae cannot grow at refrigeration temperature, they are quite resistant to freezing, and may survive in some foods for a number of years (32).

Research with both naturally and artificially contaminated eggs has demonstrated that, in the great majority of eggs, there would appear to be little growth of *S.* Enteritidis until they have been stored at 20 °C for approximately 21 days. The principal site of contamination in eggs seems to be either the outside of the vitelline (yolk) membrane or the albumen surrounding it. *S.* Enteritidis is unable to grow until storage-related permeability changes have taken place to the vitelline membrane, which allow the organism to invade yolk contents. Once this occurs, large populations of salmonellae will be achieved in both the yolk and the albumen. Membrane changes take place more quickly at higher temperatures and at high humidity. Under simulated kitchen conditions, eggs were able to support the rapid growth of *S.* Enteritidis within a few days. Such growth does not become obvious until the population exceeds 10^9 cells/egg (24, 33).

Heat resistance

Salmonella is not a spore forming organism. It is not, therefore, a heat-resistant organism; pasteurisation and equivalent heat treatments will destroy the organism under normal circumstances. $D_{60\ °C}$ values normally range from about 1 - 10 minutes, with a z-value of 4 - 5 °C. However, high fat or low moisture (low a_w) will reduce the effectiveness of heat treatments, and appropriate heat treatments must be determined experimentally for low-a_w foods. For example, it is not possible to apply a sufficient heat process to decontaminate chocolate without causing an unacceptable change in the product. *S.* Enteritidis, *S.* Typhimurium and *S.* Agona were found to have a survival rate of 0.1% after heating to 70-90 ºC for 20-30 minutes (34). Strains vary in their ability to withstand heating; *Salmonella* Senftenberg 775W is about 10 - 20 times more heat resistant than the average strain of *Salmonella* at high a_w (32). Recent research has shown that exposure to a sub-lethal heat shock may also cause cells to show an increase in heat resistance (35).

pH

Salmonella has a pH range for growth of pH 3.8-9.5, under otherwise ideal conditions, although most serotypes will not grow below 4.5 (32). The acid used influences the minimum pH for growth to occur. For example, acetic acid (e.g. when vinegar is used in mayonnaise, rather than citric acid) will generally limit the pH minimum to about pH 5. Some death will occur at pH values of less than about 4.0, depending on the type of acid and temperature, but recent evidence has shown that an acid shock can trigger a complex response in cells allowing them to survive in such environments (29). The optimal pH for *Salmonella* growth is between 6.5 and 7.5.

Water activity/salt

Where all other conditions are favourable, *Salmonella* has the potential to grow at a_w levels as low as 0.945, or possibly 0.93, depending on serotype, substrate, temperature and pH. Salmonellae are quite resistant to drying as demonstrated by an *S*. Agona outbreak associated with toasted oat cereal that occurred in the US in 1998 (36). Survival for many years in low moisture, high fat foods, such as chocolate, has been reported (37).

The growth of *Salmonella* is generally inhibited by the presence of 3 - 4% sodium chloride (NaCl), although salt tolerance increases with increasing temperature (38).

Atmosphere

Salmonellae can grow both aerobically and anaerobically, although growth can be inhibited by an Eh (oxidation - reduction or redox) potential below -30 mV (22).

Irradiation

Salmonella is more resistant to irradiation than other Gram-negative pathogens, and a D-value of 0.6 kGy has been reported (39). Decontamination doses for poultry of about 3 kGy are reported to give approximately a 3-log reduction in numbers of *Salmonella* (40).

The lethal effect of irradiation is enhanced by the presence of oxygen, and United States Department of Agriculture (USDA) Food Safety and

Inspection Service (FSIS) regulations state that the packaging used for irradiated poultry must be air-permeable (41).

Summary of Control of *Salmonella* in Foods

All sectors in the food industry, as well as consumers, need to be involved in the control of *Salmonella* at all points in the food chain. This demands the control of animal feed and hygiene during livestock production and processing, through to the prevention of cross contamination from raw food (especially raw poultry) to foods that are to receive no further heat treatment - during retail and catering, and in the home.

It is also important to prevent the contamination of fresh produce during cultivation, harvesting, and processing, by implementing Good Agricultural Practice (GAP), such as control of irrigation water quality, excluding livestock and wildlife from growing areas, and good hygiene during washing and packing.

Proper attention must be paid to the control of temperature to prevent the growth of *Salmonella* during food storage. The reformulation of foods - for example, by reducing the salt or acidity levels - can also give rise to conditions suitable for survival or growth.

The Hazard Analysis and Critical Control Point (HACCP) approach should be applied to ensure that proper controls are in place to destroy or prevent contamination with *Salmonella* during food manufacturing, processing and handling. It is particularly important to ensure that salmonellae are not present in ready-to-eat (RTE) foods in view of the potentially low infective dose.

Although the prevalence of *Salmonella* in eggs had been successfully reduced by the vaccination of laying flocks and other measures, the UK Egg Products Regulations 1993 required egg products sold or used in the preparation of food to comply with specified requirements as to heat treatment, sampling, storage and transport. These regulations have been superseded by EU regulations 853/2004 Hygiene Rules for Food of Animal Origin and 1234/2007 Common Organisation of Agricultural Markets, however neither of these regulations make any reference to the control of *Salmonella*. The UK Department of Health advises all consumers to avoid eating raw eggs, and vulnerable individuals only to eat eggs that are thoroughly cooked. The ACMSF also recommended that eggs should be consumed within three weeks of date of lay and should be labelled with a “use by” date. Once purchased, either by the caterer or the consumer, they

should be refrigerated. The use of pasteurised, rather than shell eggs, is also encouraged (24).

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STAPHYLOCOCCUS AUREUS

Although there are records of the illness possibly attributable to *Staphylococcus aureus* as early as 1830 the organism was not recognised as a foodborne pathogen until sometime between 1878 and 1880.

Staphylococcal food poisoning is generally caused by the ingestion of a toxin preformed in contaminated food. Although the illness produced is not considered to be particularly serious, it is quite common on a worldwide basis and occurs either sporadically or in significant outbreaks. The principal species involved in cases of food poisoning is *Staphylococcus aureus*.

The Organism *Staph. aureus*

Staph. aureus is a Gram-positive, catalase-positive, oxidase-negative, non-motile, non-spore forming coccus, belonging to the family Micrococcaceae, which is capable of producing heat-stable toxins (enterotoxins) in food. Cells form characteristic clumps resembling bunches of grapes. It can grow either aerobically or anaerobically (1, 2, 3).

In addition to *Staph. aureus* there are at least 29 other species belonging to the genus *Staphylococcus* (4). However, *Staph. aureus* is the species that is of main concern to food microbiologists. Between 40 and 50% of staphylococcal isolates from healthy humans are capable of producing enterotoxins. *Staph. aureus* can also produce coagulase and thermonuclease (3, 5).

Other coagulase or thermonuclease-positive staphylococci (certain strains of *Staphylococcus hyicus* and *Staphylococcus intermedius*) can produce enterotoxin (5, 6). In a food related outbreak in the United States (US) during 1991, *Staph. intermedius* was implicated as the aetiological agent. In this outbreak there were over 265 cases associated with the consumption of a 'butter-blend spread' (6, 7). It has also been demonstrated more recently that some coagulase and thermonuclease-negative staphylococci are also capable of producing enterotoxin (5, 8).

It is the enterotoxins produced by the staphylococci that are known to be the emetic cause of food poisoning. These enterotoxins are heat stable, hygroscopic, water-soluble proteins. There are 11 distinct staphylococcal enterotoxins: A, B, C1, C2, C3, D, E, G, H and I. Most food poisoning strains are found to produce A, with D being the second most frequent. The fewest number of outbreaks is associated with E (2, 5).

Staph. aureus Food Poisoning

When consumption of food has resulted in staphylococcal food poisoning, at least two errors have been made in its manufacture. The first is contamination with the organism, and the second is that conditions favourable for growth and toxin production occurred at some stage during production or storage (9).

The organism can reach numbers to produce sufficient enterotoxin to cause illness, but then may be killed in further processing or die out under the conditions of storage.

Incubation time

The usual symptoms develop abruptly within 1-7 hours. The length of incubation time, and severity of illness, depends on the amount of enterotoxin ingested (10).

Symptoms

Symptoms normally include nausea and vomiting, retching, with occasional abdominal cramping, and diarrhoea. Headache and muscle cramping and sometimes, later, dehydration, may occur in more severe cases (2).

Mortality

Recovery is normally rapid, within a few hours or a day or so. However, deaths amongst children and the elderly have occurred, but rarely (2, 3).

Infective dose

As already mentioned, no live organisms of *Staph. aureus* need to be ingested. However, *Staph. aureus* generally needs to grow to levels of 10^5-10^6/g in food for sufficient toxin to be produced. A toxin dose of 1 µg or less is sufficient to cause food poisoning (6, 11). In a well-documented outbreak associated with chocolate milk, the quantity of enterotoxin was estimated at 144 ng +/- 50 (2, 3, 12).

Foods involved

Foods involved in *Staph. aureus* food poisoning are commonly those that have been physically handled and temperature abused prior to consumption. Thus, cooked meat and poultry products (where the number of competing micro-organisms has been reduced through cooking), milk and cream, custard- or cream-filled pastries, butter, salads containing potato, egg or prawns, and other moist foods, such as cold sweets, are susceptible to the growth of *Staph. aureus*, and have been involved in *Staph. aureus* food poisoning (1, 3, 11).

Salted meat, such as ham, has also quite commonly been implicated. This meat product is partly preserved by the presence of salt, which inhibits spoilage bacteria. *Staph. aureus*, however, is able to tolerate relatively high levels of salt concentration, so is able to grow in the absence of competitor organisms if temperature abuse occurs (6, 13).

Other foods sometimes implicated include cheese - especially where there has been a starter culture failure (leading to insufficient/too slow acid production).

During the period 1969-1990, poultry and meat products were implicated in 75% of incidents of staphylococcal food poisoning reported in the United Kingdom (UK), with ham and chicken most commonly implicated. The most frequently reported place where food poisoning occurred was in the home, followed by restaurants and shops (14).

In the UK, between 1992-1993, there were 8 outbreaks due to staphylococcal food poisoning, and the foods implicated were chicken, corned beef, pork, ham/bacon, chicken salad and cheese flan (15).

In Japan, rice balls are the major item involved in staphylococcal food poisoning (3).

A variant of *Staph. aureus*; methicillin-resistant *Staph. aureus* (MRSA), has been associated mainly with hospital-acquired infection. Recently,

however, the organism has been found to colonise intensively farmed animals (such as pigs, chickens, and other livestock) and people in contact with the animals; raising concerns about the possible role of the animals as reservoirs of MRSA for human infection. The European Food Safety Authority (EFSA) has evaluated the possible risk of such an association and found no evidence to indicate that there is increased risk of humans becoming healthy carriers or infected with the bacteria (16).

Incidence of *Staph. aureus* Food Poisoning

The nature of the food poisoning caused by this organism, i.e. relatively mild symptoms and rapid recovery time, means that it is not commonly reported. Estimates in the US suggest that only between 1 and 5% of cases are reported (16). The incidence of the disease in different countries varies according to geography and eating habits. In the US between 1983 and 1987, 47 (8%) outbreaks (3,181 cases) of the total 600 bacterial outbreaks were due to *Staph. aureus*, whereas in England and Wales for the same time period 54 (2%) outbreaks out of a total of 2,815 bacterial outbreaks were recorded (6, 18). During the period 1969-1990, there was an annual average of 10-15 incidents of *Staph. aureus* food poisoning reported in England and Wales. Since 1980, the number of reported cases in the UK has not exceeded 189 per annum (2). Half of these incidents occurred during the summer months, and hot summers saw a doubling in the average annual incidence (14). Figures for 1996 showed that in England and Wales, five general outbreaks and a total of 150 individual cases were reported. Reported numbers of outbreaks and cases have been very low since 2000 with no cases at all reported for 2000, 2003 and 2005 (source: Health Protection Agency). A case in Japan in the year 2000 involved 13,420 individuals (19).

Sources

Humans

In contrast to other major types of food poisoning, humans (viz. food handlers) play a major role in the transmission of the causative organism.

Humans are an important source of *Staph. aureus* - the organism can be carried in the hair, throat and nose (it can be associated with ‘sore throats’ or colds), on the skin, and transferred to foods, especially via the hands. It has

been reported that about 40% of healthy people are nasopharyngeal carriers of *Staph. aureus* (20).

Infected cuts and sores on hands and faces can also serve as a source of the bacterium for foods.

Animals and environment

Animals can act as a source of *Staph. aureus* - through raw milk in the case of mastitic cows (mastitis can be caused by *Staph. aureus*), or through raw meat (particularly pig meat), which is commonly contaminated with *Staph. aureus* (5). *Staph. aureus* is also quite resistant to drying, and may persist for long periods on processing equipment such as meat slicers, or in dust within air handling equipment.

Staphylococci can be found in air, dust, water, and human and animal waste.

Foods

A high proportion of raw meats, including beef carcasses, ground beef, salami, pork sausage, and pork carcasses are contaminated with staphylococci. These organisms are also often present on raw poultry, and various types of seafood (7).

In general, any foods that are of animal origin or that have been physically handled and not subsequently given a bactericidal treatment may carry the risk of being contaminated with *Staph. aureus* (5).

Growth/Survival Characteristics of the Organism in Foods

The growth of *Staph. aureus* is more limited under anaerobic than under aerobic conditions. The physicochemical limits for toxin production are also narrower than for growth.

Temperature

Under otherwise ideal conditions *Staph. aureus* can grow within the temperature range 7-48.5 °C (19), with an optimum of 30-37 °C (19). Enterotoxin production has been reported in the range 10-45 °C, with an optimum of 35-40 °C (3), and is inhibited more by lower temperatures of

food storage. Longer storage at lower (10 °C), but still abusing, temperatures can result in sufficient toxin production to cause illness (3).

Freezing and thawing have little effect on *Staph. aureus* viability, but may cause some cell damage (1).

Heat resistance

Heat resistance depends very much on the food type in which the organism is being heated (conditions relating to pH, fat content, water activity (a_w), etc.). As is the case with other bacteria, stressed cells can also be less tolerant of heating.

Under most circumstances, however, the organism is heat-sensitive and will be destroyed by pasteurisation (2, 13).

Examples of reported D-values

In:

Phosphate buffer (pH 7): $D_{56\,°C}$ 1-2 minutes (z-value 8-10 °C)

Meat: $D_{60\,°C}$ 2-20 minutes (depending on a_w)

Milk: $D_{60\,°C}$ 1-6 minutes (z-value 7-9 °C)

Egg pasta: $D_{60\,°C}$ 3-40 minutes (depending on a_w)

Oil: $D_{120\,°C}$ 3-6 minutes.

The enterotoxins are quite heat resistant. Destruction depends on temperature, pH and toxin type. In general, heating at 100 °C for at least 30 minutes may be required to destroy unpurified toxin (3, 13). Enterotoxins may potentially survive thermal processes applied in the sterilisation of low-acid foods (4).

pH

The pH at which a staphylococcal strain will grow is dependent on the type of acid (acetic acid is more effective at destroying *Staph. aureus* than citric

acid), a_w and temperature (sensitivity to acid increases with increasing temperature). Most strains of staphylococci can grow within the pH range 4.2 - 9.3 (optimum 7.0 - 7.5) (3, 19) under otherwise ideal conditions.

The pH range supporting enterotoxin production is narrower (pH 5.2 - 9.0) than the one supporting growth, namely pH 4.5 - 9.3 (19). The optimal pH for the production of enterotoxin is between 7.0 - 7.5, depending on the strain and type of toxin (3).

Water activity/salt

Staph. aureus is unusual amongst food poisoning organisms in its ability to tolerate low a_w. It can grow over the range 0.83 - >0.99 a_w aerobically under otherwise optimal conditions. However, a_w of 0.86 is the generally recognised minimum in foods (5).

The a_w level supporting toxin production is dependent on temperature, pH, atmosphere, strain and solute. In general, the range is 0.87 - >0.99.

Staphylococci are more resistant to salt present in foods than other organisms. In general, *Staph. aureus* can grow in 7 - 10% sodium chloride (NaCl), but certain strains can grow in 20%. An effect of increasing salt concentration is to raise the minimum pH for growth. Enterotoxin production has been demonstrated in the presence of about 10% NaCl. The yield of toxin decreased with increasing salt concentration dependent on pH and temperature (5, 21).

Atmosphere

Although *Staph. aureus* is capable of anaerobic growth, it grows best in the presence of air.

There is little or no toxin produced under anaerobic conditions, especially vacuum-packed (VP) foods (21).

Summary of Control of *Staph. aureus* in Foods

Control against *Staph. aureus* food poisoning is essentially dependent on two factors:

1) minimisation of physical handling
2) control of temperature

Every effort should be made to prevent the contamination of foods - especially processed/cooked foods - through the poor personal hygiene of handlers. Where handling is unavoidable, scrupulous attention should be paid to hand hygiene. Ideally, people with colds, or with infected cuts/sores on their hands, should be excluded from directly handling processed or ready-to-eat (RTE) foods.

The control of temperature is essential at all times. Susceptible foods must be kept either sufficiently cold (≤4 °C) or hot (≥63 °C) to prevent the growth of *Staph. aureus*. Ideally, if food is to be stored it should be in shallow, covered containers.

Note:

Decisions about the significance of detecting staphylococci in foods need to be made with care. The mere presence of the organism in foods, especially raw foods, such as raw milk or meat, need not always be cause for concern. The organism needs to grow to large numbers in order to form toxin and present a hazard. This requires the abuse of temperature and the lack of competitor organisms (*Staph. aureus* does not compete well against spoilage bacteria unless they outnumber competitors initially).

Nevertheless, if foods containing *Staph. aureus* are used as ingredients in other foods that may offer a favourable environment for the growth of *Staph. aureus*, this may represent a hazard. Furthermore, it must always be remembered that staphylococcal enterotoxins are very heat-resistant, and will withstand the usual cooking/reheating practices.

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VIBRIO

There are some eight species of *Vibrio* that are pathogenic and have been shown to be associated with foods. The main species of *Vibrio* that is of concern to food microbiologists is *Vibrio parahaemolyticus*. However, because of recent concerns, mention will also be made of the organisms *Vibrio cholerae* and *Vibrio vulnificus*.

The Organism *V. parahaemolyticus*

V. parahaemolyticus (originally known as *Pasteurella parahaemolytica*) is a non-sporing, Gram-negative, oxidase and catalase positive, facultatively anaerobic motile rod belonging to the family Vibrionaceae. Cells are often curved or comma-shaped. A particularly significant feature of this organism is the fact that it is a halophile, i.e. it requires a minimum level of salt for growth. It is, therefore, almost exclusively associated with marine environments and seafoods (1, 2, 3).

Amongst strains of *V. parahaemolyticus*, there appear to be two groups, partly defined by their source and their pathogenic potential. These are the so-called "Kanagawa-positive" and the "Kanagawa-negative" strains. It is the Kanagawa-positive strains that cause *V. parahaemolyticus* food poisoning (2, 4, 5).

V. parahaemolyticus Food Poisoning

V. parahaemolyticus was first implicated as causing a food-poisoning-type outbreak after the consumption of a fish product in Japan in 1950. Since this time it has become well known as a cause of illness associated with sea fish and other seafoods, especially in the summer months in Japan, where the consumption of raw seafood is a common practice.

Although predominantly associated with Japan, *V. parahaemolyticus* is a potential health hazard associated with seafoods throughout the world.

However, food poisoning from this organism in the United Kingdom (UK) is a relatively rare phenomenon (the reasons for which will become clear on further reading); where incidents have been reported, they have been largely associated with imported seafoods and foreign travel.

A thermostable hemolysin and toxin are considered major virulence factors of *V. parahaemolyticus*. This haemolysin can be pre-formed in food and is heat-resistant (2, 5, 6).

Incubation time

The incubation period for *V. parahaemolyticus* food poisoning is between 4 and 96 hours (most frequently between 12 and 24 hours).

Symptoms

Food poisoning caused by *V. parahaemolyticus* is usually a mild form of gastroenteritis. However, it can begin with violent pains, and diarrhoea occurs in 98% of cases. Nausea also commonly occurs, and other less frequent symptoms include vomiting, headache, mild fever and chills. In severe cases mucus and blood can occur in the stools.

Mortality

The illness is usually self-limiting, lasting about 3-5 days (range 1-7 days), with death being rare.

Infective dose

Volunteer studies indicate that large numbers ($2x10^5$ to $3x10^7$) of Kanagawa-positive *V. parahaemolyticus* cells need to be ingested to induce food-poisoning symptoms (4).

Food involved

As already indicated, foods associated with *V. parahaemolyticus* food poisoning are those of marine origin - fish, shellfish and other seafoods. Gastroenteritis is most commonly associated with the consumption of raw

fish, which is a common practice in Japan, or with under-cooked seafoods. Foods cross contaminated by raw seafoods can also be associated with incidents of gastroenteritis caused by this organism (1, 2, 3, 6).

Because studies indicate that Kanagawa-positive *V. parahaemolyticus* generally occur only in low numbers in foods, it has been concluded that mishandling at temperatures that allow growth of the organism is normally a prerequisite of gastroenteritis caused by this organism (4, 6, 7, 8).

Incidence of *V. parahaemolyticus* Food Poisoning

In the UK, food poisoning caused by *V. parahaemolyticus* is rare, infections in the main being acquired abroad. It was first reported in England and Wales in 1972, but only between 9 and 19 laboratory-reported isolates were recorded each year by the Communicable Disease Surveillance Centre (CDSC) between 1980 and 1989 (9). Between 1989 and 1991 there were 69 sporadic cases and over 80% indicated recent foreign travel, with four cases reporting having eaten shellfish while abroad. No cases were reported in Scotland during the 1980s (10).

Other *Vibrio* species (principally *V. cholerae*, other than *V. cholerae* O1) were reported to be the cause of 156 sporadic cases of infection in England and Wales between 1989 and 1991. A similar proportion of these also involved foreign travel. One case of *Vibrio fluvialis* infection was associated with shellfish (10).

As already mentioned, the incidence of foodborne illness associated with this organism is much greater in countries where the consumption of raw fish is common, especially when water temperatures encourage the incidence of vibrios in waters where seafoods are harvested. Thus, in the summer months, the incidence of *V. parahaemolyticus* food poisoning increases in these countries.

In Japan, *V. parahaemolyticus* accounts for over 30% of all annual reported cases of food poisoning. Between 1973 and 1998, a total of 40 outbreaks of *V. parahaemolyticus* infection in the United States of America (USA) were reported to the Centres for Disease Control and Prevention, with >1,000 persons involved (8). They also estimated a 126% increase in the incidence of *Vibrio* infections during the period 1996-2002 (2). This has been partly attributed to the arrival of a new pandemic strain of *V. parahaemolyticus* (O3:K6) between 1996 and 1998. A *V. parahaemolyticus* (O3:K6) outbreak in 1998 was linked to the consumption of raw oysters and involved 416 persons in 13 USA states (11).

Levels of reported *Vibrio* infection have continued to rise in the USA between 2006 and 2009 (24).

Sources

Humans

V. parahaemolyticus can be isolated from the stools of asymptomatic individuals in the summer months in Japan, as a transient part of the faecal flora. It can also be excreted for one or two weeks by individuals recovering from *Vibrio*-associated gastroenteritis.

Animals and the environment

V. parahaemolyticus is a halophile and has been isolated from the marine environment throughout the world. Occasional isolations from freshwater or non-marine fish have been reported, but it is thought that this relates to elevated levels of salt, possibly through pollution (7).

Both the incidence and levels of *V. parahaemolyticus* are higher in the summer months; the organism is infrequently found in waters having temperatures less than 10 - 13 °C. However, the organism may survive the winter months by attaching to chitinous material from plankton (1, 3).

It is important to note, however, that most strains of *V. parahaemolyticus* found in the environment are Kanagawa-negative (1).

Foods

Marine shellfish and fish constitute the most important reservoir of *V. parahaemolyticus*. Freshly caught samples may harbour only low numbers of the organism, although in Japan, in the summer, counts of 10^3 - 10^4/g have been reported. However, most isolates are Kanagawa-negative (7).

Growth/Survival Characteristics of the Organisms in Foods

The most notable characteristic relating to the survival of *V. parahaemolyticus* is its requirement for salt (NaCl) for growth. It is also capable of remarkably rapid growth where conditions are favourable; a

generation time as short as 9 - 13 min has been recorded under optimal conditions (12).

Temperature

The minimum and maximum temperatures reported for growth are 5 °C and 44 °C, with an optimum of 30 - 35 °C. It fails to grow at 4 °C (1, 3, 12).

The organism is easily destroyed by drying, and is sensitive to refrigeration temperatures, declining in numbers during storage (7), but is only moderately sensitive to freezing (3).

Heat resistance

V. parahaemolyticus is not a heat resistant organism and is readily destroyed by cooking at temperatures of >65 °C (3, 6). D-values at 47 °C ranging from 0.8 - 65.1 minutes have been reported. (1)

pH

This organism is tolerant of high pH values, but less so of low pH values; its pH range for growth is 4.8 - 11, with an optimum of pH 7.6 - 8.6 (1)

Water activity/salt

The optimum concentration of NaCl equates to water activity (a_w) of approximately 0.98. *V. parahaemolyticus* will grow at a_w levels as low as 0.922, depending on solute (1).

V. parahaemolyticus is unable to grow in the absence of salt; its upper and lower limits for growth are 8% (although some reports suggest 10) and 0.5% NaCl, respectively, but the optimum concentration is 2 - 4% (1, 3).

Atmosphere

V. parahaemolyticus can grow in either vacuum- or aerobically packed foods (7).

Irradiation

Doses of 3kGy of gamma irradiation is sufficient to eliminate *Vibrio* from frozen shrimps (4).

Summary of Control of *V. parahaemolyticus* in Foods

Seafood harvested from waters of sufficiently high temperature (>10 °C) must be considered to be contaminated with *V. parahaemolyticus*. This organism is able to grow extremely rapidly under conditions of temperature abuse; control, therefore, relies on the strict maintenance of low temperatures.

Adequate cooking of seafood to an internal temperature of >65 °C will destroy any vibrios present.

The prevention of cross contamination from raw seafoods to prepared foods that are likely to be exposed to temperature abuse is also of paramount importance.

The Organism *V. cholerae*

Like *V. parahaemolyticus*, *V. cholerae* belongs to the family Vibrionaceae; it is a Gram-negative, catalase-positive, oxidase-positive, short, straight or curved motile, non-spore forming rod, capable of anaerobic growth.

There are a number of serogroups of *V. cholerae*, but until recently it was thought that *V. cholerae* serogroup O1 was the only cause of the disease cholera. However, in mid-1993, it became apparent that a further serogroup was capable of causing cholera; serogroup O139 (4, 13). This serogroup has caused large outbreaks in India and Bangladesh, with unusually severe symptoms and is responsible for the eighth pandemic of cholera (1, 14).

V. cholerae Illness

V. cholerae causes the illness cholera, which has led to epidemics and pandemics and can be a significant cause of illness and mortality in areas with poor sanitation and hygiene. It is endemic in the subcontinent of India, and in other parts of Asia as well as in Africa. The disease remains a major public health problem in these countries, whereas in western countries the

incidence remains low. The recent epidemic in South America is the first epidemic in South America this century (13, 15).

The incubation period can range from several hours to five days. The ingestion of sufficient numbers of *V. cholerae* can lead to multiplication of the organism in the intestine and production of cholera toxin (CT) that is an enterotoxin. This results in profuse, watery diarrhoea, involving the excretion of large numbers of organisms. Initially, the stool is brown with faecal matter, but thereafter becomes pale gray with a fishy odour. Mucus in the stool imparts the characteristic 'rice water' appearance. Vomiting is often present. Water loss can be extreme, and resulting dehydration and salt imbalance can, in severe cases, lead to death. Death may occur in as high as 50 - 70% of the cases (2). However, other illnesses can be produced by strains that lack the ability to produce cholera toxin. In these cases, mild diarrhoea is common (4, 13, 16).

The infective dose is thought to be usually 10^6 - 10^8, but can be lower under certain conditions (13, 14, 17).

Incidence of *V. cholerae* Food Poisoning

In Europe, between January and October 1994, there were 2,339 reported cases of cholera and 47 deaths. This represented a 30-fold increase compared with the previous year (13).

The Pan Health Organisation estimates that during 1991-1992 there were 750,000 cases of cholera in South and Central America with 6,500 deaths. More recently, there was a marked increase in cholera cases associated with *V. cholerae* O139 in Bangladesh between March and May 2002, when an estimated 35,000 cases occurred (2).

Sources

Humans can be short-term carriers of *V. cholerae*. Studies have also revealed that household animals like cows, dogs and cats can be sporadic carriers of *V. cholerae* O1 (4).

V. cholerae is normally associated with poor hygiene and faecal contamination; person-to-person or waterborne transmission occurs particularly where there is poor sanitation and/or untreated water supplies. However, food can occasionally become contaminated (4, 18).

Fruit and vegetables and other foods can become contaminated by polluted water, the use of 'night soil' as a fertiliser, or through food handlers, the use of contaminated containers, or contamination from flies.

Like *V. parahaemolyticus*, *V. cholerae* is part of the natural marine flora in certain areas, especially estuarine environments. Thus, fish or shellfish may be contaminated if they are harvested from contaminated waters. In these instances, the consumption of raw shellfish can be hazardous.

It is difficult to separate contaminated water from food as a real source of cholera in outbreaks of disease, but several foodborne outbreaks have been reported. These have involved mainly crabs, shrimp, raw fish, mussels, cockles, squid, oysters, clams, rice, raw pork, millet gruel, cooked rice (after three hours without reheating), cooked meat, frozen milk and dairy products, raw fruits and vegetables, and iced candies (4, 13, 15, 18). Other types of foods may also be contaminated with *V. cholerae*, for example, food bought from street vendors, raw seaweed, frozen fresh coconut milk, palm fruit, cooked potatoes, eggs, pasta and spices (2).

Growth/Survival Characteristics of the Organism in Foods

Unlike *V. parahaemolyticus*, *V. cholerae* may not have an absolute requirement for NaCl, although its growth is enhanced by the presence of low concentrations of salt. It will grow in up to 4% NaCl (16).

Its optimum temperature for growth has been reported as 30 - 37 °C, with a temperature range for growth of 10 - 43 °C. Like *V. parahaemolyticus*, it is tolerant of high pH values, but not acid conditions; it has a pH range for growth of pH 5 - 9.6 with an optimum value of 7.6 (13, 21).

The organism can grow in some foods, including seafood, certain cooked foods (where there are minimal competitors) and certain raw vegetables. *V. cholerae* can survive in moist, low-acid chilled foods for 2 or more weeks (it has been reported to survive on shellfish for 2-6 weeks, depending on temperature, and will survive for long periods on frozen shellfish); survival in high-acid foods is usually less than 1 day, and in dry foods less than 2 days (15).

The organism is sensitive to the usual methods of disinfection used in food processing and preparation, including heat, drying and chlorination. It is not a heat resistant organism, being killed by pasteurisation temperatures, and cooking to 70 °C is normally adequate to ensure the destruction of *V. cholerae* (18).

Drying and exposure to sunlight is also an effective means of killing *V. cholerae*. *V. cholerae* can survive domestic freezing and can be found after a long period in a frozen state (2). Treatment of fresh and frozen frog legs with >50 krad of ^{60}Co is known to eliminate *V. cholerae* (4).

Note: Not all strains of serogroup O1 and O139 produce CT, cause cholera or are involved in human illness. Therefore, in assessing public health significance, two critical properties must be determined. Firstly, the production of CT and secondly, the possession of the O1 and O139 antigen (4).

The Organism *V. vulnificus*

This organism does not currently appear to be causing any significant concern in the UK, although some cases have been reported in Europe. It has, however, received considerable attention in the USA because of the high fatality rate (95%). *V. vulnificus* infections have been associated with certain raw seafoods, especially oysters (8, 12, 19).

V. vulnificus Food Poisoning

V. vulnificus is highly invasive, and can cause septicaemia, which has a high associated mortality rate (approximately 60%), amongst susceptible people, through the ingestion of contaminated raw seafood (8). Wound infections can occur if seafood handlers cut themselves while cleaning or harvesting oysters in such a way that the wound becomes contaminated with sea water (4).

The incubation period for septicaemia after ingestion of the organism is generally between 16 and 38 hours, and symptoms most commonly involve fever and chills. Nausea, abdominal pain, vomiting, diarrhoea and hypotension occur less frequently. Progression of the illness can be very rapid, from asymptomatic to death within 24 hours. Secondary lesions often occur, especially in the extremities, which may require major surgical attention (4, 8, 19).

Individuals who suffer septicaemia after the ingestion of *V. vulnificus* almost always have some underlying chronic disease, especially a liver- or blood-related disorder, which may be linked with iron metabolism. Most infections occur in males over the age of 50, during the summer months (1, 4, 19).

Illness caused by *V. vulnificus* may be associated with the production of a toxin or haemolysin, but other virulence factors, including a protease and other enzymes, as well as haemagglutinating activity and a polysaccharide capsule may also be inloved. The role of the capsule in the pathogenesis of human infection appears to be clearly established (4, 12, 20).

Sources

Principal reservoirs for *V. vulnificus* are coastal seawater and brackish water (12).

V. vulnificus has been detected in clams and oysters taken from the Gulf, East and Pacific coasts of the USA, and from around the world. Raw oysters constitute a major food vehicle for the transmission of *V. vulnificus* infection in the USA (20, 21).

Growth/Survival Characteristics of the Organism in Foods

V. vulnificus is a halophile, having an absolute requirement for salt. Its optimum NaCl concentration for growth is 2.5%, and it is able to grow at concentrations of 0.5 - 5.0% NaCl (20) but not at 0% or 8%.

The lack of association between the consumption of cooked oysters and illness suggests that *V. vulnificus* is heat-sensitive. In fact, research has shown that cooking oysters for 10 minutes at 50 °C should ensure the destruction of the organism (22). D-values at 47 °C for 52 strains of *V. vulnificus* average 78 seconds (4).

It is also susceptible to ionisation and high hydrostatic pressure. A dose of 1kGy is known to decrease *V. vulnificus* populations in shell stock oysters by greater than 5 log cfu/g. Higher doses of 1.5 kGy completely inactivate *V. vulnificus* (4).

The optimum growth temperature for this organism is 37 °C, although growth may occur within the range 8 - 43 °C. *V. vulnificus* is sensitive to low temperatures; its presence has been observed mainly during warm months, rarely in cold waters or in chilled seafood, such as iced oysters. However, research suggests that this organism enters a ‘viable but non-culturable state’ in the cold environment, so that failure to isolate *V. vulnificus* from cold samples may not be a reliable measure of its absence (8, 12).

The organism may be a part of the normal flora of certain estuarine environments, and its presence does not appear to correlate with the presence of indicator bacteria (21). Its numbers are related to water

temperature, and a 1994 survey found it to be common in Danish marine environments during an unusually warm summer, indicating that it is probably ubiquitous (23).

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PATHOGENIC *ESCHERICHIA COLI* (VTEC)

Escherichia coli is part of the normal flora of the intestinal tract of both man and warm-blooded animals. The presence of *E. coli* in water or foods, therefore, may indicate faecal contamination, although their consumption may not lead to any apparent ill effects on health. Certain serotypes of *E. coli*, however, may cause diarrhoeal disease, or more serious forms of illness. These can be divided into several virulence groups, described as enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), diffusely adherent (DAEC), enteroaggregative (EAggEC) or vero cytotoxigenic (VTEC) (also described as Shiga-like toxin-producing (STEC)). The last group includes those serotypes described as enterohaemorrhagic (EHEC).

In developing countries, diarrhoea amongst infants and young children caused by different types of pathogenic *E. coli* is a major cause of morbidity and mortality. In developed countries, where standards of hygiene are high, the pathogenic *E. coli* are of relatively minor importance; little diarrhoeal illness has been associated with any *E. coli*, other than 'travellers' diarrhoea', in which enterotoxigenic *E. coli* are important (1). Vero cytotoxigenic *E. coli*, particularly serogroup O157, however, is causing considerable current concern in developed countries. The increasing incidence of *E. coli* O157 infections, including several very serious foodborne incidents in the UK, has increased concerns over the organism in this country. Further details in this book will, therefore, be restricted to this organism - vero cytotoxigenic *E. coli* (VTEC).

VTEC (vero cytotoxigenic *Escherichia coli* or verotoxigenic *E. coli*) was first implicated in infectious disease in 1982, when it was associated with two outbreaks of haemorrhagic colitis (Riley *et al*. 1983) involving hamburger patties in sandwiches. Since then, it has caused and continues to cause considerable concern in developed countries; there have been several serious foodborne outbreaks in the UK and in other developed countries.

The Organism VTEC

VTEC in common with other *E. coli* are facultative anaerobic, Gram negative non-spore-forming, catalase-positive, oxidase-negative, motile, rod shaped members of the family Enterobacteriaceae. It can be broken down into a number of serotypes, that produce their own particular toxin.

The term 'vero cytotoxigenic *E. coli*' (VTEC) encompasses those strains of *E. coli* that are capable of producing one or more toxins that are cytotoxic to Vero cells - a tissue cell culture line established from the kidneys of an African Green monkey. These toxins are known as vero cytotoxins (or verotoxins). They are also sometimes referred to as 'Shiga-like' toxins, particularly in North America (2).

More than 200 VTEC serotypes have been isolated from humans (source: WHO), but in many countries the main serogroup that has been demonstrated to produce symptoms associated with illness caused by VTEC is *E. coli* O157, termed O157 VTEC. The main serotype of concern in this group is O157:H7. O157 VTEC can also cause haemorrhagic colitis and can therefore be described as enterohaemorrhagic *E. coli* (EHEC). Not all VTEC serotypes are EHEC, but other important non-O157 EHEC serogroups associated with foodborne illness include O26, O111, O103 and O145 (3,4).

Recently, antibiotic resistant strains of VTEC have been isolated from foods. Investigation into the relationship between antibiotic resistance and death and growth kinetics in various foods, showed no difference in growth kinetics, but in acid foods (yoghurt and orange juice) the multiple antibiotic resistant strains died off faster (5).

Vero cytotoxigenic *E. coli* Food Poisoning

The organism *E. coli* O157:H7 was first associated with foodborne illness in 1982, in the United States of America (USA). In this incident, it was discovered in stool samples from patients suffering haemorrhagic colitis, who had eaten hamburgers at a fast-food restaurant. It was also isolated from a hamburger sample taken from the implicated lot (6).

Since 1982, VTEC has been increasingly linked with incidents of haemorrhagic colitis (HC - a severe illness involving bloody diarrhoea) and haemolytic uraemic syndrome (HUS - a kidney disease). VTEC infection involving progression to HUS is most common in infants and young children.

Incubation time

The incubation period is unusually variable, and may range from 1 - 14 days, but is typically between 3 - 4 days.

Symptoms

Illness caused by VTEC can take the form of relatively mild diarrhoea only. However, it can cause serious illness, particularly in vulnerable groups. Typical illness begins with non-bloody diarrhoea and severe abdominal cramps. Vomiting can occur. In about half of cases, diarrhoea becomes bloody (HC) by about two days after onset of illness; this may be more blood than stool and may be mistaken for intestinal bleeding. A small proportion of VTEC cases (between 2 and 7%, but up to 30%) may go on to develop HUS. A further condition that may result from infection by VTEC is thrombotic thrombocytopaenic purpura (TTP), an extension of HUS with additional fever and neurological symptoms. TTP mainly affects adults rather than children and can be very serious.

Mortality

VTEC is the leading cause of acute renal failure in children, which can be fatal. Also, fatalities have been reported in elderly patients with underlying medical problems. Those at greatest risk from VTEC are those between 2 and 10 years, and those over 60 years of age. The average mortality rate as a result of VTEC-associated HUS infection is reported as 1% (7, 8).

Infective dose

The infective dose for VTEC is thought to be very low, and has been reported as between 2 - 2,000 cells for *E. coli* O157:H7 (9) and less than 100 cells (Health Protection Agency). Intact packages of salami associated with a foodborne outbreak were found to contain 0.3 - 0.4 *E. coli* O157 cells per gram (10). In another outbreak associated with salami, samples of the incriminated product were found to contain *E. coli* O111 at a level of less than one cell per 10 gram (11).

Foods involved

In recent years, foodborne outbreaks of VTEC infection, particularly by serogroup O157, have become a serious public health problem. Although foodborne outbreaks have occurred in a number of countries, the incidence of infection seems to be greater in North America, the United Kingdom (UK) and mainland Europe. Large outbreaks have also been recorded in Japan and South Africa, and Latin America (particularly Argentina) reportedly has a high incidence. VTEC strains of serogroup O157 were first isolated in the UK in 1983 following an HUS outbreak. Since then, there have been at least 150 general outbreaks in England and Wales up to the end of 2001 (source: Health Protection Agency (HPA)). Epidemiological evidence suggests that many of these resulted from the consumption of contaminated foods, including beef, cooked meat, raw and pasteurised (recontaminated) milk, cheese and raw vegetables. Laboratory reports of O157 VTEC infection rose steadily after 1983, peaking at 1,087 cases in 1997. Numbers of cases have remained between approximately 800 and 1,000 between 1997 and 2008 (source: HPA) (9). Similar increases have been seen in North America. The most serious outbreak to date occurred in central Scotland in late 1996. The outbreak resulted in 512 cases and 22 deaths, and was associated with cooked meats supplied by a local butcher (12). A similar incident occurred in Wales in September 2005 where 157 cases were identified and 1 five year old boy died. The source of this outbreak was traced to cooked meats supplied by one butcher to Welsh schools in several counties.

In North America, VTEC infection has been linked mainly with undercooked ground beef and, to a lesser extent, raw milk. Thus, in the USA and Canada, VTEC is generally considered to be of (dairy) bovine origin. In a major outbreak in January 1993, undercooked, contaminated beef burgers led to an outbreak involving 732 reported cases and four deaths, centred on Washington State (13). However, the most recent outbreak involving VTEC in the USA was 2009, again associated with ground beef products. This resulted in 26 confirmed illnesses and two deaths. Outbreaks associated with spinach (2006) and frozen pepperoni pizza (2008) have also been reported in the USA (Source: Communicable Disease Centre). Seventy six people were infected with VTEC in the USA in 2009 after consumption of raw refrigerated pre-packed cookie dough (Toll House). The manufacturers have since started using heat treated flour in the product, but strongly advise their consumers to bake the product before consumption.

In Japan, a major outbreak (the largest recorded) occurred in 1996, in which over 9,000 people may have been infected, with 10 subsequent deaths. Most of those involved were children and the outbreak was associated with radish sprouts used as a component of school lunches (14).

Other foods that have been associated with outbreaks in several countries include yoghurt, mayonnaise, unfermented apple cider, unpasteurised apple juice, cantaloupe, salami and alfalfa sprouts (15). One hundred and thirty five cases were recorded in Sweden in 2005 after lettuce was irrigated with water from a VTEC contaminated stream.

Incidence of VTEC Food Poisoning

The incidence of VTEC infection in the UK shows a geographical variation. Between 1990 and 1996, the average annual rate in England and Wales was approximately 1.5/100,000, but in Scotland the average rate was over 5/100,000 for the same period. Between 1998 – 2008 the average annual rate in England and Wales remained at approximately 1.5/100,000, while the Scottish rate decreased to approximately 4.0/100,000 therefore remaining above the number of English and Welsh cases (Source: DEFRA Zoonoses report 2008) The incidence in England and Wales increased steadily until reaching a peak of 1,087 cases in 1997. Since then, the number of reported cases remained at approximately 800-1,000 per year until 2002, when only 595 cases were recorded. Reported cases in Scotland averaged 220 cases per year between 1999 – 2008 (source: HPA). The UK generally has a higher reported incidence of O157 VTEC infection than other EU countries.

High rates have also been recorded in the USA and Canada. The US incidence of O157 VTEC infection in 2001 was 1.6 cases per 100,000 people, but this represented a decrease of 21% compared with the previous four years (16). The 'Healthy People 2010' target has been met in the US and cases in 2009 were <1 per 100,000 people (34). In Canada, an isolation rate of 5.2/100,000 was reported in 1987; this increased to 8.8/100,000 in 1989, but has since decreased and the incidence in 1995 was reported as 5.1/100,000 (17). By 2000 the incidence had increased again to 10.85/100,000. Levels by 2002 had dropped to 8.38/100,000 (Source: Canadian Institute for Health).

Sources

Humans

There is evidence of person-to-person spread in such places as institutions and child-care centres, and some secondary transmission from person-to-person may occur during foodborne outbreaks. Some individuals may also act as asymptomatic carriers (3). It is reported that people who are professionally exposed to cattle can develop resistance to VTEC infection (18).

Animals and environment

There is strong evidence that cattle are a major reservoir for O157 VTEC. In the UK, a study reported in 1997 showed that 15.7% of rectal swabs from 4,800 cattle were positive for the organism. It was also found in 2.2% of sheep, but not in pigs or poultry (19), although other studies have isolated the organism from these species. A comparison of strains from cattle and local human cases in the Sheffield area showed a strong correlation (20). Faecal shedding of VTEC by animals appears to be seasonal. A recent study showed that *E. coli* O157:H7 could be isolated from the faeces of 38% of cattle in the spring, but this figure fell to 4.8% in winter (11). Between 2005 and 2006 the UK Food Standards Agency found 3.4% of sheep sent for slaughter at 4 Scottish slaughterhouses to be carriers of *E.coli* O157. The highest risk of carriage occurred between July and September (35).

A wide range of other animals have also been found to excrete VTEC, including goats, horses, dogs, cats, deer, rats and seagulls. *E. coli* O157:H7 has even been isolated from houseflies (21). There have been cases of infection and outbreaks associated with direct contact with animals, often linked to petting zoos.

It has been shown that bovine hides are a significant vector for the transmission of VTEC onto prepared carcasses within abattoirs (22).

There have also been a number of large outbreaks associated with water supplies, particularly wells contaminated with animal faeces. In 2000, a major outbreak in Canada resulted in over 1,000 cases and a number of deaths, and was linked to a contaminated mains water supply (23).

Foods

North American studies have revealed VTEC - including, occasionally, O157 - in a small proportion of various raw meat samples, and indicated a low incidence in raw milk. The food from which *E. coli* O157:H7 has been most frequently isolated is raw ground beef, especially dairy beef. It has been noted particularly in Canada, where the incidence of *E. coli* O157:H7 infection is notably high. Results from the US Department of Agriculture (USDA) microbiological testing programme for raw ground beef products show an increase in the number of positive samples from 0.06% in 1995 to 0.8% in 2001. Of the 171 positive samples recorded from 1994 - 2001, 114 were recorded during the period from 2000 - 2001 (source: USDA Food Safety and Inspection Service).

In the UK, *E. coli* O157 appears to be rare in foods. A survey reported in 1996 showed that O157 VTEC was found in 3.4% of frozen beefburgers sampled and in 2.0% of fresh minced beef, but it was not found in fresh sausages (24). A second survey, carried out in 1996-7, found that O157 VTEC could be isolated from 1.1% of raw beef products and 2.9% of raw lamb products, especially lamb sausages and lamb burgers, although it was suspected that these may have been contaminated by other added ingredients (25).

Survey results of animal carcasses and meats to date generally suggest that the organism in the UK is principally of bovine origin. Thus undercooked beef and raw milk are likely to be the prime 'high-risk' foods in this country. However, the variety of foods implicated in outbreaks indicates that many products may potentially become contaminated. A summer peak in infections also suggests a possible link with barbecued (undercooked) meat products and/or a higher rate of infection in cattle associated with pasture feeding.

Growth/Survival Characteristics of the Organism in Foods

Temperature

Under otherwise favourable conditions, the lowest reported temperature for growth of *E. coli* O157 in laboratory media, is approximately 7 °C, and the highest is 45 °C, with an optimum of 37 °C (2). (Note *E. coli* O157:H7 grows poorly at 44 - 45 °C and does not grow within 48 hours at 45.5 °C. Therefore, traditional detection methods for *E. coli* in foods cannot be relied

upon to detect *E. coli* O157:H7.) Other non-O157 VTEC strains, particularly O26, are also reported to be inhibited by high temperatures (26).

The organism appears to survive well at low temperatures and to resist freezing.

Heat resistance

E. coli O157 is not a heat-resistant organism. $D_{57\ °C}$ and $D_{63\ °C}$ values of approximately 5 minutes and 0.5 minutes, respectively, in meat, have been reported (27). Anaerobic growth, reduced water activity (a_w), high fat content and exposure to prior heat shock may result in higher D-values. The cooking of beefburgers to 70 °C for 2 minutes or equivalent in all parts of every beefburger has been recommended in the UK (28).

pH

The minimum pH for growth of *E. coli* O157:H7, under otherwise optimal conditions, is reported to be 4.0 - 4.4 (29). The minimum value is affected by the acidulant used, with both acetic and lactic acids being more inhibitory than hydrochloric acid (2). *E. coli* O157 is unusually acid-tolerant and survives well at low pH values, as witnessed by its survival in American unfermented 'apple cider' (pH 3.6 - 4.0) and mayonnaise (reported to be pH 3.8) - two apparent vehicles of infection in two outbreaks in the USA - especially at chill temperatures (15).

Water activity/salt

Current published data suggest that *E. coli* O157 grows well at sodium chloride (NaCl) concentrations up to 2.5% and may grow at NaCl concentrations of at least 6.5% (w/V) (a_w less than 0.97) under otherwise optimal conditions (30). A recent outbreak associated with dry fermented meat (a_w approximately 0.9) demonstrated the ability of the organism to survive the fermentation and drying processes used in the manufacture of such products (15). *E. coli* O157:H7 is highly resistant to desiccation (31).

Atmosphere

E. coli O157 is a facultative anaerobe; it grows well under aerobic or anaerobic conditions. Modified-atmosphere packaging (MAP) has little effect on the growth or survival of this organism (32).

Irradiation

VTEC strains do not differ significantly from other Gram-negative pathogens in their resistance to gamma irradiation. Typical D-values of 0.24-0.39 kGy have been recorded in chilled meat and poultry (33).

The use of irradiation to control O157 VTEC in beef products has been proposed in the USA. In 2000, this treatment was permitted by the United States Department of Agriculture (USDA) for chilled and frozen red meat, and maximum dose levels were specified. Irradiated ground beef products have recently gone on sale at some retail outlets.

Summary of Control of VTEC in Foods

Control measures against VTEC are similar to those for other vegetative pathogens, such as *Salmonella* and *Listeria*. The fact that it appears primarily to be associated with bovine sources means that greatest attention should be paid to the avoidance of unpasteurised milk and to the hygiene practices in beef processing. Contamination with faecal material must be minimised; cross-contamination from raw to cooked products must be avoided and adequate cooking should be employed (where appropriate) to ensure the destruction of any VTEC present. As in the case of other pathogens, the HACCP approach should be used to assist in these measures. There is still a need for further research into the effect of food processes on the survival and growth of VTEC.

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YERSINIA

Yersina enterocolitica was initially named *Bacterium enterocoliticum*, and the genus *Yersinia* was then proposed in 1944. However, the allocation of *Yersinia* to the family Enterobacteriaceae was only established in 1964. *Yersinia* includes three well-established pathogens – *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Y. enterocolitica* – and several non-pathogens (1, 2). *Y. enterocolitica* is the most common cause of illness; however, *Y. pseudotuberculosis* may have been the cause of some outbreaks in Europe and Japan (3, 4). This section only considers *Y. enterocolitica*.

The Organism *Y. enterocolitica*

Yersinia are Gram-negative, oxidase negative, catalase positive non-sporing rods (occasionally coccoid) (2). Like other members of the family Enterobacteriaceae, *Yersinia* is a facultative anaerobe that ferments glucose. They are non-motile at 35 - 37 °C, but motile at 22 - 25 °C; however, some human pathogens strains of serovar O:3 are non-motile at both temperatures (1, 2).

There are 11 currently recognised *Yersinia* species but, apart from *Yersinia pestis* (causative agent of bubonic and pneumonic plague), the *Yersinia* species of most significance to human health is *Y. enterocolitica*. Within this species, only certain strains that carry a virulence plasmid are generally considered to be pathogenic. If this plasmid is lost, this renders the strain non-virulent; virulence is also temperature-dependent (3, 5, 6). Elements encoded by the chromosome are also necessary for maximum virulence. Other pathogenic factors of *Yersinia* species include an enterotoxin and the capacity for cellular invasion (2, 3, 7).

There are many serotypes amongst the species *Y. enterocolitica*. Serotypes O:3 and O:9 and, to a lesser extent, O:5,27 are the predominant pathogenic serotypes in the United Kindom (UK) and Europe. In the United States of America (USA), serotype O:8 has been the most commonly reported cause of outbreaks of yersiniosis followed by O:5,27, but has now

been overtaken by O:3, currently the most common serotype worldwide (2, 8).

Yersinia Food Poisoning

Y. enterocolitica has only been recognised as a foodborne pathogen since about the mid-1970s. Yersiniosis is the gastrointestinal illness caused by the consumption of food containing viable pathogenic *Yersinia*.

Many strains of *Yersinia*, including serotypes O:3, O:8 and O:9, are capable of producing a heat-stable enterotoxin. However, this toxin may not play an important part in inducing yersiniosis.

Incubation time

The incubation period may range from 1 - 11 days (2, 5). The illness is usually short-lived, but it can persist for 1 - 3 weeks (9), or even several months if there are complications.

Symptoms

Yersinia can cause a wide range of clinical symptoms, depending on the strain involved, the dose and the age and susceptibility of the host. However, acute gastroenteritis - diarrhoea and abdominal pain - is the most common manifestation of infection.

Babies and children under five are most susceptible to yersiniosis. In babies, children and adolescents, the main symptoms are gastroenteritis and inflammation of the lymph glands; in adults, the predominant symptoms are abdominal disorders and diarrhoea. Complications include arthritis and skin disorders. Pharyngitis is also common in all age groups (2, 6, 7).

Intestinal pain from yersiniosis, especially amongst adolescents and adults, can be so severe as to be confused with appendicitis. Fever - and less commonly, vomiting - can also occur.

Mortality

Death is rare, but can be associated with septic infections amongst persons with underlying disease.

Infective dose

The severity of symptoms is thought to be dose related; however, the actual infective dose is not known. Certain reports suggest that it might be very high in adults, probably $>10^4$ cells (7), but lower in infants and the immunocompromised.

Foods involved

The most frequently reported mode of transmission of yersiniosis has been foods and water. However, the exact role of food in yersiniosis remains unclear.

In most countries milk appears to have acted as a vehicle of yersiniosis more often than any other food. Two outbreaks in Canada in the mid-1970s were associated with the consumption of raw milk. In the US, an outbreak during 1976, which affected 220 grade school children, was linked to the consumption of chocolate milk (3). Reconstituted milk and pasteurised milk have also been identified as the cause of other outbreaks in the USA.

The main exception to milk as the vehicle of infection is in countries where pork is commonly eaten raw, such as in Belgium, where yersiniosis is relatively common (10, 11).

Other foods implicated as sources in outbreaks include soyabean curd (tofu) packed in untreated spring water, and bean sprouts (2).

During the period 1986 - 1988, there were a total of 10 laboratory-reported suspected incidents of foodborne yersiniosis in England and Wales. One – a family outbreak – was associated with the consumption of pork; the other sporadic cases were linked with milk (12).

Incidence of *Yersinia* Food Poisoning

The incidence of yersiniosis is not known, but it is often reported in the cooler regions of Europe and North America (1, 2, 13). It is of high prevalence in Belgium and the third most common bacterial infection in Sweden (6). In certain countries, such as Canada, Holland and Australia, *Y. enterocolitica* has surpassed *Shigella* as a cause of foodborne disease (8, 13, 14).

Laboratory reports of *Y. enterocolitica* infection increased in England and Wales from 45 in 1980 to about 570 in 1989. The reported incidence also increased in Scotland. Since 1989, numbers have decreased in England and

Wales from under 700 in 1991 to 12 (provisional data) in 2006. During the period 1986 - 1988, there were only 10 incidents that were suspected as being foodborne. As with other forms of foodborne illness, the true incidence of infection is likely to be considerably higher than the reported incidence suggests (10).

Sources

Humans

Strains of *Y. enterocolitica* (mostly non-pathogenic) may be carried in low numbers as part of the transient intestinal flora of apparently healthy humans. A food handler was implicated in one outbreak of yersiniosis in the USA. Pathogenic strains have also been isolated from apparently healthy food handlers in Japan (15).

Person-to-person transmission, by the faecal-oral route, of serotypes O:3 and O:9 has been reported .

Animals and environment

The organism *Y. enterocolitica* is ubiquitous; it is present in a wide range of animals, especially pigs. The organism is present in the oral cavity, particularly the tongue and tonsils, lymph nodes, in the intestine and faeces (2). Environmental sources, including water, may act as a direct or indirect vehicle for *Yersinia* spp. However, it is important to note that most of these 'environmental' strains are not pathogenic.

It has been concluded that no food-producing animal other than pigs appears to be a common reservoir of pathogenic *Y. enterocolitica* (the organism being harboured mainly in the throat/tonsil area) (4, 7, 16).

Spread of yersiniosis by handling infected pigs, dogs, cats and rodents has been reported. Fleas may also be implicated (6).

Foods

The role of foods in the transmission of yersiniosis is not clearly understood, because most isolations from foods are through surveys, and not in response to an outbreak, so the isolates do not correspond with those from human illness. Foods found to be contaminated with *Y. enterocolitica* include milk

and non-ripened/non-fermented dairy products, various meats and poultry, seafood, vegetables and miscellaneous prepared foods, including salads. Milk and pork have received most attention in surveys because of their known association with outbreaks (2, 4, 17, 18, 19, 20).

Growth/Survival Characteristics of the Organism in Foods

Temperature

Yersinias are psychrotrophic organisms, being capable of growth at refrigeration temperatures; doubling times at 10 °C are reported as 5 hours and at 1 °C, approximately 40 hours. Extremely slow growth has been recorded at temperatures as low as 0 °C to -1.3 °C. However, the optimum temperature for growth of *Y. enterocolitica* is 28 - 29 °C with the reported growth range of -2 - 42 °C (1, 2, 5). The maximum temperature where growth has been recorded is 44 °C (3, 5).

The organism is quite resistant to freezing and has been reported to survive in frozen foods for extended periods (1, 2).

Heat resistance

The organism is sensitive to heat, being easily killed at temperatures above about 60 °C. D-values determined in scaling water were 96, 27, and 11 seconds at 58 °C, 60 °C and 62 °C, respectively (2). Internal temperatures of 60 °C in beef roasts inactivate up to a million cells of *Yersinia* per gram, whereas 51 °C leaves some survivors (21). Therefore, it should be destroyed by milk pasteurisation and other commonly applied heat processes, such as cooking, boiling, baking and frying temperatures (1).

pH

Y. enterocolitica is sensitive to pH values of less than 4.6 (more typically 5.0) in the presence of organic acids, e.g. acetic acid. *Y. enterocolitica* are not able to grow at pH <4.2 or >9.0. A lower pH minimum for growth (pH 4.1 - 4.4) has been observed with inorganic acids, under otherwise optimal conditions. Its optimum is pH 7.0 - 8.0.

Water activity/salt

Yersinia may grow at salt concentrations up to about 5% (*ca*. water activity (a_w) 0.96), but no growth occurs at 7% (a_w <0.945). Growth is slowed in foods containing 5% salt (5, 7).

Atmosphere

Carbon dioxide exhibits some inhibitory effect on the growth of *Y. enterocolitica*. Vacuum packaging (VP) can slow its growth to a lesser extent. Growth has been demonstrated in vacuum-packed and modified atmosphere packaged (MAP) meats (2, 22).

Irradiation

Y. enterocolitica is among the most sensitive bacteria, needing the lowest radiation dose for elimination (D10 ~0.20kGy) (2).

Summary of Control of *Y. enterocolitica* in Foods

Because refrigeration is relatively ineffective in preventing the growth of *Y. enterocolitica* in foods, measures should be taken to minimise contamination with this organism in foods, especially those that are chilled. Appropriate heat treatments, where possible, should be carried out to ensure the destruction of the organism. Raw pork represents a particular hazard and should be treated as such. Special care should be taken when handling the carcass to prevent cross-contamination.

Additional note:

The mere presence of *Y. enterocolitica* in foods cannot give any real indication of its ability to cause illness; the organism would need to be tested further before there could be any certainty of its public health significance. Nevertheless, its presence in cooked foods indicates inadequate heat processing or post-process contamination and cannot be justified.

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OTHER BACTERIA THAT MAY BE FOODBORNE AND HAVE HEALTH IMPLICATIONS

SHIGELLA

Shigellas are Gram-negative, non-motile, non-spore forming, oxidase-negative, catalase-positive rods with a facultatively anaerobic metabolism (although with some exceptions), belonging to the family Enterobacteriaceae, and are closely related to *Escherichia coli*. There are a number of species involved in the disease shigellosis, but *Shigella sonnei* accounts for the greatest proportion of cases in developed countries. *Shigella flexneri*, *Shigella boydii* and *Shigella dysenteriae* are also important species (1, 2).

Foodborne shigellosis is rare in the United Kingdom (UK) with an average of approximately 1,200 cases reported per year in England and Wales (Source: Health Protection Agency), but no data available as to how many of those cases were as a result of eating contaminated food rather than infection from contaminated water. Shigellosis is a human disease; it is host-adapted to humans, and its transmission is mainly via person-to-person contact by the faecal-oral route. It is not indigenous in foods. However, water, milk and occasionally food can be a vehicle for the organism where hygiene is inadequate, usually in developing countries, and especially amongst children, particularly those under six years of age. In developed countries, transmission can occur through infected food handlers, and it is a major cause of illness in the United States of America (USA). Most foodborne incidents of shigellosis involve poor personal hygiene amongst handlers, especially the failure to observe hand-washing requirements after using the toilet before handling food (1, 3, 4).

Most foods involved in foodborne shigellosis (in the USA) are salads; however, a number of other food types have been implicated, most involving infected food handlers (1, 3, 4).

In 1994, there was an outbreak of *S. sonnei* infection traced to iceberg lettuce imported from Spain, and cases of the illness were detected in several European countries, including the UK (5, 6). The lettuces had been contaminated with faecal material, but the source of the contamination is unknown (6). There has also been a recent *S. flexneri* outbreak in the UK

associated with fruit salad obtained from a supermarket salad bar (7). Another outbreak occurred with fresh parsley in the USA and Canada, traced back to a farm in Mexico (8) and in 2010 over 100 people were infected with *Shigella* after eating at a branch of Subway in Illinois, USA.

Shigellosis can range from an asymptomatic infection (asymptomatic carriers can be a source of the organism) to mild diarrhoea, to dysentery. Blood and/or mucus and pus may be apparent in the stools in severe cases, and other symptoms can include dehydration, vomiting and fever. Shigellosis is highly infectious, involving only a very small infective dose (possibly as few as about 10 cells) (1). The incubation period can range from 12 hours to 7 days, usually 1-3 days. The illness persists for up to 2 weeks.

Shigella is a relatively fragile organism; it is easily destroyed by heat (e.g. 63 °C for 5 minutes) (1); some strains can grow at temperatures between 7 ºC and 46 °C (the optimum is 37 °C); its reported pH range for growth is about pH 5 - 8 (depending on acid type, etc.), but *Shigella* will not survive for long in acid foods, although there is some evidence that it can survive 13 - 92 days in mayonnaise-containing salads, and some cheese products (9). The organism dies only slowly at reduced water activity (a_w) and survives in both frozen and chilled foods (10).

Proper attention to personal hygiene, as well as the control of the temperature of foods, will prevent the contamination and growth of *Shigella* in foods.

AEROMONAS

Aeromonas species are Gram-negative, oxidase and catalase positive, non-spore forming, facultatively anaerobic rods, of variable motility, belonging to the family Aeromonadaceae, although formally within the Vibrionaceae, like *Plesiomonas*. There is still some dispute over the placing of this genus. While it is well known that certain aeromonads can cause infections in both fish and man, the role of *Aeromonas* spp. in foodborne illness remains unclear. However, since the 1970s there has been increasing evidence implicating *Aeromonas* spp. in incidents of human gastroenteritis, and a few incidents indicating a food vehicle (11, 12).

Its purported involvement as a foodborne pathogen is based on several factors: its isolation in faeces from patients suffering food poisoning in the absence of any other pathogen, an immunological response after infection,

work on pathogenicity in laboratory animals, and its presence in the types of foods commonly associated with food poisoning outbreaks.

The three *Aeromonas* species of potential significance in relation to food safety are the motile species of *Aeromonas*: *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* (11, 12).

The most common type of gastroenteritis involving *Aeromonas* is a cholera-like illness (watery stools and mild fever); less common is a dysentery-like illness (blood and mucus in the stools). The illness is usually mild and self-limiting, but can be severe, especially in children, those over 50 years of age, and the immunocompromised.

The ability of a given strain of *Aeromonas* to cause illness appears to be linked with its ability to produce a number of 'virulence factors'. These include relatively heat-sensitive enterotoxins, which may or may not need to be pre-formed in food to cause illness (this is not yet clearly established), β-haemolysin, haemagglutinins and other factors. The ability of aeromonads to produce these virulence factors also appears to be linked with temperature; isolates from chilled foods or water may not be capable of growth or the production of virulence factors at body temperature. Thus foodborne isolates may or may not be capable of causing infection (11, 12, 13).

The main source of *Aeromonas* species is generally accepted to be water (hence the name, *A. hydrophila* - water-loving), including chlorinated and potable water. There have been a number of well-documented waterborne outbreaks, and water may contribute to the contamination of foods with *Aeromonas*. The organism is also widely distributed in nature, in a wide range of animals, including cows, and is occasionally carried by humans (11, 12, 13).

Aeromonas is a very common contaminant in foods, including seafoods, meat and poultry, milk, vegetables and salads. Numbers can occasionally exceed 10^5/g. It would seem, therefore, that *Aeromonas* is commonly consumed with food, with no apparent detrimental effect on health. There are only a few reports of 'food poisoning' from *Aeromonas* in foods, and these have involved mainly prawns or oysters (3, 12).

The infective dose for *Aeromonas* has yet to be established, although it is likely to be relatively high, given the information above.

Aeromonads vary in their ability to grow at different temperatures. Potentially, the organism has a wide temperature range for growth and many strains are psychrotrophic, being able to grow at 5 °C and some as low as 2 °C (11, 12, 13), with an optimum at about 28 °C (14). *Aeromonas* is very heat-sensitive, being readily destroyed by pasteurisation or equivalent heat treatments. At 48 °C, D-values for different isolates heated in raw milk

ranged from 3.20 - 6.23 minutes (11). Under otherwise optimal conditions, it has potential to grow over a pH range of *ca* 4 - 10 (12), and most strains are sensitive to >4.5% sodium chloride (NaCl) (11).

PLESIOMONAS SHIGELLOIDES

Plesiomonas is a facultatively anaerobic Gram-negative, catalase- and oxidase-positive, non-spore forming, mostly motile rod belonging to the family Vibrionaceae, although very closely related to *Aeromonas*. A small number of infections among humans have been associated with this organism, mainly involving raw oysters. It is also naturally present in fresh and marine waters and as part of the natural flora of finfish and shellfish, especially in warmer countries. It has been isolated at low rates (<1%) from healthy humans in Japan, and from various mammals, including cattle and pigs, as well as from birds (3, 15, 16).

Symptoms associated with *Plesiomonas shigelloides* gastroenteritis include diarrhoea, abdominal pain and nausea, with fever, headache and vomiting being less common. The symptoms usually appear within 48 hours, and the duration is usually 1 - 9 days (15, 17).

The major distinction, in practical terms, between *Aeromonas* and *Plesiomonas* is growth temperature; *Plesiomonas* is not a psychrotroph - some strains can grow at 8 °C, but not at 5 °C. (The upper temperature limit for growth appears to be about 45 °C.) Adequate refrigeration, therefore, is effective in limiting the growth of *Plesiomonas* in foods. The organism is also heat-sensitive, being unable to survive heating at 60 °C for 30 minutes (15).

The infectious dose is unknown, but presumed to be $>10^6$ cells (18) and may be higher as 10^9 cells are reported in piglets (28)

Other characteristics of interest include the ability of both clinical and environmental isolates of *Plesiomonas* to grow in 5% NaCl (28) and at pH 4.5 - 8.5 (15, 16).

PSEUDOMONAS AERUGINOSA

Pseudomonads are Gram-negative, motile, catalase-positive, oxidase-positive or negative, strictly aerobic non-spore forming rods, and members of the family Enterobacteriaceae. *Pseudomonas aeruginosa* has been shown to be capable of producing enterotoxins and has, infrequently, been linked with food poisoning. *Pseudomonas cocovenenans* (recently reclassified as *Burkholdaria cocovenenans*) can also produce toxic substances, and has been associated with hypoglycaemia and death from the Indonesian food tempeh bongkrek (19, 20, 21, 22).

Ps. aeruginosa is a classic example of an opportunistic pathogen. It rarely causes problems in healthy individuals; however, in infants, hospital patients and immunocompromised persons, the organism can cause a range of serious illnesses. Thus, infection from *Ps. aeruginosa* can be of particular concern in hospitals and, in infants, profuse diarrhoea can lead to death. Illness from *Ps. aeruginosa* in otherwise healthy adults takes the form of mild enteritis. Intestinal illness from *Ps. aeruginosa* appears to involve a large infective dose ($>10^6$) (19).

The main route of transmission is person-to-person, but *Ps. aeruginosa* can occasionally be transmitted through water or food.

Pseudomonads are ubiquitous in moist environments such as soil, animals, plants and water - their natural habitat is soil, polluted water and sewage. Several surveys carried out in hospitals have indicated contamination of vegetables, meats and frozen foods with *Ps. aeruginosa* (19).

Pseudomonads are sensitive to heat and drying but are resistant to disinfectants (19).

It has been concluded that the presence of *Ps. aeruginosa* in foods does not justify specific attention for healthy people.

OTHER MEMBERS OF THE ENTEROBACTERIACEAE

There are a number of genera besides those better-known members of the Enterobacteriaceae such as *Salmonella* and *E. coli* that may cause ‘opportunistic’ gastroenteritis when ingested in large numbers. These

include *Edwardsiella tarda*, *Klebsiella* spp. and *Proteus* spp. *Providencia* spp. and *Enterobacter* spp. have also been inconclusively suggested as being involved in foodborne enteritis (19, 20), though there is increasing evidence that they may occasionally cause food poisoning (18).

A severe outbreak of gastroenteritis, followed by haemolytic uraemic syndrome, was associated with the consumption of green butter (i.e. butter containing parsley) sandwiches. Isolates of identical verotoxigenic *Citrobacter freundii* were recovered from the parsley and patients' faecal samples (23). As *C. freundii* is widely distributed in the environment, this incident indicates the potential importance of this organism in causing foodborne illness.

STREPTOCOCCI/ENTEROCOCCI

The streptococci are Gram-positive, catalase negative, non-motile, non-spore forming facultatively anaerobic coccoid-shaped bacteria. They are mainly harmless, but a few are pathogenic to humans. These pathogenic species are haemolytic and some produce toxins. Some of the pathogenic types may be foodborne and have been particularly associated with milk and dairy products. However, since the broad application of milk pasteurisation, hazards are largely restricted to places where milk is still consumed raw or to where there has been a failure in pasteurisation. Brief mention will be made of three types of streptococci that have raised or are currently raising questions in relation to food safety (19).

Streptococcus pyogenes

Streptococcus pyogenes is the characteristic species of the so-called Lancefield Group A streptococci (GAS), the conventional serological classification scheme for these bacteria. Its natural reservoir is humans where it can live without causing infection (although it has also been isolated from cases of mastitis in cows), and it is one of the most common bacterial agents associated with upper respiratory tract and skin infections.

Str. pyogenes is a common cause of pharyngitis, and can also cause septic sore throat, tonsilitis, and scarlet fever. Most illness is transmitted via person-to-person contact or through airborne spread. However, these non-gastrointestinal diseases can be carried to other individuals via food. In

addition, some cases of gastroenteritis have been attributed to *Str. pyogenes*; symptoms may be dose-dependent (3, 19, 20).

An extremely large outbreak (involving over 10,000 cases and 200 deaths) occurred in the USA in 1912, when milk handlers suffering pharyngitis transmitted the organism to milk (24), which was not subsequently pasteurised because of equipment failure. Other foods involved in the transmission of scarlet fever or septic sore throat caused by *Str. pyogenes* or similar GAS include seafood and other salads, ice cream, custards, macaroni cheese and sandwiches. These infections commonly involved multiple control failure with infected food handlers, as well as inadequate temperature control. There have also been associations made between skin infections, meat or poultry handling and *Str. pyogenes*.

In most situations, control against infection with *Str. pyogenes* can be assured by food handler hygiene, coupled with thorough cooking/heat treatments where appropriate and by rapid chilling of foods to below 7 °C.

Other streptococci

The Lancefield Group C organism *Streptococcus zooepidemicus* - a cause of mastitis in cows - was reported to cause a milk-borne food poisoning outbreak in humans, involving seven deaths, in the UK in 1984. This occurred after consumption of contaminated unpasteurised milk (20). An outbreak occurred in Finland in 2006 caused by the consumption of goats cheese made from unpasteurised milk (source: Health Protection Agency).

Other types of streptococci have also been implicated in incidents of skin infections amongst meat handlers, as well as with various other extra-intestinal infections, which are infrequently linked with foods. A discussion of these bacteria and the involvement of foods in their transmission can be found in Stiles (19), and Hajmeer and Fung (18).

Enterococci

Probably more familiar to food microbiologists are the enterococci, otherwise known as Lancefield Group D streptococci or faecal streptococci. These bacteria encompass a small number of species, including the species formerly known as *Streptococcus faecalis* and *Streptococcus faecium* (now known as *Enterococcus faecalis* or *Enterococcus faecium*) (3, 19, 20).

The enterococci are normally present in the faeces of mammals, including man, but may also be found in a wide range of environments, such

that their presence in foods may not be a reliable indicator of direct faecal contamination. They are relatively heat-resistant, so they can be the only surviving bacteria (apart from spore-formers) in pasteurised foods. They are also relatively resistant to drying (19).

The role of enterococci in foodborne illness remains uncertain. It is recognised that enterococci can cause a variety of extra-intestinal infections, and they have been implicated in infant diarrhoea in developing countries. They have also been implicated in a small number of apparent foodborne outbreaks, where large numbers of enterococci have been reported in the absence of other better-known foodborne pathogens. In such cases, mild 'food-poisoning symptoms', including nausea, pains and diarrhoea, have been reported. Results from human volunteer studies where large numbers of enterococci have been ingested are somewhat conflicting. It is possible that certain strains are pathogenic under certain conditions, and more studies are needed to improve our understanding of the pathogenicity of foodborne isolates (3, 18, 19, 20).

Enterococci can be found in a wide range of foods, but, as indicated, their significance in foods is not clear. Enterococci have been shown to have a high level of adaptability and since the mid 1980s certain strains of *E. faecium* and *E. faecalis* have emerged that are resistant to the antibiotics vancomycin and teicoplanin. These strains are known as glycopeptide-resistant enterococci (GRE) or vancomycin-resistant enterococci (VRE) (Source: Health Protection Agency).

MYCOBACTERIUM AVIUM SUBSP. *PARATUBERCULOSIS*

Mycobacterium avium subsp. *paratuberculosis* is an aerobic, usually gram-positive, catalase and oxidase-positive, non-motile, non-spore forming curved or straight rod belonging to the family Mycobacteriaceae. It has recently been suggested that *Mycobacterium avium* subsp. *paratuberculosis* (MAP) may be a potential foodborne pathogen. Although it is normally associated with disease in cattle, there is a growing body of evidence that it may have a role in the development of a chronic inflammatory bowel condition called Crohn's Disease. In part, this is because Crohn's disease in humans has a very similar aetiology to Johne's disease in cattle, which is caused by MAP. The link is not currently proven, but concerns that MAP

may survive milk pasteurisation because of the relatively high heat resistance of the organism have led to recommendations for extended high temperature/short time (HTST) milk pasteurisation treatments of 72 ºC for 25 seconds instead of the standard 15 seconds (25, 26, 27), although this enhanced treatment may not completely destroy all strains of the organism.

Limited data is available concerning the prevalence of MAP from environmental sources, although it has been isolated occasionally from water; milk still appears to be the main source of the organism. MAP has also been found in beef when either the meat has been contaminated from the carcass during slaughter or via dissemination of the bacterium into the tissues of infected animals (29). It has been shown that cooking beef to a core temperature of 75 ºC (well done) is the only way to render the meat free of MAP. Lower cooking temperatures, despite having an effect, only reduce the numbers of MAP present (29). MAP has been found to attach to soil after contamination with faecal matter from MAP-infected cattle. It is thought that the organism may attach to the upper layers of the soil thereby becoming available for ingestion to other grazing animals such as sheep and providing a means of transportation of the pathogen to water sources. Contamination of water sources is of great concern as routine chlorine disinfection methods would not eliminate MAP (30).

Ultimate control is therefore by ensuring that the herd is free of the disease.

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FOODBORNE VIRUSES AND PROTOZOA

VIRUSES

Viruses are an important cause of gastroenteritis and, although most illness is caused by person-to-person spread, food plays a part in the transmission of viral gastroenteritis. Many of the incidents of food poisoning that are of 'unknown aetiology' (where no food poisoning bacteria are detected) are likely to be caused by viruses (1). However, the actual role of viruses in the incidence of 'food poisoning' is little understood, mainly because it is virtually impossible to detect foodborne viruses with current techniques.

Viral gastroenteritis was first recognised in a school outbreak in Norwalk, Ohio, in 1969. The virus was detected in stool samples and became known as the 'Norwalk agent'. Since this time, the role of viruses in foodborne illness has become increasingly recognised, although most cases probably go unreported and the incidence of foodborne transmission of illness remains poorly understood (2).

The first confirmed outbreaks of foodborne viral gastroenteritis in the United Kingdom (UK) occurred in the south of England in 1976 and 1977 from contaminated cockles. This was followed by an outbreak of hepatitis A in 1978 in the Midlands and north of England attributed to mussels. In all incidents, the shellfish had been harvested from sewage-polluted waters and were inadequately cooked. Also, viral gastroenteritis arising from the consumption of oysters has been a major problem (2) and it has been reported that razor clams have been involved.

The viruses that are known to be important to food safety (i.e. they are known to be foodborne) fall mainly into two distinct groups: 1) the group that causes gastroenteritis – now correctly termed noroviruses (previously referred to as Small Round Structured Viruses (SRSVs), the Norwalk group, or Norwalk-like viruses (NLV)) and known as the 'winter vomiting virus' due to its seasonality and symptoms; and 2) hepatitis A virus (HAV) - the cause of 'infectious hepatitis' or viral hepatitis.

Foods are occasionally involved in the spread of other viruses such as rotaviruses, tick-borne encephalitis virus, astroviruses, coronaviruses and

enteroviruses (1, 3). However, this publication will only consider noroviruses and HAV.

The Virus

Viruses are unique in nature. They are the smallest of all replicating organisms and are characterised by their ability to pass through filters that retain even the smallest bacteria. They consist solely of a small segment of nucleic acid encased in a protein shell (4).

Noroviruses are human enteric caliciviruses and are able to replicate only in the gastrointestinal tract of humans (4). Hepatitis A viruses belong to the family Picornaviridae (5).

Food Poisoning from Noroviruses

Noroviruses have accounted for 85% of non-bacterial gastrointestinal outbreaks in Europe between 1995 and 2000 (6). The number of laboratory reports of norovirus infection in England and Wales for 2009 was 7,677, 10% of which are estimated to have been foodborne (Source: Health Protection Agency). Testing sensitivity has increased greatly since 2006 which partially explains the increase in the number of cases since then. It should be noted however that norovirus remains non cultivable at this time (30). Two principle genogroups of norovirus, GI and GII exist. GI appear to be mostly associated with food, principally shellfish, and GII with person to person transmission (31). Foodborne gastroenteritis often referred to as 'gastric or stomach flu', caused by noroviruses usually has a rapid (commonly 'explosive') onset of vomiting, abdominal pain and non-bloody diarrhoea, fever, nausea, chills, weakness, myalgia, and headache, accompanied in specific cases by a self-limiting course of illness (1, 4) after an incubation time of about 24 hours (range 15 - 48 hours, depending on the agent; and on dose). Symptoms typically last about 24 hours (range 12 - 60 hours). Attack rates (the proportion of victims amongst those eating an implicated food) are usually high (in excess of 50% (28)). Virus is shed in vomit and in faeces for perhaps a week after onset (1) and are highly infectious. Numbers of virus particles in faecal material or vomit (which is often 'projectile' and uncontrollable) can be very high, so food and environment can easily become contaminated by an infected food handler, especially in catering (4). There is also frequent secondary spread from those infected to family and other contacts, so that the final number of people

involved in an outbreak can be very large (numbers of cases occasionally reaching thousands) (2, 4, 6, 7, 8).

The largest recorded outbreak of norovirus attributable to one restaurant occurred in the UK in 2009. Fifteen percent of diners (529 cases) became ill with norovirus after eating at the restaurant between January and February 2009. In addition 6 cases occurred as a result of secondary spread of the virus to household members, and 6 members of staff tested positive for the virus. The outbreak is believed to have been caused by contaminated oysters and razor clams that were either served raw or inadequately cooked. The outbreak was then exacerbated by ongoing transmission at the restaurant due to staff working when ill or returning to work too quickly after illness (27).

Viral gastroenteritis can occur in all age groups but it is more common in adults than in children. An immunity acquired to norovirus is very short lived.

Food Poisoning from Hepatitis A

Viral hepatitis transmitted by the enteric (faecal-oral) route (1) can be quite mild or even symptomless in children, where it is most common. The severity of the illness is greater in adults and increases with age (2, 5).

The incubation time is normally about 4 weeks (15 - 50 days, median near 28 days) (1, 5), and symptoms involve the gradual development of anorexia, malaise, pyrexia and vomiting, followed later by jaundice (1, 9). Recovery is usually within a few weeks, but may take several months. Death has occurred (particularly amongst the elderly), but the fatality rate is low <1% (2, 5, 7, 9). Infection with Hepatitis A has become unusual in the UK during the 21st century (source: Health Protection Agency).

Viral infective dose

Viruses need a ‘host’ in order to multiply - they are not capable of growing (or producing toxin) in foods. However, they may be extremely infectious, such that only a few particles (perhaps fewer than 10) need to be ingested to cause illness (4, 5, 7, 30).

Foods involved

The main food type involved in reported incidents of illness involving foodborne viruses in the UK - whether hepatitis A or gastroenteritis - is molluscan shellfish, particularly raw oysters, as well as mussels and cockles. These shellfish are filter feeders that inhabit shallow coastal/estuarine waters, commonly near sewage outlets. The molluscs extract particles, including bacteria and viruses, from the large quantities of water passing over their gills. Depuration procedures that may be effective in eliminating bacterial pathogens cannot be relied upon to eliminate viruses, so the consumption of raw or inadequately cooked molluscs can lead to viral disease. The heat treatment of cockles to an internal temperature of 85 - 90 °C maintained for 1.5 min has been recommended for the destruction of HAV (1), and appears to have been effective in preventing outbreaks from norovirus in cooked molluscs (2, 6, 9).

The other group of foods that are associated with foodborne viral disease comprises foods that are handled or otherwise contaminated by infected food handlers, and that are not subsequently cooked, particularly in catering. Salads are especially vulnerable, as well as ice, prepared fruits, such as melons, soft fruits, cold desserts, cakes, sandwiches, green onions, savoury snacks, soups etc., that may be heavily garnished and/or handled during preparation by hands that may be carrying viruses (2, 4, 6, 10).

Sources of Foodborne Viruses

The original source of all foodborne viruses is the human intestine - the contamination of foods with either noroviruses or HAV is ultimately of human faecal origin (1, 8, 9, 11).

Viruses can be carried in sewage-contaminated water, or on fresh produce such as vegetables harvested from soils treated with sewage sludge or polluted water. Faecal or vomit contamination of foods can also take place at the time of preparation or serving by infected food handlers (6, 8, 9, 11).

Noroviruses can be excreted shortly before onset of symptoms, can be present in large numbers in stools and vomit during apparent illness, and decrease rapidly after recovery (usually within 24 hours). HAV can be present in stools for up to 2 weeks before the onset of symptoms, but can be absent by about a week after the onset of jaundice (1, 5, 11).

Human ‘carriers’ of noroviruses or HAV have not been conclusively demonstrated (11).

Survival Characteristics of Foodborne Viruses

Because of the difficulties in studying foodborne viruses, especially noroviruses, very little is known about their survival characteristics in foods. However, norovirus has been shown to remain infective after exposure to pH 2.7 for 3 hours, and has remained intact even after a 30 minute exposure to pH 2 at body temperature (7). The virus may be inactivated by temperature treatments at 63 °C for 30 minutes, 70 °C for 2 minutes and within seconds at 100 °C (4). Also, like most viruses, they are resistant to refrigeration and freezing (1, 8).

HAV is destroyed by heating at temperatures above 85 °C; it is resistant to acidic conditions (pH 3), as well as refrigerated and frozen storage (1, 2, 7, 9, 10).

Summary of Control

The lack of knowledge on these organisms means that the only practical means of control are adequate cooking of foods, prevention of cross-contamination and practising good personal and food hygiene.

Norovirus-infected personnel should be excluded from handling food for at least 2 days after recovery from viral gastroenteritis. The exclusion of infected food handlers is difficult in the case of HAV infection because the shedding of HAV particles in stools may have peaked well before jaundice symptoms become evident. However, it has been recommended that food handlers should be excluded for 7 days after the onset of jaundice in viral hepatitis (12).

PROTOZOA

Protozoa are single-celled organisms. They have compartmentalised organelles and the cysts or oocysts (dependent upon the species); infectious bodies that are shed by an infected host, are environmentally resistant due to their thick cell walls. They are obligate intracellular organisms and do not multiply in the environment with many requiring a vertebrate host in order to complete their lifecycle. Thus studies of food matrices and determination of the safety of some products can be challenging. In addition, protozoa cannot be cultured or cryopreserved in the way that bacteria can.

Furthermore, cysts and oocysts may easily be mistaken, under microscopic examination, for other bodies, such as yeasts or mould spores. For these and other reasons, like viruses, protozoa are probably under-recognised as causes of foodborne and waterborne illness. Nevertheless, these parasites are becoming increasingly identified as a cause of gastroenteritis, especially in the immunocompromised, and as a cause of 'travellers' diarrhoea'.

Protozoa can be blood borne or transmitted via a vector, such as a mosquito. Others are acquired via water and can be found in soil after being shed by an infected host. Some cysts and oocysts may be present in potable water supplies from time to time, and present a problem for those in the food industry who use mains water in the preparation of foods that do not receive further heat treatment. The increased recognition of problems of contamination of water supplies with parasites, and consequent 'boil notices', led to the need to consider additional protection measures (for the purposes of 'due diligence') where mains water is used in food (and beverage) manufacture.

CRYPTOSPORIDIUM

The Organism

Cryptosporidium spp. belong to the family Cryptosporidiidae. They were considered coccidian protozoan parasites. The most important species of *Cryptosporidium* for humans are *Cryptosporidium hominis* and *Cryptosporidium parvum*. *C. hominis* almost exclusively infects humans, whereas *C. parvum* infects humans and livestock (type I exclusively humans and type II, humans and cattle). Its usual host is food animals, especially lambs and calves, where it grows in the intestinal lining and is passed as oocysts (the spore-like transmissible stage, size *ca* 4 - 6 microns) in the faeces. The life cycle (sexual and asexual) is completed in the gut of a single host (13, 14, 15, 16, 17, 29).

Human Cryptosporidiosis

This is often a zoonotic disease, but direct person-to-person spread is common, especially amongst children. In the UK, infection is most common in children under six years old. Epidemiological evidence suggests that

Cryptosporidium may also be transmitted via contaminated water, milk or food (13, 14, 15, 17).

Incubation time

This depends on the size of the infective dose, but is typically 5 - 7 days (range 2 - 14 days).

Symptoms

Typically, symptoms comprise foul smelling, watery or mucoid diarrhoea, which may be accompanied by abdominal pain, vomiting, malabsorption, fever and loss of appetite and weight. Rarely, cryptosporidiosis may involve extra-intestinal organs including the gall bladder, lungs, eyes and vagina.

Mortality

In otherwise healthy individuals, symptoms commonly persist for 2 to 3 weeks and are self-limiting. However, hospitalisation is sometimes required and the illness may be life-threatening to the severely immunocompromised, such as AIDS patients.

Cryptosporidiosis has been associated with increased child mortality in developing countries (15).

Infective dose

The infective dose for humans is not known but is thought to be as low as 10 oocysts, or even lower. (Infectivity studies with lambs have indicated a probable minimum infective dose of one oocyst.) (14, 16)

Incidence of *Cryptosporidium* Infection

C. parvum is responsible for more infections in Europe and Kuwait and *C. hominis* is responsible for most human infections in the rest of the world.

The reported incidence of cryptosporidiosis appears to be fairly steady in England and Wales with approximately 4,500 cases reported each year

between 1989 and 2008 (source: Health Protection Agency). It is not known how many of these can be attributed to water- or foodborne infection (18).

Sources

Humans

Person-to-person transmission is now considered to be common, primarily via the faecal-oral route. Oocysts may be excreted - initially in very large numbers - for from two weeks to two months after symptoms of infection cease. Repeated exposure may lead to immunity and asymptomatic infection; little is known about asymptomatic carriage but it is thought that asymptomatic cases may excrete low numbers of oocysts. Control is therefore reliant on scrupulous personal hygiene (14, 17).

Animals and environment

Cryptosporidium spp. have been reported in more than 40 species of mammals, birds, reptiles and fish. *C. parvum* is not host-specific and may be transmitted to man from a wide range of animals. The parasite develops in the gut and oocysts may be passed in extremely large numbers in the faeces (up to 10^6 daily), in a fully infective form. It is particularly common in young calves and lambs; an association has, therefore, been made between children bottle-feeding lambs and other farm-visit experiences. Pets appear to play a minor role in the transmission of the disease (14).

The disposal of animal excreta (or even human sewage) on farm land, e.g. through the practice of muck and slurry spreading, can lead to the contamination of water sources and supplies, as well as causing the direct contamination of food crops. Unusually heavy rainfall may contribute to the levels of oocysts in water supplies and exacerbate the above effect.

Food

Largely because cryptosporidia cannot be cultured in the laboratory, food has only rarely been linked directly to incidents of cryptosporidiosis. However, raw sausages, offal and raw milk have been suggested as possible vehicles (17) and an outbreak occurred in the USA in 2006 associated with non fermented apple cider. Fresh produce and shellfish that have been in

contact with contaminated water are also possible vehicles of the organism. This is probably an important mode of transmission in travellers' diarrhoea (13, 14, 17).

However, it is generally considered that food is unlikely to be a major source of infection in comparison with animal-human (zoonotic), waterborne or person-to-person transmission.

Water

Oocysts are environmentally resistant and retain their infectious potential for considerable time in moist environments; they have been reported to survive for up to a year in seawater. Current water treatment systems cannot guarantee the complete absence of oocysts in mains water at all times (14, 15).

In Britain, outbreaks of disease considered to be associated with inadequately filtered or contaminated drinking water have occurred in several parts of the country, some outbreaks involving hundreds of confirmed cases. A very large outbreak in Milwaukee, in the United Sates of America (USA), affected an estimated 403,000 people, when the city water supply became contaminated with this parasite (19).

Air may also occasionally act as a vehicle for transmission of *Cryptosporidium*. The inhalation of oocysts held in aerosols in animal processing plants and during certain farming practices (such as muck spreading) may contribute to the occurrence of cryptosporidiosis.

Survival Characteristics of the Organism in Foods

Temperature

Oocysts can survive at refrigeration temperatures, but are killed by freezing (below -20 °C) (14).

Heat resistance

Oocysts are not heat resistant, being sensitive to holding for 20 minutes or more at temperatures above 45 °C (14); they are readily destroyed by pasteurisation or heat treatments equivalent to 5 - 10 minutes at 65 - 85 °C

(15). In the event of a 'boil notice', water need only be raised to boiling point and allowed to cool; a prolonged holding time is not required.

The organism is sensitive to desiccation, requiring moisture for survival. Air drying for a few hours at room temperatures should ensure complete destruction of any oocysts present. Oocysts do not survive freeze drying (14).

pH, water activity and atmosphere

Little is known about the effects of pH, water activity (a_w) and gas atmosphere on the survival of *Cryptosporidium* oocysts. However, extremes in pH appear to have a significant effect on survival.

Summary of Control of *Cryptosporidium*

As indicated, these parasites are not able to grow in food or water, requiring an animal or human host. However, the oocysts may remain viable outside the host for long periods under cool, moist conditions. They are also resistant to most disinfectants, such as chlorine; prolonged exposure to between 8,000 and 16,000 parts per million (ppm) chlorine may be necessary to kill oocysts. Filtration is necessary for their removal from water - 1 micron pore size or slow sand filtration with flocculation is recommended. Alternatively, an appropriate heat treatment, or treatment with 10 vol. hydrogen peroxide, chlorine dioxide, or ozone (e.g. 2 ppm for 10 minutes) may represent options, but each has its practical limitations. Irradiation appears to require exposures greater than those achievable by conventional treatment (14, 15).

GIARDIA

Like *Cryptosporidium*, *Giardia* causes gastroenteritis in humans; in fact it is now considered to be one of the leading protozoal causes of gastroenteritis worldwide affecting millions or people via sporadic and epidemic outbreaks (29).

The Organism

The species that is known to be pathogenic for man and animals is *Giardia lamblia* (also known as *Giardia intestinalis* or *Giardia. duodenalis*). *Giardia* spp. are binucleate flagellate protozoa, with both trophozoite (the active motile stage in the protozoan life cycle, 10 - 20 microns in size) and cyst (4 - 11 micron) stages. This organism is capable of infecting more than one host species. The importance of sheep and cows as sources of *Giardia* in the UK is not known, but it has been demonstrated in these animals, especially in calves and lambs. Cats and dogs may also be infected with *Giardia*, although the significance of this for humans is uncertain.

Giardiasis

Infection with *Giardia* is generally associated with poor hygienic conditions, including poor water quality (17).

Infection results from the ingestion of cysts, which may remain viable in cool, moist environments for long periods outside the host (13, 16, 18, 20).

Incubation time

Symptoms occur after 1 - 4 weeks and generally last for about 2 weeks (range 5 days to several months if untreated), during which there may be shedding of cysts (cysts appear in stools after 3 - 4 weeks) and, less commonly, trophozoites in the stools.

Symptoms

Symptoms of illness include diarrhoea, malabsorption, abdominal pain, flatulence, loss of appetite and sometimes fever. Hospitalisation is occasionally required. Infection may also, commonly, be asymptomatic.

Mortality

Mortality has been reported only twice.

Infective dose

The infective dose is 10 or fewer cysts. Trophozoites are virtually non-infective owing to their susceptibility to gastric acid (13, 16, 18).

Sources

Three routes of transmission have been identified: food, water and person-to-person transmission. Only a small number of outbreaks, none of which has been identified as being foodborne, have been reported in the UK. These waterborne outbreaks were caused by post-treatment contamination of the water with sewage or faeces (18, 20). Likewise, in the UK, it is not yet clear whether potable water is an important vehicle for the transmission of *Giardia* (18, 21, 22) although *Giardia* cysts are resistant to standard chlorine treatment.

Child-care facilities, where poor personal hygiene is coupled with close physical contact between children, are a major site of person-to-person transmission.

In the USA, reported foodborne transmission, generally involving inadequate personal hygiene of exposed/infected food handlers, has implicated noodle and fruit salads, canned salmon and taco ingredients (17, 23).

In spite of the fact that shellfish could concentrate *Giardia* cysts, to date, molluscs have not been involved in foodborne outbreaks. They could be a potential source of foodborne outbreaks though, especially when eaten raw (20).

Incidence of *Giardia* Infection

Outbreaks of giardiasis have been reported most often in the USA, where it is the most commonly recognised cause of waterborne outbreaks. It is estimated that 15% of the U.S population is infected with this organism (23).

In the UK, *Giardia* is rarely associated with large outbreaks, usually being reported from numerous apparently unconnected family or group cases. The incidence rose from 4,613 cases in 1983 to about 7,000 cases in 1992, but has since declined to just under 3,000 cases in 2006 (Source: CDSC).

Growth and Survival Characteristics of the Organism in Foods

Giardia cysts are easily killed by boiling, but they can survive freezing for a few days. They are, however, resistant to low pH values, above about pH 3. The cysts are susceptible to inactivation by ozone and halogens; however, the concentrations of chlorine used for drinking water may not inactivate *Giardia* cysts. Inactivation by chlorine requires prolonged contact time and filtration.

OTHER PROTOZOA

This section deals with other protozoa that may be foodborne or waterborne and have health implications.

Entamoeba histolytica

Entamoeba histolytica (cyst size 10 - 20 micron), is primarily a human parasite; dogs, cats and other mammals have been reported to be infected only rarely. The organism is anaerobic requiring glucose or galactose as its main respiratory substrate (23, 24).

Incubation period is 2 - 4 weeks. Initially, infection causes amoebic dysentery (bloody or mucoid diarrhoea). Later, symptoms consist of abdominal pain, fever, severe diarrhoea, vomiting and lumbago; it may resemble shigellosis. Pregnant or nursing women may experience fulminating amebiasis with ulceration of the colon and subsequent toxicity. Asymptomatic infection is more common, but is still accompanied by cyst shedding. There appear to be pathogenic and non pathogenic 'variants' of *E. histolytica*. These are morphologically indistinguishable, but it is now thought that they may represent different species (24). *E. histolytica* is estimated to be responsible for 50,000–100,000 deaths per year worldwide, however, less than 100 cases were reported per year in England and Wales in 2005 and 2006 (source: Health Protection Agency).

If trophozoites (12 - 60 μm) of pathogenic *Entamoeba* enter the bloodstream, they may enter the liver (or, rarely, other organs), with serious consequences (13, 23, 24).

Transmission of the cyst is primarily through the person-to-person faecal-oral route. However, food or water can become contaminated with cysts by

food handlers, where human excrement is used as fertiliser for crops, or where foods that are not further cooked are exposed to sewage-polluted water (encysted forms can survive as long as 3 months in sewage sludge) (13, 23).

The cysts are less resistant to chlorine than *Cryptosporidium*, and are also sensitive to heat and freezing. As with *Giardia*, trophozoites are virtually non-infective (13).

Toxoplasma

The coccidian parasite, *Toxoplasma*, contains one species, *Toxoplasma gondii*. Its definitive host is the cat family but nearly all warm blooded animals can be infected and serve as intermediate hosts. Oocysts may be excreted by cats in large numbers and may survive for over a year under cool, moist conditions. Initially, they are not infective, but become infective after 24 - 48 hours exposure to air. Human infection can follow the ingestion of as few as 100 oocysts (incubation period in adults is 6 - 10 days while in infants it is congenital); waterborne outbreaks have also been associated with *Toxoplasma*. Other animals, such as livestock (sheep, goat, chicken and pig), can become infected through exposure to pasture or feed that has been contaminated by cat faeces. Human infection can then follow ingestion of raw or undercooked meat from such animals when they enter the food chain (13, 21, 23, 25).

It is thought that foodborne infection in man is uncommon. The parasite is sensitive to heat (>61 °C for 3.6 minutes) and freezing (-13 °C) (20). However, handling raw meat and the consumption of raw meat dishes may represent a hazard, particularly to pregnant women as it can be passed on from mother to child, *in utero*. (20, 23, 25). It is estimated that between 7 - 34% of people in the UK have been infected with *T. gondii*, however, the vast majority of these people will not have noticed any symptoms (source: Health Protection Agency).

Infection in humans can be asymptomatic or can occur as a mild ‘flu-like’ illness with rash, headache, muscle aches and pain and swelling of the lymph nodes However, congenital infections or infection in the immunocompromised can be serious. Latent, asymptomatic infections can become active and life-threatening upon loss of immunocompetence (due to medications or disease, especially AIDS) (23).

Balantidium coli

Balantidium coli are ciliated protozoa (70 µm diameter) associated with a variety of hosts, especially pigs and non-human primates. Although found worldwide it is not a particularly prevalent organism. Infection in man follows ingestion of cysts (45 - 65 microns in size); symptoms include diarrhoea or dysentery, tenesmus, nausea, vomiting, anorexia and headache. Insomnia, muscular weakness and weight loss has also been reported. It can also cause ulcerative colitis. Ulcers differ from those caused by *Entamoeba histolytica* in that the epithelial surface is damaged, but with more superficial lesions compared to those caused by amoebae. The infection can be transmitted from person to person or via food (13, 20, 24).

Sarcocystis

Sarcocystis are predominantly coccidian parasites of cattle and pigs belonging to the family Sarcocystidae. Infection in man is acquired by the consumption of raw or undercooked meat (especially beef or pork) that contains the oocysts. Studies have shown that symptoms occur within 3 - 48 hours (depending on the species) and consist of abdominal pain, fatigue, dizziness, nausea, vomiting, diarrhoea and malaise. Muscle ache is another symptom associated with Sarcocystis infection (13, 23, 26).

Microsporidia

Microsporidia are obligate intracellular spore-forming parasites found in mammals, fish, crustaceans and insects. This parasite is transmitted by the faecal-oral route by means of small spores (*ca* 1 micron); the source is not known but the faecal excretion route provides the potential for water- or foodborne transmission. Infection occurs mainly in AIDS patients with enteritis being the most common clinical manifestation of microsporidiosis. Diarrhoea is typically chronic and intermittent, loose to watery, large volume, and non-bloody. Anorexia, weight loss and dehydration are common, and abdominal cramping, nausea and vomiting may occur. Other clinical features include keratoconjunctivitis, mycositis, nephritis, hepatitis, sinusitis and pneumonia. (20).

Cyclospora

Cyclospora spp. are coccidian protozoa that belong to the family Eimeriidae. These parasites are common in a variety of mammals and cause disease when infectious oocysts are ingested; although the definitive host and the infective dose, for the species infecting man, is not yet known. The route of transmission is faecal-oral. Infection in otherwise healthy individuals can cause diarrhoea, malaise, lack of energy and appetite, and weight loss lasting for 4 - 8 weeks. Incubation period ranges between 2 and 11 days. Infection occurs most commonly in those travelling to developing countries. Infections have been reported from such places as Nepal, India, Pakistan, Indonesia, Southeast Asia, Papua New Guinea, Middle East, North Africa, the United Kingdom, the Carribean, the United States, and Central and South America, although the true prevalence in any population is unknown. There is some evidence for waterborne transmission but this is controversial. There have been a number of well documented outbreaks in the USA associated with imported fresh produce like berries, basil, lettuce and snow peas. (20, 23, 26).

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FOOD-SPOILAGE BACTERIA

INTRODUCTION

Microbial Food Spoilage – a Brief Overview

Food spoilage can be defined as any change in the characteristics of a food that renders it unacceptable. The cause of such a change may be a physical process, such as moisture loss, a chemical process, such as rancidity, or a biological process, such as insect infestation or microbial action. In some cases, spoilage may be the result of a combination of two or more of these. However, it can be said that the most common cause of food spoilage is microbiological activity.

The economic cost of microbial food spoilage is difficult to estimate, but it is certainly large. Despite the benefits of modern preservation and storage technology, considerable quantities of food have to be discarded each year as a result of the actions of micro-organisms. Even so, spoilage receives relatively little attention in comparison with food safety, and the processes and interactions involved are often not well understood. Microbial food spoilage is also important in many foods as a warning of the possible growth of pathogens, and, although the difference between spoiled and unsafe foods may be clear to microbiologists, it is worth remembering that the consumer does not necessarily make such a distinction. In any case, products that spoil before the end of their shelf-life may have to be withdrawn from sale, since the consumer will not buy spoiled food. A scan of recall notices in the press will reveal that a high proportion of the recalls due to microbiological problems are concerned with spoilage, not food pathogens.

Microbial food spoilage is usually due to the presence, survival and eventual growth of bacteria, yeasts, or moulds in a food product. Spoilage may then be apparent as visible growth, discoloration, gas production, or changes in texture, but is more usually due to ‘off odours’ or taints from the by-products of microbial metabolism.

There are few foods that are inherently resistant to microbiological spoilage. Frozen foods will not support microbial growth, and neither will

products with very low water activities, although adequate storage conditions are essential to maintain low moisture levels. Consumers now favour lightly preserved and minimally processed foods; microbial spoilage is therefore a potential problem with most products. Whether spoilage will occur, and how rapidly, are essentially questions of microbial ecology and are determined by a combination of environmental and other factors. In fact, there are now more instances of spoilage in these types of foods, and raw ingredient quality and processor hygiene are now more important than ever.

Spoilage of unprocessed foods

Unprocessed foods, such as meat, fish, raw milk or vegetables, usually support a varied, natural microflora. Many species are likely to be present, and some of these will be able to exploit the environment provided by the food more effectively than others. These organisms will eventually predominate, even though they may have been only a minor component of the initial population, and will normally become the major causes of spoilage. Since the initial population of unprocessed foods is naturally diverse, these principal spoilage species are always likely to be present, and, for this reason, the composition of the microbial population at the point of spoilage is quite predictable. The microbial populations that develop during the spoilage of many unprocessed foods have been extensively studied and reported. For example, large numbers of *Pseudomonas* spp. are almost always found in spoiled, chilled, fresh meats.

Sometimes, however, the major microbial causes of spoilage do not predominate. For example, it has been estimated that the main species of spoilage bacteria in chilled fresh fish make up only about 30% of the total population when spoilage becomes apparent. The predominant organisms in this case are not really involved in spoilage. This is a reflection of the particular nature of the biochemical changes that cause the fish to become unacceptable. The interactions between the spoilage microflora and the biochemical processes that occur during spoilage are not fully understood for many foods.

For some foods, it has been possible to identify specific spoilage organisms (SSOs) within the general spoilage population. For example *Photobacterium phosphoreum* has been reported to be primarily responsible for spoilage in certain fish products, although it may not predominate. For many foods, SSOs have not yet been found, but this is a potentially

interesting area for future research, since it may be possible to target the SSO in a particular product as a means of extending shelf-life.

Microbial spoilage does not usually become apparent until the population reaches quite high levels (typically $>10^7$ per gram for bacteria). This is because individual bacteria have only a very small potential to cause biochemical changes in foods, and the collective activity of a large population is required before those changes become detectable. However, there is evidence that this is not always the case. For example, relatively small populations (approximately 10^3 per gram) of *Alicyclobacillus acidoterrestris* have been shown to be sufficient to produce a taint in fruit-based beverages. Very small starting populations of *Zygosaccharomyces* spp. can also grow to sufficient levels to produce gas and cause problems in beverages.

Spoilage of processed foods

Processed foods are likely to have been subjected to treatments designed to delay the onset of spoilage, such as pasteurisation, modified-atmosphere packaging or drying. In other words, the factors that affect the growth of micro-organisms have been manipulated. Very often this results in eventual spoilage that is less immediately predictable. The initial microbial population of a processed food may be reduced and potential spoilers may have been eliminated (e.g. by heating). Therefore, other organisms, not normally regarded as spoilers, may be able to predominate and cause spoilage. For example, heat-resistant lactobacilli may cause spoilage of vacuum-packed, cooked meats, and psychrotrophic bacilli may spoil pasteurised milk products.

Spoilage bacteria

Although spoilage of foods by yeasts and moulds is important, particularly in acid and low-moisture products, bacterial spoilage is potentially a more serious problem with highly perishable products, especially chilled foods. Bacterial spoilage may be caused by a diverse range of species, may give rise to varied spoilage symptoms, and is not always predictable in processed foods. Therefore, it is important to understand something of the physiology and ecology of potential spoilage bacteria. This knowledge provides a possible basis for the identification of potential spoilage problems in new products, and the investigation of problems with existing ones. This section

of Micro-Facts is intended to provide concise summaries of the key characteristics of a range of important spoilage bacteria.

In some cases, very little has been published about the attributes of some of the bacterial species involved in spoilage. Non-pathogenic bacteria in foods have been much less extensively studied than those that cause food poisoning. Nevertheless, the following pages represent an attempt to compile the key points about each genus, as far as is currently possible.

ACETIC ACID BACTERIA

The Organisms

Acetic acid bacteria are aerobic, Gram-negative, catalase and oxidase-negative, non-motile and non-sporeforming rod or ellipsoidal-shaped cells that can typically oxidise ethanol to acetic acid. They belong to the family Acetobacteraceae comprising 14 genera, of which *Acetobacter*, *Asaia*, *Acidomonas*, *Gluconacetobacter*, *Gluconobacter* and *Kozakia*, have been associated with food. Some species of *Gluconacetobacter* (a recently described genus) were formerly classified as *Gluconobacter* or *Acetobacter* species.

Acetic acid bacteria have been defined as bacteria capable of acetification, i.e. the aerobic conversion of ethanol to acetic acid. *Acetobacter*, but not *Gluconobacter*, can cause over-oxidation of formed acetic acid, to produce carbon dioxide and water.

Key Species in Foods

Acetobacter spp. and *Gluconobacter* spp. are well known contaminants of alcoholic beverages, and *Gluconobacter* is often associated with soft drinks and juices. *Acetobacter* and *Gluconobacter* are also associated with fruit spoilage problems, such as pink disease in pineapples, and rots in apples and pears.

Acetic acid bacteria are used commercially in the vinegar production industry (usually *Acetobacter* spp.).

More recently, the newly described *Gluconacetobacter sacchari* and *Asaia* spp. have been isolated from fruit-flavoured bottled water.

Sources

Environment

Acetic acid bacteria are widespread in nature and their natural habitats are sugared and alcoholised niches such as flowers and fruit. *Gluconobacter* have been isolated from the nectar bearing parts of flowers, as well as from bees and their hives. *Acetobacter* can also be found in garden soil and canal water. Although both *Acetobacter* and *Gluconobacter* have been isolated from fruits, *Acetobacter* are usually found when fermentation is more advanced because of their preference for alcohol-enriched environments.

Apart from flowers and fruit, *Asaia* have also been found in fermented glutinous rice, and *Gluconacetobacter* spp. have been isolated from various plant materials such as sugar cane and coffee plants.

Food

Gluconobacter and *Acetobacter* are associated with alcoholic beverages such as beer and wine. *Gluconobacter* spp. have been isolated from low pH, high sugar soft drinks and juices, as well as fruit.

Spoilage Characteristics

In soft drinks, particularly still drinks, spoilage by acetic acid bacteria classically causes turbidity in the product and a characteristic sour odour. In these products, spoilage by acetic acid bacteria is usually caused by *Gluconobacter* spp. because of their preference for high-sugar environments. Sometimes off-flavours can be the only indication that spoilage has occurred and some strains can be present in high numbers but have no effect on the taste or odour of the product. Turbidity and off-flavours in fruit flavoured bottled water caused by *Asaia* spp. and *G. sacchari* have also been described.

In beer, wine, sake and, to a lesser extent, cider, acetic acid bacteria can cause vinegary off-flavours, turbidity, discoloration and ropiness (or slime) as a result of extracellular cellulose formation. The surface contamination they cause is often apparent as an oily or mouldy film. *Acetobacter* is of the most importance in these products because of its preference for alcohol-enriched niches and, in wines, *Acetobacter pasteurianus* can cause a vinegary or a mousy, sweet-sour taste.

A visual defect in stored vinegar, known as 'mother of vinegar', is caused by *Acetobacter* species.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

The optimum for growth of acetic acid bacteria is 25 - 30 ºC. However, some *Acetobacter* strains are recorded as growing at 37 ºC and above, and certain *Gluconobacter* strains may be able to grow at temperatures as low as 4 ºC.

Acetic acid bacteria are not particularly heat-resistant, and are not reported as surviving mild pasteurisation processes.

pH

The optimum pH for growth of acetic acid bacteria is around pH 5 - 6, with a minimum pH for growth in the range of 3 - 3.5, for most acetic acid bacteria. Many *Gluconobacter* strains can remain viable at pH 2.4. *Acetobacter acidophilus* can grow at pH 2.4 and some industrial strains of *Gluconobacter* can tolerate acetic acid concentrations up to 13.5%.

Water activity

Although growth of these organisms does not occur at low water activity (a_w), they may survive in concentrates and cause spoilage once the concentrate is diluted. The minimum a_w for growth is likely to be dependent on many factors including the solute present. As previously noted, *Gluconobacter* spp. have a preference for high sugar niches, whereas *Acetobacter* favours alcohol-enriched environments.

Atmosphere

Acetic acid bacteria are obligate aerobes and are usually not a problem in highly carbonated beverages because of their sensitivity to elevated levels of dissolved carbon dioxide.

Preservatives and biocides

Strains of *Gluconobacter* and *Acetobacter* resistant to sorbate and benzoate have been reported, and acetic acid bacteria have the potential to cause the spoilage of products that rely solely upon these preservatives for their stability.

Control

The main means of control of the acetic acid bacteria is the use of mild heat treatments. The use of preservatives can prove effective, particularly in combination with other hurdles such as reduced a_w and titratable acidity. However, resistant strains can develop. Care should be taken in choosing raw materials of good microbiological quality and to ensure good hygiene in processing equipment when producing products that may be susceptible to acetic acid bacteria spoilage.

ACINETOBACTER

The Organism *Acinetobacter*

Acinetobacter spp. are Gram-negative, non-motile, non-sporeforming rods. They can become spherical during the stationary phase of the growth curve. They are aerobes, with a strictly respiratory metabolism, and are oxidase-negative and catalase-positive. The genus *Acinetobacter* is quite heterogeneous, belonging to the Moraxellaceae family.

Key Species in Foods

Currently there are 17 genomic species, but the species that have been most commonly isolated from foods include *Acinetobacter lwoffi*, *Acinetobacter johnsonii* and *Acinetobacter calcoaceticus*.

Sources

Environment

Acinetobacter spp. are common and widely distributed in soil, water and sewage. They are also found as components of the normal skin microflora of humans and animals.

Foods

These organisms may be isolated from a wide variety of foods, particularly chilled fresh meat, fish, eggs, poultry, milk and vegetables. They have also been found to cause spoilage of soft drinks.

Spoilage Characteristics

Acinetobacter spp. are an important part of the normal spoilage flora of fresh meat stored under aerobic conditions. They predominate under conditions of temperature abuse, but are less important at chill temperatures, where Pseudomonads predominate. They produce 'off odours' as a result of amino acid metabolism and may also cause souring of ham. They are common spoilage organisms of poultry and shellfish and may cause 'colourless' rots in eggs.

Spoilage of soft drinks by *A. johnsonii* is characterised by white filamentous growth.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Some strains are psychrotrophic and growth at 4 °C may occur, but *Acinetobacter* spp. do not compete well under these conditions. Strains grow well between 20 °C and 30 °C, the optimum temperature for most strains being 33 - 35 °C.

pH

It has been reported that growth is reduced on meats with a pH of <5.7 and the optimum pH for growth seems to be 5.5 - 6.0. In some cases *Acinetobacter* has been able to tolerate low pH; growth has been reported in soft drinks with pH 3.3.

Water activity

A minimum a_w value permitting growth of 0.96 has been reported for a strain identified as *Acinetobacter* spp. but a value as high as 0.99 has been reported for a meat-spoilage isolate. No *Acinetobacter* were isolated in a study on fish fillets treated with 10% brine.

Atmosphere

Acinetobacter spp. are aerobic organisms and are not normally involved in the spoilage of vacuum-packed or similar products. Despite this fact they have been isolated in low numbers from chilled vacuum-packed meats.

Preservatives and biocides

There is very little published information on the effect of antimicrobials on *Acinetobacter* spp. They are known to withstand exposure to 0.7% hydrogen peroxide and 10 parts per million ozone.

Control

Acinetobacter spp. are normally present on the surface of fresh meat and poultry carcasses, and may cause significant spoilage under aerobic conditions, particularly if the temperature is allowed to rise above 8 °C. Spoilage is therefore best controlled by careful temperature control. As they do not tolerate high levels of salt and are not resistant to heat, growth can be controlled by increasing the salt concentration and by pasteurisation. Prevention of cross-contamination from the skin during processing, by good hygienic practices, is also important.

ALICYCLOBACILLUS

The first *Alicyclobacillus* species was isolated in 1982 and was initially classified as a *Bacillus*. It has since been reclassified into the new genus *Alicyclobacillus* partly because of the presence of ω–alicyclic fatty acid in their cell membrane.

The Organism *Alicyclobacillus*

Alicyclobacillus spp. are Gram-positive, thermophilic and acidophilic sporeforming (terminal or subterminal with or without swollen sporangia), catalase-positive, oxidase-negative, aerobic, motile rods belonging to the family Alicyclobacilliaceae. *Alicyclobacillus* spp. are often referred to as thermophilic acidophilic bacteria (TAB), particuarly in the soft drinks industry.

Key Species in Foods

Of the currently recognised seventeen species of *Alicyclobacillus* only five species, *Alicyclobacillus acidoterrestris*, *Alicyclobacillus pomorum*, *Alicyclobacillus herbarius*, *Alicyclobacillus acidocaldarius* and *Alicyclobacillus acidophilus* have so far been isolated from fruit juices and beverages. Of these *A. acidoterrestris* is the most important species as it is widespread, has strong spoiling potential and is the strain most commonly found associated with spoilage incidents.

Although *Alicyclobacillus acidocaldarius* has been isolated from foods, it is thought to be mainly associated with hot springs.

Sources

Environment

Soil is the probable natural habitat of strains of *Alicyclobacillus* associated with food spoilage.

Foods

The organism has been isolated from low pH (e.g. <3.8) canned or hermetically sealed products such as fruit juices, juice-based beverages and tomato-based products. It has also been isolated from more diverse shelf-stable products, including spoilage of carbonated fruit drinks and iced tea. It is probably present on the surface of whole fruits as a result of soil contamination. It has been found in white grape juice but never red, the reasoning thought to be that phenolic compounds in the red grape skins inhibit the organism.

Water is also a possible source of contamination; in particular, water used for beverage manufacturing processes and some sweeteners, for example high fructose corn syrup.

Spoilage Characteristics

Spoilage by *A. acidoterrestris* can be very difficult to detect visually. Spoilage takes the form of an 'antiseptic', 'medicinal' or 'phenolic' taint in fruit beverages, particularly in apple-based, pasteurised, shelf-stable fruit drinks. The offensive taste or odour has been attributed to guaiacol (formed from vanillin or tyrosine), bromophenols (2,6-dibromophenol) and chlorophenols (2,6-dichlorophenol); these are potent tainting compounds. A slight sediment may also be present, but no gas is produced and therefore no obvious swelling of the packaging (flat-sour spoilage). Spoilage can result from very low initial levels of contamination, and taints can be produced by populations as low as 10^3 cfu/ml.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

A. acidoterrestris is essentially a thermophile, but isolates seem able to grow over a very wide range of temperatures; growth at temperatures as low as 12 ºC and as high as 80 ºC has been reported, but growth is very slow at the extremes of this range. The optimum growth temperature seems to be 42 - 60 ºC.

The spores of the organism are very resistant to elevated temperature and are able to survive pasteurisation processes applied to fruit juices. D-values at 90 ºC of 16 - 23 minutes have been reported for a spoilage strain. Heat

resistance is also much affected by the pH of the heating medium, and a small increase in pH may produce significantly higher resistance.

pH

A. acidoterrestris is an acidophile and is able to grow within the pH range 2.0 - 7.0, with an optimum pH of 3.5 - 5.0.

Water activity

The organism does not seem to grow at reduced water activities. Inhibition by sugar concentrations of 18 ºBrix has been reported, and growth does not occur in fruit juice concentrates. Spores do however remain viable in fruit juice concentrates with 70 ºBrix.

Atmosphere

A. acidoterrestris is aerobic, but has been reported to grow in sealed beverage containers.

Preservatives and biocides

A concentration of 6% ethanol inhibits growth, but preservatives added to juices, such as benzoic acid and sorbic acid have also been shown to prevent spoilage. The bacteriocin nisin has been shown to reduce the thermal resistance of spores of *A. acidoterrestris* and is therefore an effective inhibitor of the growth and germination of these spores.

Control

As pasteurisation cannot guarantee the complete inactivation of spores of *A. acidoterrestris*, it has been recommended that control requires the monitoring of raw materials for contamination by *A. acidoterrestris*, so that contaminated fruit can be excluded from vulnerable products. Effective washing of fruit surfaces has also been suggested as a control measure, including the use of chlorine dioxide as an oxidising agent. Hot filled products should be cooled quickly to prevent the germination and growth of

any remaining spores. In addition, storage of fruit juices below 20 °C and preferably in refrigerators and reducing head space will slow growth rate of Alicyclobacilli. Spoilage by this organism is rare, but can be a serious problem.

BACILLUS

The genus *Bacillus* is large and diverse, and includes a number of species important in foods. Several are pathogens and some are important in food spoilage.

The Organism *Bacillus*

They are Gram-positive, endospore forming rods, and most are motile. They are aerobic or facultatively anaerobic, catalase-positive and oxidase-positive or negative members of the family Bacillaceae. They are able to carry out a wide range of metabolic processes, and the genus includes psychrotrophic and thermophilic species. Gas is not usually produced from glucose. Endospores are resistant to environmental factors and may remain viable for many years.

Key Species in Foods

Some of the species known to cause foodborne disease are also important in food spoilage, particularly *Bacillus cereus*, *Bacillus subtilis* and *Bacillus licheniformis*. Other species, such as *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) and *Bacillus circulans*, are also isolated from foods. *Bacillus coagulans* is an important spoilage organism affecting milk, vegetables, fruit and pasteurised acid foods, and the thermophilic species *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) is important in canned foods.

Sources

Environment

Bacilli are natural inhabitants of the soil and water, and are also found in plant matter. Their resistant endospores can be passively distributed very widely and they can be isolated from many sources.

Foods

Bacillus spp. can be found mainly in low-acid foods (pH >4.6) that have received a low temperature pasteurisation and are then chilled, or are commercially sterilised (e.g. ultra-high temperature or UHT-treated milk), or acid foods that have received pasteurisation and are stored at ambient temperature. Raw materials such as vegetables, cereals, eggs and meats are also contaminated. The resistance of the endospores means that bacilli are important contaminants of heat-processed and dried foods.

Spoilage Characteristics

The bacilli are involved in a variety of specific types of spoilage in a diverse range of foods. For example, psychrotrophic species produce serious off-flavours such as bitter, putrid, stale, rancid, fruity, yeasty and sour tastes in a wide range of dairy products such as milk, cheese and cream. *Bacillus* spp. such as *Bacillus sphaericus*, *B. subtilis* and *Bacillus lentus* have been associated with flavour defects. They cause problems in pasteurised milks, particularly *B. cereus* strains that produce lecithinase. This enzyme causes a defect known as ‘sweet curdling’, or ‘bitty cream’. ‘Flat sour’ spoilage of evaporated milk is caused primarily by *G. stearothermophilus* and *B. coagulans* but can be caused by *B. subtilis*, *B. licheniformis* or *Bacillus macerans*. *B. coagulans* has also been associated with spoilage of evaporated milk, resulting in firm coagulation. It has also been discovered that highly heat-resistant spores belonging to a recently-identified species, *Bacillus sporothermodurans*, may cause spoilage in UHT-processed milks. Psychrotrophic bacilli also cause souring in cooked meat products.

Aciduric, thermoduric species, such as *B. coagulans*, can cause spoilage in acid, heat-processed products such as tomato pastes and sauces, asparagus, corn, lima beans and peas. *B. licheniformis* may grow

anaerobically in processed tomato products, causing a localised rise in pH sufficient to allow *Clostridium botulinum* to grow.

Thermophilic species produce 'flat sour' spoilage of low-acid, canned food. *B. subtilis*, *Bacillus pumilus*, *Bacillus megaterium* and *B. cereus* are associated with the formation of 'rope', a mucoid, stringy mass, in bread. Rope is characterised by the development of fruity or sweet odours, or rotting fruit/bad cheese. This problem has become more common again recently with the trend away from the use of calcium propionate as a preservative in bread.

B. subtilis is known to produce non-volatile acidity in wines.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

The *Bacillus* species found in foods are able to grow over a wide range of temperatures. Psychrotrophic strains of *B. cereus* are shown to have a lower limit of growth at 2.5 ºC, some strains of *B. circulans* are able to grow at 2 ºC and *B. macerans/B. subtilis* are reported to have a lower limit of growth at 0 ºC. Many mesophilic species are able to grow at 50 ºC, and some thermophiles grow at 65 ºC or higher, but show little growth at 35 ºC.

Heat resistance of endospores is variable, with the psychrotrophic strains showing less resistance than mesophilic or thermophilic strains. For example, psychrotrophic *B. cereus* isolates record D-values of 5 - 8 minutes at 90 ºC, but mesophilic strains show similar values at 100 ºC. At 100 ºC, a *B. subtilis* strain showed a D-value of 11 minutes and *B. coagulans* isolates varied from 0.7 - 7.0 min, dependent on pH. Spores of *G. stearothermophilus* are very heat-resistant, and D-values of 1.0 - 5.8 min at 121 ºC have been recorded.

pH

Growth of aciduric species, such as *B. coagulans*, has been recorded at pH 3.8, but minimum values of >4.0 are more common for these organisms. *B. cereus* has been reported to grow at pH values between 4.3 and 9.3 and *B. subtilis* from 4.5 - 8.5.

Water activity

Most *Bacillus* spp. do not grow below a_w values of 0.93 - 0.95, although some strains of *B. subtilis* have been shown to grow at 0.90. Endospores are extremely resistant to drying and survive for very long periods. Salt tolerance is variable, and may be between 2.5% and 10% for species associated with foods.

Atmosphere

Facultatively anaerobic species such as *B. licheniformis* grow well in anaerobic environments, but even aerobic species may show limited growth in sealed containers. Many species are strongly inhibited by carbon dioxide.

Preservatives and biocides

The endospores are generally quite resistant to sanitisers and biocides and are difficult to eradicate from processing environments. *B. subtilis* in bread is inhibited by propionate, but acetic acid is less effective. Bacilli show variable sensitivity to other food preservatives.

Control

Bacillus spp. are very common and widely distributed in foods, but tend not to compete well with other spoilage microflora in fresh foods. They are more likely to cause problems in heat-processed foods and products relying on hurdles such as reduced pH and water activity for their stability. Control of flat sour spoilage in canned foods is achieved by minimising cooling times and storage temperatures. Monitoring of ingredients quality is an important control for *Bacillus* spoilage, as is adequate temperature control in chilled foods and good hygiene practice. The bacteriocin nisin is active against bacilli, and may be useful in canned vegetable products.

Radiation doses between 10 - 50 kGy are required to inactivate bacterial spores, with typical D-values of 1 - 4 kGy, depending on the organism. The D-values for vegetative organisms are generally below 0.8 kGy.

BROCHOTHRIX

Brochothrix spp. are related to *Listeria* and to *Corynebacteria*. The genus was proposed in 1976 to accommodate organisms previously classified as *Microbacterium thermosphacta*.

The Organism *Brochothrix*

Brochothrix spp. are Gram-positive, non-motile, non-sporeforming rods, but become pleomorphic in older cultures, that is occur either singly or in chains. They are facultative anaerobes, catalase-positive and oxidase-negative, belonging to the Listeriaceae family.

Key Species in Foods

There are two species in the genus, but only *Brochothrix thermosphacta* is significant in foods. *Brochothrix campestris* is normally found in soil and water.

Sources

Environment

The sources of *B. thermosphacta* are not well understood, but it probably comes from soil and water. It possibly also comes from animal faeces as it is a common inhabitant of the intestinal tract of animals. It is a very common contaminant in meat-processing facilities and can be readily isolated from plant and equipment.

Foods

B. thermosphacta is commonly found in meat and poultry products, but has also been isolated from modified atmosphere packaged (MAP) fish, frozen vegetables and dairy products. The organism is a particular problem in chilled, vacuum-packed (VP) and MAP fresh meat products.

Spoilage Characteristics

Spoilage of fresh and cured meat may occur in both aerobic and anaerobic conditions, and is usually apparent as an 'off'-odour, sometimes described as a sour, sweaty or acidic, malty, dairy or cheesy smell. It results from the formation of a variety of metabolic by-products from glucose fermentation, including acetoin, diacetyl, and traces of iso-butyric and iso-valeric acids. Spoilage is often not apparent until some time after the population has reached its maximum level of about 10^5 cfu/g. The production of lipase and protease are other factors contributing to food spoilage. *B. thermosphacta* utilises amino acids, but hydrogen sulphide (H_2S) is not produced. The organism may grow on fat surfaces, especially in pork and lamb. Lipases are not produced in detectable amounts at temperatures below 20 ºC. Production of proteases has been found to be less at 6 ºC than at 10 ºC. The ecology of spoilage is complex and is affected by pH, gaseous atmosphere and the presence of competing microflora.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

B. thermosphacta is a psychrotroph, able to grow at temperatures as low as -0.8 ºC. Most isolates will not grow above 30 ºC and have an optimum of 20 - 25 ºC. However, growth may be quite rapid at temperatures above 5 ºC.

The organism is not heat-resistant and will not survive even mild pasteurisation treatments. D-values at 55 ºC of 1 minute have been recorded in skimmed milk and buffers, although resistance is likely to be higher in meats.

pH

B. thermosphacta is favoured by high pH values and has an optimum pH for growth of 7.0, within a range of 5.0 - 9.0. Spoilage of high pH meat (>6.5) is much more rapid than for more acid substrates.

Water activity

B. thermosphacta has been reported to grow at a_w values down to 0.94 at temperatures of 20 - 25 ºC in an aerobic atmosphere, and some strains are able to tolerate 10% salt.

Atmosphere

The organism is a facultative anaerobe and will grow in vacuum-packed meats, but it grows more rapidly in air. It will also grow in MAP packs containing elevated oxygen or carbon dioxide levels. It can tolerate up to 50% carbon dioxide, provided some oxygen is present.

Preservatives and biocides

B. thermosphacta can tolerate 100 parts per million nitrite at >pH 5.5 and 5 ºC aerobically in the presence of 2 - 4% salt. Some resistance to sulphur dioxide has been reported. Immediate reductions in bacterial counts have been observed in meat products tested with nisin (when combined with lysozyme and EDTA in a gelatin coating).

Control

Since it is so widespread in meat plants and therefore likely to contaminate meat products during processing, effective cleaning and sanitation procedures are essential to control *B. thermosphacta* in MAP and vacuum-packed fresh meats. In addition, as it requires a small level of oxygen to be present, its growth could be inhibited in MAP products where oxygen scavengers are used to reduce the level of oxygen to below 0.2. Its relative sensitivity to heat means that it is rarely a problem in cooked meats, providing that significant recontamination does not occur. It should be inactivated by a heat treatment of 63 ºC for 5 minutes. *B. thermosphacta* is sensitive to radiation of 2.5 kGy.

CLOSTRIDIUM

The genus *Clostridium* contains a large number of species, many of which are important in foods, both as causes of foodborne disease and as spoilage organisms.

The Organism *Clostridium*

They are Gram-positive, motile or non-motile rods, producing oval or spherical endospores that usually distend the cell. They are catalase-negative, oxidase-negative, obligate anaerobes, although oxygen tolerance varies widely. Most species have a metabolism that is predominantly either proteolytic or saccharolytic, and there are both psychrophilic and thermophilic species. They belong to the family Clostridiaceae.

Key Species in Foods

Those species responsible for foodborne disease, *Clostridium botulinum* and *Clostridium perfringens*, are not considered here although they can cause spoilage of foods e.g. produce gas and have proteolytic activity.

Many species may be involved in food spoilage, but some of the most significant are *Clostridium sporogenes*, *Clostridium butyricum*, and *Clostridium tyrobutyricum*. These are considered non-pathogenic. *C. butyricum* and *Clostridium pasteurianum* are referred to as butyric anaerobes. Other mesophilic clostridia that have been associated with spoilage of foods include *Clostridium barati*, *Clostridium bifermentans*, *Clostridium beijerinckii*, *Clostridium pseudotetanicum*, *Clostridium fallax*, *Clostridium sordellii* and *Clostridium felsinium*.

Note that there are reports of botulinum neurotoxin production and associated illness in *C. butyricum* and *C. barati*; for more information see the *C. botulinum* chapter.

Some recently recognised psychrotrophic and psychrophilic species, often referred to as ‘blown pack clostridia’, such as *Clostridium laramie*, *Clostridium aligidicarnis* and *Clostridium estertheticum*, seem to be important in certain types of vacuum-packed meat spoilage. The thermophilic species *Clostridium thermosaccharolyticum*, important in low acid canned food spoilage, has recently been reclassified as a species of *Thermoanaerobacterium*, and the organism still sometimes called

Clostridium nigrificans, which causes ‘sulphide stinker’ spoilage in canned foods, is more accurately known as *Desulphotomaculum nigrificans*.

Sources

Environment

Clostridia are ubiquitous in the environment and are found in soil, decaying vegetable matter, animal faeces and marine sediments. The spores are persistent and may be passively distributed.

Foods

Clostridia are present as contaminants in many foods, particularly meat and dairy products. Their heat-resistant endospores may survive in heat-processed foods, and may be able to germinate and grow in sealed packs where anaerobic conditions develop.

C. sporogenes is responsible for causing spoilage of a number of foods including cheese, condensed milk, cooked meat, canned vegetables and canned fish. It is a common *Clostridium* species found in milk and meat. *C. butyricum* is responsible for causing spoilage of cheese, condensed milk and acid canned foods, in particular tomatoes and other fruits (e.g. pear and apricot juices, and canned pineapples) together with *C. pasteurianum*. *C. butyricum* is also associated with spoilage of wines. *D. nigrificans* causes spoilage of canned sweetcorn, peas, mushroom, mushroom-containing high pH foods and canned baby clams. *Thermoanaerobacterium thermosaccharolyticum* can cause spoilage of acid and acidified products such as tomato and other fruit-containing products, including those containing starchy (farinaceous) ingredients, such as spaghetti. Other products affected include canned sweet potatoes, pumpkin, green beans, mushrooms, asparagus, and vegetable soup.

Spoilage Characteristics

The type of spoilage depends very much on the product and the species involved. Spoilage is manifested by changes in product pH (caused by organic acid production), gas production and production of foul odours (e.g. through volatile acids).

C. sporogenes, the only proteolytic and lipolytic clostridia associated with spoilage, may cause putrefactive spoilage in under processed canned foods, and the thermophilic species *T. thermosaccharolyticum* can cause hard swells in cans cooled too slowly or stored at high temperatures. Resulting products are found to be sour, fermented, or have a butyric odour. Putrefactive spoilage of large meat joints may occur if clostridia are present in deep tissue, and grow before the temperature is adequately reduced, or before curing salt concentrations have reached inhibitory levels. The condition known as 'bone taint' in meat carcasses is also thought to be caused by growth of clostridia in deep tissue. Psychrotrophic and psychrophilic clostridia (*C. laramie*, *C. aligidicarnis* and *C. estertheticum*) have been found to cause spoilage of chilled, vacuum-packed fresh and cooked meats, producing gas and/or offensive odours. *D. nigrificans* causes black spots on cured meats. *C. pasteurianum*, *C. butyricum*, *C. sporogens* and *C. tyrobutyricum* cause late gassiness of cheese. Of these, *C. tyrobutyricum* is the principal species involved in 'late blowing' of brine-salted hard and semi-hard cheeses, as a result of butyric acid and gas production. *D. nigrificans* cause production of hydrogen sulphide, sometimes resulting in blackening of the product.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

The clostridia are able to grow over a wide range of temperatures. Most are mesophilic, with optimum growth temperatures of 30 - 37 ºC, and are able to grow within the range 10 - 50 ºC. Some are psychrotrophic (maximum growth temperature between 32 ºC and 40 ºC) and able to grow slowly at 4 ºC. The psychrophilic species *C. laramie* (maximum growth temperature below 20 ºC) may be able to grow at temperatures as low as -3 ºC, and has an optimum of 15 ºC and a maximum of 21 ºC; it has been found to cause spoilage of meat stored at 2 ºC. Thermophilic species have optimum growth temperatures of 50 - 60 ºC; they may grow at 65 ºC or higher, but little, if any, growth occurs at 37 ºC.

The heat resistance of endospores varies widely. For example, *C. butyricum* spores have a reported D value at 80 ºC of 5 minutes ($D_{100\,^{\circ}C}$ of 0.1 - 0.5 minutes), whereas an extensively studied isolate of *C. sporogenes*, PA3679, has a D-value at 121 ºC of 0.1 - 1.5 minutes ($D_{100\,^{\circ}C}$ of 80 - 100 minutes, z value of 9 - 13 ºC). *T. thermosaccharolyticum* spores are

extremely heat-resistant, and D-values of 3 - 4 minutes have been recorded at 121 ºC ($D_{100\,^{o}C}$ of 400 min, z value of 12 - 18 ºC).

pH

Growth at pH values as low as 3.55 have been recorded for species such as *C. butyricum* and *C. pasteurianum* (butyric anaerobes), but minima of > 4.2 are more common for these organisms. The majority of species are inhibited by pH values below 4.5.

Water activity

Most clostridia are not able to grow in low-moisture foods, and a_w of 0.93 - 0.94 would prevent growth of most species. Some are able to tolerate salt concentrations of up to 10%.

Atmosphere

Although the clostridia are obligate anaerobes, some species may tolerate low oxygen concentrations and demonstrate growth in environments that would not normally be considered anaerobic.

Preservatives and biocides

The endospores of clostridia are relatively resistant to many sanitisers and biocides. Nitrites have been used to control clostridia in meat products and cheese, and a bacteriocin, nisin, has also been used to control 'late blowing' in cheese. Phosphates and antimicrobial enzymes like lysozyme are also found to be active against clostridia.

Control

In heat-processed foods that are intended to be commercially sterile, the thermal process is often calculated to destroy the spores of spoilage species, such as *C. sporogenes*, that are heat-resistant. The spores of thermophilic species may be too resistant to be controlled simply by a thermal process, but sufficiently rapid cooling and storage at temperatures below 30 ºC will

prevent their growth. The risk of thermophilic spoilage can also be reduced by applying strict microbiological standards to ingredients likely to contain thermophilic spores, such as sugars and starches. In chilled foods, adequate temperature control is important, as are quality of raw materials and good hygiene practice. In meat processing, it is important to avoid deep incisions or punctures that may inoculate spores into deep tissues.

Dose required for 90% inactivation of *Clostridium* spores is 3.5 kGy. Radiation values of 1.5 kGy have been found to destroy spores of *C. butyricum*.

ENTEROCOCCUS

The genus *Enterococcus* was formed in 1984 and replaced an earlier classification based on the classical 'faecal streptococci' and Lancefield serological group D streptococci. Nineteen species of *Enterococcus* are currently recognised, but not all of them are of faecal origin and many species are rarely found in foods.

The significance of enterococci in foods is the subject of debate. Some strains are opportunist pathogens, and may cause foodborne disease, though very rarely. They are also cited as indicator organisms (*Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus durans* and *Enterococcus hirae*), but their presence in food is usually not a consequence of faecal contamination. They are sometimes used as starter cultures in food production or as a probiotic. However, some species are important spoilage organisms, and this aspect is considered below.

The Organism *Enterococcus*

Enterococci are Gram-positive, non-sporeforming ovoid cells, occurring singly or in pairs and short chains. Within the chains, the cells are frequently arranged in pairs and are elongated in the direction of the chain. Those species found in foods are non-motile (with the exception of *Enterococcus gallinarium* and *Enterococcus casseliflavus*). They are facultatively anaerobic, oxidase-negative and catalase-negative, and have a homofermentative carbohydrate metabolism, producing mainly lactic acid. They have complex nutritional requirements and are resistant to environmental stress. They are easily distinguished from other Gram-

positive, catalase-negative homofermentative cocci, such as streptococci and lactococci, in that they are able to grow at 10 ºC and 45 ºC, in 6.5% sodium chloride (NaCl), in the presence of 40% bile at pH 9.6. They belong to the family Enterococcaceae.

Key Species in Foods

By far the most common species found in foods are *E. faecalis* and *E. faecium*. *E. durans* is also occasionally found in milk, and *E. casseliflavus* has been reported to cause yellow discoloration in vacuum-packed cooked meats.

Sources

Environment

Enterococci are found in soil, surface waters, sea water, waste waters and municipal water treatment plants, on plants and in the gastrointestinal tract of animals. *E. faecalis* and *E. faecium* are principally associated with the digestive tracts of humans and other mammals, including poultry, pigs and cattle whereas yellow-pigmented *Enterococcus mundtii* and *E. casseliflavus* are typically associated with plants. The natural distribution of other species is not well understood.

Foods

Due to their intestinal habitat in food animals, Enterococci are common contaminants of milk and fresh/processed meat, and may be isolated from numerous other foods. They are also present on fresh produce such as celery, cilantro, mustard greens, spinach, collards, parsley, dill, cabbage and cantaloupe, and possibly originate from the use of untreated irrigation water or manure slurry. They are used as starter organisms in some traditional southern European cheeses.

Spoilage Characteristics

Spoilage usually takes the form of souring as a result of lactic acid production during growth. This may occur in pasteurised milk and dairy

products, and in meats, particularly cooked, cured meats. Enterococci may also produce surface slime and cause greening in the core of some cooked meats. They may be involved in the production of biogenic amines such as histamine in some foods.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

The enterococci found in foods are able to grow over a wide range of temperatures. Many strains are psychrotrophic and some isolates of *E. faecium* have been shown to grow at temperatures as low as 1 ºC. Maximum growth temperature has been reported as around 50 ºC, with an optimum for most strains of approximately 37 ºC. These organisms are also notably resistant to freezing.

The enterococci are well known for their heat resistance and are able to survive mild pasteurisation processes. *E. faecium* is notably more heat-resistant than *E. faecalis*, and D-values at 70 ºC of 1.4 - 3.4 minutes, and 0.02 - 0.6 minutes, respectively, have been obtained. *E. faecium* has been associated with spoilage of pasteurised canned ham.

pH

Enterococcus spp. are able to grow over a wider pH range than most bacteria, and growth between 4.4 and 10.6 has been recorded.

Water activity

The minimum a_w value for growth varies with the type of solute present, but is reported as 0.93 for *E. faecalis*. Most strains are able to tolerate salt at a concentration of 10% and are resistant to drying.

Atmosphere

Enterococci are facultative anaerobes, but those found in food, especially *E. faecalis*, are well adapted to grow in aerobic conditions.

Preservatives and biocides

The enterococci, particularly *E. faecium*, are generally quite resistant to environmental stress, and are therefore persistent, but are not especially resistant to preservatives and biocides. However, multiple antibiotic resistance in isolates of these organisms is causing increasing concern.

Control

Enterococci are commonly present in milk and meat products, and their heat resistance means that they can be a particular problem in pasteurised foods. It is therefore important to ensure that such products receive an adequate heat process. Their persistence and ability to grow in a wide range of environments also mean that they may be able to colonise plant and equipment. Thorough cleaning and sanitising regimes are therefore important in their control.

FLAVOBACTERIUM

The genus was first named in 1923 to describe bacteria forming yellow (*Flavus*) or orange pigmented colonies in culture media.

The Organism *Flavobacterium*

The genus *Flavobacterium* is very heterogeneous, and contains a variety of organisms with certain characteristics in common. They are all Gram-negative, aerobic, non-motile, non-sporeforming, non-fermentative rods belonging to the family Flavobacteriaceae. *Flavobacterium* spp. are catalase and oxidase-positive, and usually have low nitrogen requirements. Recent revisions have created new genera (*Weeksella*, *Bergeyella*, *Chryseobacterium* and *Empedobacter*), into which many former flavobacteria have been placed, but their importance in foods is uncertain. The genus *Flavobacterium* currently contains 56 species.

Key Species in Foods

Flavobacteria are difficult to characterise, and the position of many is taxonomically uncertain. Therefore, isolates from foods are rarely identified to species level. *Flavobacterium aquatile* is the type species. Species reported in foods include *Flavobacterium breve* and *Flavobacterium odoratum*.

Sources

Environment

Flavobacterium spp. are ubiquitous and may be easily isolated from fresh water and soil. They are often associated with plant material, and may also be part of the normal skin microflora of animals.

Foods

These organisms have been isolated from a wide variety of chilled foods, including fresh meat and poultry, unpasteurised milk, fish and shellfish, uncarbonated mineral waters and alcoholic beverages produced from grain, where they are a component of the autochthonous or indigenous flora. They are also commonly found in injection brines for cured meat products. They are able to grow and cause spoilage in margarines, emulsions and butter due to their lipolytic properties. Other dairy products like rice pudding, cream, cheddar cheese and milk-based canned goods are also susceptible to spoilage by *Flavobacterium*.

Spoilage Characteristics

Flavobacterium spp. are rarely the major cause of spoilage but may be components in the spoilage flora in some foods, such as fish and shellfish, eggs, and vegetable products. Some strains produce pectinases causing degradation of plant material, and heat stable proteinases and lipases may be produced in milk. They may be involved in the spoilage of pasteurised milk and egg as post-process contaminants. Strains identified as *Flavobacterium maloloris* were reported to produce a surface taint and ‘apple or putrid cheesy odour’ in butter, possibly as a result of growth in the cream used in

its production. Certain strains may also cause bitterness in dairy products as a result of phospholipase production. They give a vinegary flavour and off-odour to alcoholic beverages.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Many strains are psychrotrophic and growth at 5 ºC or below may occur. The maximum temperature for growth is usually about 30 ºC, but some strains will grow at 37 ºC. Some isolates are reported to remain viable for long periods in frozen foods.

Flavobacterium spp. are normally considered heat-sensitive and are not usually expected to survive pasteurisation. However, there have been reports of survival in pasteurised egg, and D-values in skimmed milk at 63 ºC of 4.3 - 9.6 minutes were recorded for strains isolated from butter.

pH

There is little published information on minimum values permitting growth. They are not reported to be capable of growth at low pH values, and probably have an optimum pH of 6.0 - 7.0. Only 83% reduction on levels of *Flavobacterium* was observed in biofilms on stainless steel after exposure to a solution of water at pH 11 for 20 minutes.

Water activity

There is very little information on minimum a_w values that will support growth of *Flavobacterium* spp. Reports of salt tolerance are variable; some strains are inhibited by a concentration of 1%, but others are normally found in meat injection brines or associated with sea fish.

Atmosphere

Flavobacterium spp. are aerobic organisms and are normally inhibited by anaerobic conditions. Some strains have been reported to be sensitive to elevated concentrations of carbon dioxide.

Preservatives and biocides

There is very little published information on the effect of antimicrobials on *Flavobacterium* spp., but individual strains have been found to be inhibited by nitrite at a concentration of 100 - 200 parts per million at pH 6. Destruction by chlorine is reported to be rapid.

Control

Flavobacterium spp. are normally present on the surface of fresh meat and poultry carcasses, but usually die out during storage. They are also likely to be present in milk, fish and vegetables, and have occasionally been shown to become established in food-processing environments. Therefore, effective hygiene and sanitation procedures are important in the control of spoilage. These organisms can also be controlled by pasteurisation, but adequate post-process hygiene is important to prevent recontamination.

A 4 log reduction in levels of *Flavobacterium* on stainless steel has been achieved by exposure to 200 parts per million solution of chlorine adjusted to pH 6.5 for 20 minutes. *Flavobacterium* are also found to be sensitive to essential oil compounds.

HAFNIA

The Organism *Hafnia*

Hafnia spp. are members of the Enterobacteriaceae. *Hafnia* is a motile, Gram-negative, non-sporeforming, catalase-positive, oxidase-negative rod. They are facultative anaerobes, possessing both respiratory and fermentative metabolism, and both acid and gas are produced from glucose.

Key Species in Foods

There is currently only one species in the genus, *Hafnia alvei*.

Sources

Environment

H. alvei is present in animal and human faeces, but is not always an indicator of faecal contamination. It can be isolated from both soil and water.

Foods

The organism is found in a wide range of refrigerated foods, particularly meat, fish and seafood, and vegetables. It is also found in eggs and milk.

Spoilage Characteristics

Gaseous and putrefactive spoilage of chilled vacuum-packed meats has been reported, and hydrogen sulphide may be produced. *H. alvei* may also be associated with the production of histamine in scombroid fish, and with spoilage of eggs.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Some strains are psychrotrophic and growth may be quite rapid at 7 ºC. However, reports of minimum growth temperatures are variable; for example, a value of 2.5 ºC has been obtained in laboratory media, but the minimum for growth in foods is uncertain. Rapid growth at 43 ºC has also been reported.

Heat resistance is not particularly significant. A D-value at 55 ºC of 0.36 minutes has been recorded in peptone water, with a value of 0.74 minutes being found in smoked fish.

pH

There is little published information on the effect of pH on the growth of *H. alvei*. However, it would be reasonable to expect the organism to grow over a range similar to that of other Enterobacteriaceae (4.5 - 9.0 is not untypical).

Water activity

There is little published information on the effect of water activity on growth, but related organisms tend to grow at minimum a_w value of approximately 0.95.

Atmosphere

The organism is a facultative anaerobe and will grow in vacuum-packed meats. Under aerobic conditions, *H. alvei* does not compete well with obligate aerobes.

Preservatives and biocides

H. alvei has been reported to be relatively resistant to sulphur dioxide used as a preservative in fresh sausage, but there is little published information on its general resistance to antimicrobial compounds.

Control

The importance of *H. alvei* in spoilage is limited mainly to vacuum-packed meat products. Effective cleaning and hygiene procedures are important to minimise the initial level of contamination of these products. Mild heat processes are also effective.

LACTOBACILLUS

Lactobacillus is a highly heterogeneous genus that contains over 50 species, many of which are important in food spoilage. Some of these species have been placed in a new genus, *Weissella*, including *Lactobacillus viridescens*, a species that causes greening in meat.

The Organism *Lactobacillus*

The lactobacilli are Gram-positive, non-sporeforming rods or coccobacilli, and are usually non-motile and catalase and oxidase-negative. They are strictly fermentative, aerotolerant or anaerobic, and aciduric or acidophilic, and usually have complex nutritional requirements. Their carbohydrate metabolism may be homofermentative, producing mainly lactic acid, or heterofermentative, producing a mixture of lactic and acetic acids and carbon dioxide. They are able to grow over a wide range of temperatures and may tolerate apparently hostile environments. Some species produce bacteriocins that have an antagonistic effect on other bacteria. Lactobacilli belong to the family Lactobacillaceae.

Key Species in Foods

Many species have been isolated from foods as spoilage organisms, but also as components of the natural microflora of fermented foods. Some species are used as starter cultures in fermented dairy, meat and vegetable products.

They are divided into three groups based on fermentative features. These include:

- obligately homofermentative species such as *Lactobacillus acidophilus* and *Lactobacillus delbrueckii*
- facultatively heterofermentative species such as *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus sake*
- obligately heterofermentative species *Lactobacillus brevis*, *Lactobacillus buchneri* and *Lactobacillus fermentum*.

Sources

Environment

Most *Lactobacillus* spp. important in foods probably originate from plant material. They are usually associated with environments containing large amounts of fermentable carbohydrate. Some truly anaerobic species are associated with the mucosal membranes and digestive tracts of animals, but these are rarely found in foods.

Foods

Lactobacilli are found in a variety of foods, particularly dairy products, vacuum-packed or MAP meats and poultry products, alcoholic beverages, soft drinks, canned and acidified or fermented fruit and vegetable products.

Spoilage Characteristics

Atypical flavours, such as cheesy, sour, acid and sometimes liver-like have occurred due to acid formation. Excessive acid production in vacuum-packed cooked meat products can affect sensory attributes. Acids produced can also cause sour/acid odours in raw milk, carbonated and still soft drinks and canned vegetables, bitter flavours in cheese, unpleasant buttery flavours in juices and beer and haziness in alcoholic beverages.

Greening of meat may also occur due to the production of hydrogen peroxide by some species of *Lactobacillus*. Some Lactobacilli may cause or accelerate spoilage by formation of hydrogen sulphide gas which results in malodorous off-odours and black spots on meat. Pigment production, such as pink and orange pigments, can occasionally cause spoilage in cheese.

Some lactobacilli are associated with gas production. They have been associated with the rapid development of carbon dioxide in vacuum packed meat products. Canned vegetables may be spoiled by lactobacilli forming considerable amounts of carbon dioxide, causing blown cans. Blown cans can also occur with sour fruit products. In salad dressings the gas formed leads to bubble-filled dressing and/or blowing of the containers. Bloater damage in brined cucumbers results from an increase in gas pressure inside the cucumbers during fermentation. 'Protein swell' is an unusual type of spoilage caused by lactobacilli. It raises the pH (due to ammonia production) of the spoiled product. In this case, the proteins are decomposed and the subsequent decarboxylation of amino acids leads to enhanced gas production.

Exopolysaccharides (EPS) can be produced in meat products, cider and wine causing severe problems. *L. sake* has been found to cause 'ropy' spoilage of vacuum-packed cooked meat products, by producing the EPS dextran and H_2S. Heterofermentative lactobacilli have been associated with the ropy spoilage of wine, cider and beer.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Lactobacillus spp. are able to grow over a wide temperature range. Some species are psychrotrophic and may grow at temperatures as low as 2 ºC and some are moderately thermotolerant, growing at temperatures up to 55 ºC. Most species have optimum growth temperatures of 30 - 40 ºC. Thus growth may occur in chilled and ambient foods.

Lactobacilli are relatively heat-resistant and may survive inadequate pasteurisation processes. For example, D-values as high as 11 minutes at 70 ºC have been reported for *L. plantarum*. Heat resistance varies with pH, a_w and other factors, and may be significantly higher in low-moisture foods.

pH

Lactobacilli are all acid-tolerant, but minimum pH for growth varies with species and with the acid present. *L. brevis* will grow at pH 3.16 in the laboratory, but *Lactobacillus lactis* does not grow below 4.3. *Lactobacillus suebicus* isolated from fermented apples and pear mashes and *Lactobacillus acetotolerans* from rice vinegar are able to grow at pH 2.8 and pH 3.3 respectively. Most species are inhibited by pH values above neutral.

Water activity

The minimum a_w value for growth varies with species and with the type of solute present, but is likely to be within the range 0.93 - 0.96. Lactobacilli are not particularly sensitive to dry conditions and survive for long periods in dried vegetables.

Atmosphere

Most of the species important in foods are aerotolerant, but do not grow well in aerobic environments. Sufficient growth to cause spoilage is unlikely to occur unless the redox potential is reduced.

Preservatives and biocides

Some spoilage species are quite resistant to certain food preservatives, particularly acetic acid, nitrites/nitrates, and sulphur dioxide. *L. suebicus* is able to tolerate ethanol concentrations of 12 - 16%.

Control

Lactobacilli are very common contaminants in plant material and are often present in meat and dairy products. They are one of the few spoilage organisms that cannot easily be controlled by reducing pH, and may grow well in environments that cannot be readily colonised by other bacteria. Spoilage usually becomes detectable only slowly, especially at low temperatures, and therefore adequate temperature control of susceptible products is important in achieving the desired shelf-life. Adequate heat processing, satisfactory microbiological quality of ingredients and good hygiene practice are all important controls.

Note: A recent study has shown that *Lactobacillus hilgardii* are spoilage, high-histamine-producing bacteria in wines. Histamine is a biogenic amine that can cause headaches and allergic-like reactions in susceptible individuals.

LEUCONOSTOC

The Organism *Leuconostoc*

Leuconostoc spp. are lactic acid bacteria (and therefore members of the Lactobacillaceae family) and can often be found in association with *Lactobacillus* spp. They are facultatively anaerobic, Gram-positive, non-sporeforming, non-motile, catalase and oxidase-negative, heterofermentative cocci. They are dependent for growth on the presence of fermentable carbohydrate, and have complex nutritional requirements. End products from glucose metabolism are lactate, ethanol and carbon dioxide. Some strains produce an extracellular polysaccharide, dextran, from sucrose. *Leuconostoc* spp. are used as starter cultures in certain fermented dairy and vegetable products.

Key Species in Foods

The principal species in foods is *Leuconostoc mesenteroides*. Three subspecies of this organism have been recognised - *mesenteroides*, *dextranicum* and *cremoris* (usually associated with dairy products). Other species of importance in food spoilage are *Leuconostoc gelidum* and *Leuconostoc carnosum*, which are associated with meat products, *Leuconostoc gasicomitatum* associated with spoilage of acetic acid fish preserve, *Leuconostoc paramesenteroides* (recently placed in the genus *Weissela*) and *Leuconostoc oenos*, (now reclassified as *Oenococcus oeni*) are species associated with wine.

Sources

Environment

The primary environmental source of *Leuconostoc* spp. is plant material. They are reported to be the commonest type of lactic acid bacteria on growing vegetation. Other sources to foods include food utensils, gastrointestinal tract and animal hides.

Foods

Leuconostoc spp. are often associated with meat products, particularly vacuum-packed or MAP sliced meats, dairy products, and fermented vegetable foods, such as sauerkraut. *L. mesenteroides* strains are common contaminants of sugar-processing operations. It has also been responsible for spoilage of wine and cider. *L. carnosum* is reported to be strongly associated with vacuum-packed ham.

Spoilage Characteristics

Leuconostoc spp. are rarely the dominant organisms in spoiled products, but they may contribute to spoilage in a variety of foods. *L. mesenteroides* subsp. *mesenteroides* and *dextranicus* are involved in souring and blowing of vacuum-packed or MAP meats, and where exopolysaccharides are produced, a ‘ropy slime’ spoilage may result. *L. carnosum* is associated with spoilage of vacuum-packed ham. Acid and dextran production by

L. mesenteroides subsp. *mesenteroides* can cause serious problems in sugar processing, and sufficient dextran may be produced to block pipelines. Some strains may cause blowing in certain cheeses, and spoilage of concentrated orange juice has also been reported.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Some *Leuconostoc* strains are psychrotrophic and are able to grow at 2 ºC. *L. carnosum* is able to grow slowly at 1 ºC, but not at 37 ºC. A minimum growth temperature of 5 - 8 ºC is more common; with maxima in the range 40 - 50 ºC. Optimum temperature for dextran production is usually 20 - 25 ºC.

The heat resistance of *Leuconostoc* spp. varies, but most strains are not notably heat-resistant. A D-value at 60 ºC of 31.3 seconds has been reported for *L. mesenteroides*, but strains that produce extracellular polysaccharides may be notably more resistant and may survive brief exposure to temperatures as high as 85 ºC.

pH

Minimum pH for growth varies with species and with acidulant, but a strain of *L. mesenteroides* has been reported to be capable of growth at 3.5. Optimum pH for most species is nearer 6.0, with the exception of *L. oenos*, which is acidophilic and has an optimum of 4.2 - 4.8.

Water activity

There is little information on the effect of a_w on *Leuconostoc*. Most species are not particularly salt-tolerant, but *Leuconostoc fallax*, a species found in sauerkraut, has been reported to grow in the presence of 6.5% salt. *L. mesenteroides* subsp. *mesenteroides* grows well in sugar cane juice having a sucrose content of 15 - 20%.

Atmosphere

Leuconostoc spp. are facultative anaerobes and will grow in vacuum-packed and MAP meats. Growth in the presence of oxygen is said to be enhanced by the presence of 15% carbon dioxide.

Preservatives and biocides

L. carnosum, associated with cured meats, has been reported to show some resistance to nitrite, and *L. oenos* is quite resistant to sulphur dioxide. Extracellular dextran production may have a protective effect on cells exposed to biocides.

Control

Leuconostoc spp. can be difficult to eradicate from food-processing environments, particularly where large quantities of sugar are present. Those strains that produce dextran from sucrose show enhanced resistance to heat and other environmental stresses, and readily form biofilms in association with other spoilage organisms. For cooked meat products, an adequate heat process is required and effective cleaning and hygiene procedures are essential to prevent recontamination during operations such as slicing. Experiments using Pulsed Electric fields of 30 - 50 kV/cm at 50 ºC have resulted in 5 log cycle reductions of *L. mesenteroides* in orange juice.

MICROCOCCUS

The Organism *Micrococcus*

Micrococcus spp. are Gram-positive, non-sporeforming, non-motile cocci that occur in pairs, tetrads or clusters, but not in chains. They are both catalase- and oxidase-positive and members of the family Microccaceae. Micrococci are generally obligate aerobes with a strictly respiratory metabolism, although one species has been reported to be capable of anaerobic growth. Some species produce pigmented colonies ranging from yellow to pinkish-red. Recently, some species have been transferred to new

genera, but only the genus *Kocuria* is associated with foods. The distinction between micrococci and staphylococci is not always clear, and misidentification may occur.

Key Species in Foods

Species isolated from foods include *Micrococcus luteus* and *Micrococcus varians*. The latter has recently been reclassified as *Kocuria varians*, but is still generally referred to as *M. varians* in the literature. Micrococci isolated from foods are often not identified to species level.

Sources

Environment

The skin of animals appears to be the natural environment of most *Micrococcus* spp.; they are also common airborne contaminants and are frequently isolated in dust and on dry surfaces.

Foods

Micrococci are often found associated with dried meat products and are also common in milk and on the surface of cattle and sheep carcasses. They may be used as starter cultures in some fermented meat and dairy products.

Spoilage Characteristics

Micrococci are not generally considered to be important spoilage organisms but may contribute to spoilage of a wide range of products, particularly those subjected to pasteurisation, or intermediate-moisture foods. Extensive growth on meat surfaces may cause discoloration, and slime may be formed on the surface of cured meats. They have been isolated from spoiled pasteurised milk and liquid egg.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Data on the effect of temperature on the growth of micrococci are sparse, but some strains are psychrotrophic. The optimum growth temperature is thought to be between 25 ºC and 37 ºC. *Micrococcus* spp. have been reported to grow on meat at 4 ºC, and isolates of *M. luteus* and *M. varians* were found to grow in milk at 7.2 ºC. A strain of *M. varians* did not grow on MAP meat at 4 ºC, but did grow at 10 ºC. The same organism grew at 37 ºC, but not at 45 ºC.

Micrococci can be described as thermoduric and may survive mild heat processes. *M. varians* was found to have a D-value at 70 ºC of 0.15 minutes in a fish terrine, and has been reported to survive in milk at 68 ºC for 16 seconds. An isolate identified as *Micrococcus freudenreichii* was found to have a D-value at 62 ºC of 3.5 minutes. Survival at such temperatures can be extended greatly at reduced a_w values and in fatty products, but the effect is less marked at higher temperatures.

pH

There is little information on the effect of pH on growth, but *M. varians* was found to grow at a pH of 5.0, and some isolates of *Micrococcus* spp. have been reported to grow at 4.5.

Water activity

Micrococci are quite resistant to drying and may survive for long periods in low-moisture environments. Growth of *M. luteus* has been shown at a_w of 0.93, and some isolates are able to grow at a salt concentration of 15%. Micrococci have also been isolated from curing brines with salt concentrations of approximately 20%.

Atmosphere

Most species are strict aerobes, but *M. varians* has been shown to be capable of growth in the absence of oxygen and may be involved in spoilage of vacuum-packed meats or meat stored under carbon dioxide.

Preservatives and biocides

There is very little published information on the effect of antimicrobials on micrococci; however, a strain of *M. luteus* has been shown to be more resistant to four common sanitisers than several other Gram-positive organisms.

Control

Micrococci are often overlooked in foods, but may be troublesome in pasteurised products, especially those with a high salt content, where there are few competing organisms. They may persist for long periods on dry surfaces, and effective hygiene programmes are necessary to prevent colonisation of suitable environments. Prevention of airborne contamination by micrococci requires adequate air filtration and handling systems.

MORAXELLA

The Organism *Moraxella*

Moraxella spp. belong to the family Moraxellaceae along with *Acinetobacter*, *Psychrobacter* and other related groups. They are Gram-negative, aerobic (although some strains are weakly facultative), non-motile, non-sporeforming organisms. The genus *Moraxella* contains two distinct groups or subgenera. In the first group the organisms occur in pairs or chains and are rod-shaped. They are in the subspecies *Moraxella*. The second subgroup, *Branhamella*, contains the cocco-bacilli that occur singly or in pairs. However, they show pleiomorphic characteristics when grown in conditions of reduced oxygen and elevated temperatures. They have a strictly respiratory metabolism, are oxidase- and catalase-positive, and do not produce acid from glucose. They can be distinguished from *Acinetobacter* on the basis of their oxidation reaction.

Key Species in Foods

Species isolated from foods include *Moraxella osloensis*, *Moraxella atlantae*, and *Moraxella phenylpyruvica*, although it has recently been proposed that the last species be transferred to the genus *Psychrobacter*.

Sources

Environment

Moraxella spp. are widely distributed in soil. They are also found in marine environments and are present on the skin of humans and animals.

Foods

These organisms are often found on chilled proteinaceous foods such as fresh meat and poultry, and are usually part of the initial microflora on the surface of dressed animal carcasses. They have also been isolated from dairy products, and form the predominant flora of fish.

Spoilage Characteristics

Moraxella spp. are part of the normal spoilage flora of fresh meat and poultry stored under aerobic conditions. They may produce 'off' odours as a result of amino acid metabolism, but are often regarded as having low spoilage potential.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

The optimum growth temperature is 33 - 35 ºC. Some strains are psychrotrophic, and growth at 4 ºC may occur, but *Moraxella* spp. do not compete well under these conditions.

pH

Moraxella spp. are favoured by pH values of 6.0 or above, and do not grow well on meats at lower pH.

Water activity

A minimum a_w value permitting growth as high as 0.99 has been reported for a meat-spoilage isolate. *Moraxella* spp. tend to die out on the surface of lamb carcasses during storage, and it has been suggested that this is due to desiccation.

Atmosphere

Moraxella spp. are aerobic organisms and are not normally involved in the spoilage of vacuum-packed or similar products. However, a few strains have been reported to grow slowly under anaerobic conditions.

Preservatives and biocides

There is very little published information on the effect of antimicrobials on *Moraxella* spp.

Control

Moraxella spp. are normally present on the surface of fresh meat and poultry carcasses, and may be involved in spoilage under aerobic conditions, particularly if the temperature is allowed to rise above 8 ºC. Spoilage is therefore best prevented by careful temperature control. Prevention of cross-contamination from the skin during processing by good hygiene practice is also important. Kill values reported for radiation treatment of *Moraxella* spp. is between 0.191 and 0.86 kGy.

PHOTOBACTERIUM

The Organism *Photobacterium*

The genus *Photobacterium* spp. belonging to the family Vibrionaceae, are large, facultatively anaerobic, Gram-negative, catalase-positive, non-sporeforming rods that are usually motile; they are capable of fermentative and oxidative metabolism. Oxidase reaction is variable, and most isolates have a positive requirement for sodium ions. Some species show marked bioluminescence.

Key Species in Foods

There are currently 12 species and 2 subspecies of *Photobacterium*; however, *Photobacterium phosphoreum* is the species most often found in foods.

Sources

Environment

The principal source of *Ph. phosphoreum* is temperate marine waters.

Foods

Ph. phosphoreum is generally associated with marine fish, and can be isolated from the skin and in the digestive tract.

Spoilage Characteristics

It has recently been suggested that *Ph. phosphoreum* is the principal organism responsible for spoilage in chilled MAP and vacuum-packed temperate marine fish, particularly cod. Spoilage is usually apparent as objectionable odours, produced by the reduction of trimethyl amine oxide (TMAO) to trimethyl amine (TMA). TMA is largely responsible for the characteristic fishy odour of spoiled fish. The importance of the organism in

fish spoilage generally is still uncertain, partly because conventional culture techniques may greatly underestimate its numbers.

In addition to being a spoilage organism, *Photobacterium* has been associated with food poisoning due to histamine production. *Ph. phosphoreum* was found to be the causative organism in an incidence of food poisoning due to histamine fish poisoning from dried sardines.

Ph. phosphoreum has also been isolated in large numbers from visibly luminescent cooked shellfish. Post-cooking contamination and subsequent growth during storage were thought to have occurred.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Optimum temperature for growth is generally 18 - 25 ºC. *Ph. phosphoreum* is psychrotrophic and grows well at 5 ºC. Minimum temperature for growth is reported to be approximately 0 ºC in fish, and growth does not usually occur at 35 ºC.

Information on heat resistance is very scarce, but the organism seems to be quite heat-sensitive and may be killed by the temperatures attained in pour plate culture techniques.

pH

There is little published information on the effect of pH, but the minimum value for growth is probably approximately 5.0.

Water activity

There is very little published information about the effect of a_w on the growth of *Ph. phosphoreum*, but growth is reported to occur at a salt concentration of 6.5%.

Atmosphere

The organism is a facultative anaerobe, and growth has been demonstrated in MAP and vacuum-packed fish. It is reported to be carbon dioxide tolerant.

Reports have shown that histamine production is strongly inhibited in products packed in 40% carbon dioxide/60% oxygen.

Preservatives and biocides

There is little published information on the effect of biocides and preservatives. However, inhibition of growth in MAP fish by potassium sorbate and chelating agents has been reported.

Control

Ph. phosphoreum appears to be quite common in temperate marine fish and may be more important in spoilage than is appreciated. Its heat sensitivity may allow control by minimal thermal processes. It has also been suggested that specific inhibition of the organism in MAP fish may extend shelf-life significantly.

PROTEUS

The Organism *Proteus*

Proteus spp. are members of the Enterobacteriaceae and are Gram-negative, catalase-positive, oxidase-negative non-sporeforming rods that often display pleomorphism. They are motile and often show swarming growth on moist agar plates. They are facultative anaerobes, possessing both respiratory and fermentative metabolism, and both acid and gas are produced from glucose. Some organisms previously classified as *Proteus* spp. have recently been assigned to other genera.

Key Species in Foods

There are now only two species important in foods, *Proteus mirabilis* and *Proteus vulgaris*. *Proteus morganii*, which is an important producer of histamine in scombroid fish, has been placed in the new genus *Morganella*.

Sources

Environment

Proteus spp. are found in human and animal faeces, soil and polluted waters.

Foods

These organisms are found in vegetables, meat, poultry, fresh-water fish and raw milk. Their entry into the food chain may be associated with foods via faecal contamination; however, they are not necessarily a reliable indicator of this type of contamination.

Spoilage Characteristics

Proteus spp. may be responsible for spoilage of cured meats such as dry-cured hams, sweet-cured bacon, and vacuum-packed bacon. Spoilage may take the form of souring or tainting. The spoilage of vacuum-packed bacon is referred to as 'cabbage odour'. In cottage cheese, it causes spoilage termed as 'slimy curd' due to slime formation and production of off-flavours and off-odours. They may also cause 'custard rots' in eggs, and have been shown to produce histamine in scombroid fish.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

These organisms are usually considered to be mesophilic, but a few strains have been shown to grow psychrotrophically. *P. vulgaris* has been found to grow very slowly at 4 ºC, but growth has not been detected at 1 ºC.

Little data has been published on the heat resistance of *Proteus* spp., but it has not been reported to be very significant.

pH

Growth has been shown to occur over the pH range 4.4 - 9.2 in laboratory media.

Water activity

There is little published information on the effect of water activity on growth. Growth in cured ham was reported at a salt concentration of 5%, but not at 6%.

Atmosphere

The organism is a facultative anaerobe and will grow in vacuum-packed bacon.

Preservatives and biocides

There is little published information on the effect of antimicrobials.

Control

Proteus spp. are only really important as spoilage organisms in chilled foods under conditions of temperature abuse. Therefore, good temperature control is essential. Adequate hygiene procedures are also important controls, especially for cured meat products that are not kept below 5 ºC throughout processing.

PSEUDOMONAS

The Organism *Pseudomonas*

The genus *Pseudomonas* is large and heterogeneous, but contains several species that are very important in spoilage, particularly of chilled foods, since they are psychrotrophic. The pseudomonads are Gram-negative, oxidase- and catalase-positive, non-sporeforming small rods. They are aerobic and the cells are usually motile. Some species produce water-soluble, fluorescent pigments. They are members of the family Pseudomonaceae.

Key Species in Foods

Pseudomonas fluorescens and *Pseudomonas fragi* are the two species most commonly associated with spoilage of chilled foods, and both *Pseudomonas lundensis* and *Pseudomonas putida* are also important. *Pseudomonas aeruginosa* is an opportunistic pathogen, but is probably of more significance in water supplies than in foods.

Sources

Environment

Pseudomonas spp. are important components of the microflora of soil and water, and are widely distributed in the environment. They are frequently isolated from plant matter.

Foods

Pseudomonads are common contaminants of fresh produce including spinach, lettuce, cabbage, potatoes, and tomatoes, and are usually the predominant spoilage organisms in chilled foods with a near neutral pH and a high water activity, such as fresh meat, poultry, milk and fish.

Other foods with which pseudomonads are associated include butter, cocoa beans, cream, eggs, cereals and mushrooms.

Spoilage Characteristics

The aerobic growth of pseudomonads in chilled foods may result in spoilage due to 'off' odours produced as the organisms utilise amino acids, releasing sulphur-containing compounds, such as dimethyl sulphide, and amines, such as cadaverine and putrescine. Pseudomonad populations of 10^6 cfu/g may be sufficient to produce odours in milk. In fresh meats, surface counts of $>10^7$ cfu/cm^2 are required for odour production, and populations of 10^8 cfu/cm^2 or more produce visible slime on the meat surface. *Pseudomonas* spp. also produce extracellular proteolytic enzymes and lipases, which may have a direct role in spoilage, and which are able to survive some pasteurisation processes and affect products such as UHT milks.

They are also associated with spoilage of butter, resulting in 'off' odour formation and rancidity. Some are associated with the formation of fruity odours or black discoloration of the butter.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

The species important in food spoilage are usually psychrotrophic, and are able to grow and form colonies at 0 - 7 ºC. For example, *Ps. fragi* has been observed to produce detectable slime on fresh meat stored at 1 ºC, and slow growth has been demonstrated for *Ps. fluorescens* at 0 ºC. Mesophilic pseudomonads do not grow at 10 ºC or below. Maximum growth temperatures vary, but key food-spoilage species do not usually grow above 35 - 40 ºC.

Pseudomonas spp. are not heat-resistant and are readily destroyed by mild pasteurisation treatments. D-values at 55 ºC in the range of 1 - 6 minutes are reported for food-spoilage species.

pH

Pseudomonads are not acid-tolerant and most species are able to grow within a range of 5.0 - 9.0, with an optimum of 6.0 - 8.0.

Water activity

Growth does not occur at low a_w values, and pseudomonads are not tolerant of drying. They are found on the surface of foods with a_w 0.99 or higher. No sign of growth has been observed at a_w 0.91. The minimum a_w for growth is dependent on other factors, such as the solute present, pH, etc., but under otherwise ideal conditions, growth at 0.95 - 0.97 is possible. However, for practical purposes in foods, values below 0.97 severely inhibit these organisms.

Atmosphere

Pseudomonads are obligate aerobes and do not grow or cause spoilage in the absence of oxygen. Their presence in large numbers in foods that would not be expected to support their growth, such as vacuum-packed meats, may indicate faulty packaging.

Preservatives and biocides

Pseudomonas spp. tend to be relatively resistant to many biocides, including quaternary ammonium compounds (QAC). They are particularly difficult to eradicate from contaminated equipment if present within biofilms.

At pH 6 and following incubation at 5 ºC in nutrient broth, >10 parts per million of diacetyl inhibited *P. fluorescens*.

Control

Pseudomonads are common, and widely dispersed in foods and the environment. Their ability to grow at low temperatures and cause spoilage of a wide range of chilled foods can cause serious problems for manufacturers. Control of spoilage in susceptible products is best achieved by the use of high-quality raw materials, and the operation of thorough cleaning and hygiene regimes. Mild heat processes are a very effective means of destroying pseudomonads, but high standards of post-process hygiene are necessary to prevent recontamination.

Chemical interventions used to control *Pseudomonas* spp. include ozone, chlorine, hydrogen peroxide and organic acids. Studies have shown that exposure of *P. fluorescens* to 2.5 parts per million of ozone for 40 seconds results in a 5 - 6 log reduction in bacterial counts. 4 log reductions of surface contamination have also been achieved with hydrogen peroxide. *Pseudomonas* spp. are sensitive to chlorine and chlorine dioxide. The D10 value for *P. fluorescens* subjected to irradiation is in the range of 0.5 - 1.0 kGy.

PSYCHROBACTER

The Organism *Psychrobacter*

The genus *Psychrobacter*, belonging to the family Moraxellaceae, was proposed to include organisms previously classified as *Acinetobacter* and *Moraxella*, and also contains those organisms sometimes referred to as *Moraxella*-like. They are Gram-negative, non-sporeforming, non-motile rods or cocco-bacilli, often occurring in pairs. They are aerobic, catalase and oxidase-positive, and their metabolism is respiratory.

Key Species in Foods

The genus originally contained only one species, *Psychrobacter immobilis*, first described in 1986; since then 26 species have been described. The importance of these species in foods is as yet unknown.

Sources

Environment

The natural habitat of *Psy. immobilis* is water. It has also been isolated from soil, air and sea ice.

Foods

The organism is commonly isolated from proteinaceous foods such as meats, poultry, fish and milk.

Spoilage Characteristics

Psy. immobilis is part of the typical spoilage microflora found on chilled fresh meats and poultry. Its importance is uncertain, since it is not proteolytic and does not compete well with pseudomonads. However, it does produce some volatile metabolites and may cause lipolysis in meat if present in high numbers. It has also been found as a post-pasteurisation contaminant in spoiled pasteurised milk.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Psy. immobilis is psychrotrophic and grows well at 5 ºC. Minimum temperature for growth is reported to be approximately 1 - 2 ºC; optimum growth temperature is 20 ºC. Growth does not usually occur at 35 - 37 ºC and those strains that grow do not normally grow at 5 ºC.

Information on the effect of high temperatures is very scarce, but *Psy. immobilis* has not been reported to have any significant heat resistance.

pH

There is little published information on the effect of pH, but growth seems to be favoured by near neutral pH values above 5.5.

Water activity

There is very little published information about the effect of a_w on the growth of *Psy. immobilis*, but growth is reported to occur at a salt concentration of 6.5%.

Atmosphere

The organism is a strict aerobe; however, some strains can grow under anaerobic conditions provided there is a suitable electron acceptor.

Preservatives and biocides

There is little published information on the effect of biocides and preservatives.

Control

Selection of good-quality raw materials and mild heat processes are effective controls for *Psy. immobilis*. However, as with pseudomonads,

adequate sanitation and hygiene procedures are vital to prevent recontamination, particularly in dairies.

SERRATIA

The Organism *Serratia*

Serratia spp. are members of the Enterobacteriaceae and are motile, Gram-negative, non-sporeforming rods. They are facultative anaerobes, possessing both respiratory and fermentative metabolism, and are catalase-positive and oxidase-negative. Acid and sometimes gas is produced from glucose. Some strains produce pink or red pigments, but the colonies of others are colourless.

Key Species in Foods

The principal species of importance in foods is *Serratia liquefaciens*. Other species that may occasionally be isolated are *Serratia marcescens* and *Serratia plymuthica*.

Sources

Environment

Serratia spp. are found in soil, water and the digestive tracts of animals, and on plant material.

Foods

The organisms are found in a wide range of foods, particularly refrigerated meat and vegetables, and eggs. *S. plymuthica* is often associated with freshwater fish.

Spoilage Characteristics

S. liquefaciens is an important spoilage organism in packed meat products. It produces volatile amines such as putrescine and cadaverine in vacuum-packed meats. It grows very well in vacuum-packed, high pH meat, and H_2S is produced under these conditions. This is one reason why dark, firm, and dry (DFD) meat is not considered suitable for vacuum packing. *Serratia* spp. may also produce heat-stable lipolytic enzymes in dairy products and may be involved in egg spoilage. *S. marcescens* is sometimes associated with a condition known as 'red bread', where growth and pigment production occur within bread that has high moisture content.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Some strains of *S. liquefaciens* are psychrotrophic and growth occurs at 4 - 5 ºC. However, reports of minimum growth temperatures are variable. A value of 1.7 ºC has been obtained in laboratory media, but the minimum for growth in foods is uncertain.

There is little published information on the heat resistance of these organisms, but there are no established reports of strains that are able to survive pasteurisation processes.

pH

Growth of *S. liquefaciens* is favoured by pH values of >6.0 in meats. The pH range within which growth occurs is approximately 5 - 9.

Water activity

A minimum water activity permitting growth of 0.93 has been reported for *S. liquefaciens*.

Atmosphere

The organism is a facultative anaerobe and will grow in vacuum-packed meats. It will also grow in MAP meats and is resistant to carbon dioxide, having been shown to cause meat spoilage at concentrations of 50%.

Preservatives and biocides

S. liquefaciens is reportedly not inhibited by sorbic or benzoic acids at 0.15% in vacuum-packed meat. However, acetic acid at a concentration of 0.1% did delay growth and higher concentrations prevented it. Studies have shown that *S. liquefaciens* and *S. marcescens* are resistant to 185 - 270 parts per million sulphur dioxide.

Control

The importance of *S. liquefaciens* in spoilage is limited mainly to vacuum-packed and DFD meat products. Effective cleaning and hygiene procedures are important to minimise the initial level of contamination of these products. Mild heat processes are also effective.

SHEWANELLA

The genus *Shewanella* was proposed in 1985, for a group of organisms previously classified as *Alteromonas putrefaciens* and, before that, *Pseudomonas putrefaciens*. Originally, one species, *Shewanella putrefaciens*, was included, but this included a highly heterogeneous group of isolates from different sources, and there are now a further three accepted species.

The Organism *Shewanella*

The organisms are aerobic, Gram-negative, straight or curved non-sporeforming, motile rods. *S. putrefaciens* is catalase and oxidase-positive and has a respiratory metabolism, but is well able to grow in the absence of

oxygen using alternative terminal electron acceptors. They are members of the family Shewanellaceae.

Key Species in Foods

Although the genus *Shewanella* comprises more than 20 species, only *S. putrefaciens* is important in food spoilage. The other species are all found in marine environments. There have been suggestions that one of these, *Shewanella alga*, may be a human pathogen, but it is not known whether it can cause foodborne infection.

Sources

Environment

The principal source of *S. putrefaciens* is temperate marine waters, but it has been isolated from fresh water. It is also found in oil field wastes, surfaces of processing equipment and clinical specimens.

Foods

S. putrefaciens predominate in marine fish, particularly ice-stored, or MAP fish. However, it can also be isolated from poultry and from other meats, particularly high pH meat.

Spoilage Characteristics

Spoilage is usually apparent as objectionable odours. In fish, especially gadoid fish such as cod, *S. putrefaciens* reduces trimethyl amine oxide (TMAO) to trimethyl amine (TMA). TMA is largely responsible for the characteristic odour of spoiled fish. The organism also produces sulphur compounds including H_2S, methyl mercaptan, and dimethyl disulfide from sulphur containing amino acids. *S. putrefaciens* is also a major cause of 'greening' in high pH meat, and may grow rapidly on chicken leg meat and skin, both of which have higher pH values than breast meat.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

S. putrefaciens is psychrotrophic and capable of forming colonies at 0 - 7 ºC. They grow well at 5 ºC. Minimum temperature for growth is reported to be approximately 2 ºC. *S. putrefaciens* is unable to grow at temperatures exceeding 37 ºC.

Information on heat resistance is very scarce, but an isolate from marine fish was reported not to survive at 55 ºC for 20 seconds.

pH

Growth of *S. putrefaciens* is favoured by pH values above 6.0. It will not tolerate acid conditions, and a minimum pH value for growth of approximately 5.3 has been reported. In meat stored at low temperatures, growth does not usually occur below pH 6.0.

Water activity

S. putrefaciens are found in foods having a_w 0.99 or higher. They show no signs of growth at a_w 0.91. The minimum a_w required for their growth is in the range of 0.95 - 0.97 depending on the type of food and salts or sugars used. Inhibition of growth by salt at a concentration of 5% has been reported.

Atmosphere

Although principally an aerobic organism, the ability of *S. putrefaciens* to grow anaerobically enables it to grow very well in vacuum-packed and MAP fish and meats. Inhibition by high concentrations of carbon dioxide (up to 10%) has been reported.

Preservatives and biocides

There is little published information on the effect of biocides and preservatives. However, an isolate of *S. putrefaciens* from poultry was found

to be more resistant than *Pseudomonas* spp. to sodium hypochlorite and other sanitisers.

Control

S. putrefaciens can often be readily isolated from fish and meat processing plants, and may become established in such environments. Therefore, effective cleaning and sanitation are important controls to minimise product contamination. Reduced pH is also an effective control in foods. Ionising radiation of 1.5 kGy has been found to be effective in reducing the number of *S. putrefaciens* on the surface of beef, pork, turkey and poultry.

SPOROLACTOBACILLUS

The genus *Sporolactobacillus* were originally thought to be an intermediate form between *Lactobacillus* and *Clostridum*. *Sporolactobacillus* have since been given their own genera and moved into the family Bacillaceae and are thought to be intermediates between Bacillaceae and Lactobacillaceae.

The Organism *Sporolactobacillus*

Sporolactobacillus are microaerophilic or facultatively anaerobic, Gram-positive rods occurring singly or in pairs. They are spore-forming with terminal endospores and swollen sporangia, motile, oxidase-negative, catalase-variable (although usually negative), and homofermentative, producing lactic acid from hexose sugars.

Key Species in Foods

The type species is *Sporolactobacillus inulinus* but the genus also contains other species. There are thought to be 5 species in total but the organisms similarity to *Bacillus* and *Lactobacillus* can make classification difficult.

Sources

Environment

Found in soil, chicken feed and root crops but are distributed in low numbers. The organism has been isolated from the faeces and gut of herbivorous animals and from the waste water of an abattoir, thereby suggesting its possible presence in meat.

Foods

The relevance of the organism in foods remains unclear. Published literature suggests that little information is available regarding the physiology, characteristics or habitats of *Sporolactobacillus* as it is a rare food spoiler, however the organism does have the potential to cause spoilage should the spores survive heat treatment. *Sporolactobacillus* is thought to be a spoilage organism in soft drinks and has been isolated from orange juice.

Spoilage Characteristics

Spoilage of foods is by the conversion of sugars to lactic acid.

Growth/Survival Characteristics of the Organisms in Foods

Temperature

Sporolactobacillus are mesophilic organisms with an optimum temperature for growth of 35 °C. Minimum reported figures for growth are 10 °C and no growth has been seen above 45 °C.

Spores of *S. inulinus* are thought to have a comparatively low heat resistance, however a spore heat resistance of D-value 90 °C for 1 - 8 minutes has been recorded, the same as for other bacilli. Another study recorded a D-value of 5 minutes at 80 °C with an a_w of 0.95 and pH 7.

pH

Growth has been reported between pH 3.5 - 5.5 with an optimum of pH 4.5. No growth has been observed at pH 9.6.

Water activity

The germination of spores is inhibited by water activities below 0.955 when sodium chloride (NaCl) is the humectant. In glycerol figures of 0.880 have been recorded. Vegetative cells were inhibited by a_w of 0.955 in NaCl and 0.905 in glycerol. Growth has been reported in the presence of 5% NaCl but not at 6.5% NaCl.

Atmosphere

Sporolactobacilli are microaerophillic of facultatively anerobic.

Preservatives and biocides

Laboratory studies have shown vegetative growth of *Sporolactobacillus* to be inactivated by 5000 - 7000 µg/ml of potassium sorbate. 2000 µg/ml sodium nitrate is insufficient to inactivate the organism.

Ionising radiation of 0.350 - 0.525 kGy has been found to be effective in reducing the numbers of vegetative cells of *Sporolactobacillus* and 2.5 kGy for spores.

Control

As *Sporolactobacillus* are preservative resistant and their removal cannot be guaranteed by pasteurisation temperatures, control of the quality of raw materials is essential for the prevention of food spoilage by this organism.

OTHER BACTERIA THAT MAY CAUSE MICROBIOLOGICAL SPOILAGE

The principal genera of bacteria that are involved in the microbiological spoilage of foods are covered in the preceding pages of this section of Micro-Facts. However, this list cannot be exhaustive, and there are other bacteria that have occasionally been implicated in the spoilage of particular foods. Some examples are given below.

Aeromonas

Aeromonas spp. are Gram-negative, catalase- and oxidase-positive, facultatively anaerobic rods that produce large quantities of gas from the fermentation of carbohydrates. They are non-sporeforming, generally motile members of the family Vibrionaceae. Although considered generally aquatic organisms they have been recovered from the meat of different animals (cows, chickens, pigs, lambs), plants (broccoli, celery, spinach, alfalfa sprouts), and food products of animal origin, including raw milk. They are frequent contaminants of marine water and freshwater, but can also be found in chlorinated and unchlorinated drinking water-distribution systems, and bottled uncarbonated mineral drinking water. Seafood, such as shellfish, oyster and fish, are common sources of this organism. *Aeromonas hydrophila* is an occasional cause of foodborne disease (an enterotoxin has been identified in *Aeromonas caviae* and *A. hydrophilia*), but is also important in fish spoilage, particularly at temperatures of >5 ºC. Other species are also common, and many are psychrotrophic or psychrophilic. *Aeromonas* spp. are important in the spoilage of freshwater and farmed fish, and some processed fish products.

Alcaligenes

The genus *Alcaligenes* consists of Gram-negative, aerobic, catalase and oxidase-positive, motile, non-sporeforming, non-fermentative rods, which may produce alkali from certain substrates. They are members of the family Alcaligenaceae. Some strains are capable of anaerobic respiration in the presence of nitrite or nitrate. They can grow between 5 ºC and 37 ºC but have an optimum growth temperature of between 20 - 37 ºC. They are found in soil and water, and are common inhabitants of the intestinal tract of some animals. They are therefore considered potential contaminants of dairy products, meat and poultry. They have been found in bottled and ground spring waters, marine and fresh fish, and on raw vegetables. *Alcaligenes* spp. have been associated with spoilage of eggs, pasteurised milk as a post-process contaminant, some meat products, such as bacon, and with rancidity in butter.

Carnobacterium

The genus *Carnobacterium* was proposed in 1987 to include species of lactobacilli. They are Gram-positive, catalase and oxidase-negative, motile or non-motile, non-sporeforming, facultatively anaerobic rods belonging to the family Lactobacillaceae. These organisms are psychrotrophic, and heterofermentative, but are unable to grow at pH 4.5, unlike most lactobacilli. This non-aciduric genus is highly specific to fresh meat and mainly poultry. *Carnobacterium divergens* is associated with vacuum-packed meat products and may produce tyramine (a product formed due to the decarboxylation of the amino acid tyrosine), but is not thought to be an important cause of spoilage. *Carnobacterium maltaromaticum* (formerly *Carnobacterium piscicola*) is found in fish, and may have a role in spoilage of MAP products where again it may produce tyramine.

Corynebacterium

Corynebacteria are Gram-positive pleomorphic, non-sporeforming rods. Some species may be slightly curved or have no club ends. They are facultative anaerobic, catalase-positive, oxidase-negative, non-sporeforming members of the family Corynebacteriaceae. Some are pathogens, but those found in foods are generally non-pathogenic and associated with plants. Most are mesotrophs but a few strains are psychrotrophic. *Corynebacterium* spp. have been isolated from raw grated beetroot and are associated with spoilage of vegetables and also fish harvested from warm waters. They may be part of the initial microflora of packaged meat products, but do not seem to be important in spoilage. They are prevalent in poultry slaughtering premises where they cross-contaminate equipment and surfaces from the skin follicles.

Erwinia

Proteolyic *Erwinia* spp. are members of the Enterobacteriaceae, but are mainly associated with plants. They are facultative anaerobes, Gram-negative, catalase-positive, oxidase-negative, motile, non-sporeforming straight rods. Species such as *Erwinia herbicola* and *Erwinia carotovora* (now called *Pectobacterium carotovora*) are important in the decay and spoilage of vegetable products, particularly ‘leafy’ products, such as fresh

pre-packed salads. *Erwinia* do not grow, and induce 'soft rot' of fresh produce stored at refrigeration temperatures.

Vibrio

Vibrio spp. are Gram-negative, facultatively anaerobic, catalase-positive, oxidase-negative, motile, non-spore forming, straight or curved rod shaped members of the Vibrionaceae. Several species are pathogens, such as *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. However, other species are common in aquatic environments and are often psychrotrophic and halophilic. They are important spoilage organisms in seafood, but *Vibrio costicola* are also associated with putrefactive spoilage of cured meat products.

Enterobacteriaceae

The family Enterobacteriaceae is a large group of Gram-negative, catalase-positive, oxidase-negative, non-sporeforming, motile and non-motile, facultatively anaerobic, rod shaped bacteria that ferment glucose and produce acid and gas. They include several important human pathogens like *Salmonella*, *Shigella*, *E. coli* and *Yersinia*, but some are involved in the microbiological spoilage of certain products. Strains of some species, such as *Citrobacter freundii*, *Klebsiella* spp, *Providencia rettgeri* and *Enterobacter agglomerans* (some strains now classified as *Pantoea agglomerans*) have been associated with spoilage of foods, particularly vacuum-packed and MAP meat and poultry products, and cured meats. Other members of the Enterobacteriaceae include *Edwardsiella*, *Erwinia*, *Escherichia*, *Hafnia*, *Proteus* and S*erratia*.

Enterobacteriaceae are often used as an indicator of process failure, or post-process contamination due to poor hygienic practices.

Coliform Bacteria

Coliform bacteria are members of the Enterobacteriaceae. They are defined as Gram-negative, rod-shaped organisms which ferment lactose to produce acid and gas at 37 ºC. Coliforms are commonly used as indicators of poor sanitary practices, inadequate processing or post-processing contamination e.g. *Edwardsiella*, *Klebsiella*, or *Arizona*. Coliforms can be found in the

aquatic environment, in soil, on vegetation and also in the faeces of warm-blooded animals.

Faecal coliforms include genera that originate from faeces. They produce acid and gas from lactose between 44 ºC and 46 ºC. *E. coli* is a typical faecal coliform, and is often used as an index of faecal contamination i.e. they can indicate the presence of other enteric pathogens such as *Salmonella*. They can also be used similarly to coliforms as indicators of poor sanitary practices, inadequate processing or post-processing contamination.

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FOOD-SPOILAGE FUNGI

MOULDS AND MYCOTOXINS IN FOOD

Mycotoxin contamination of crops has been a worldwide problem for thousands of years. However, the significance of the mycotoxins present in foods, its effect on human health and its impact on the economy has been assessed only over the last few decades. Plant fungal pathogens or food spoilage moulds are the source of this type of toxin (1). The Food and Agriculture Organisation (FAO) estimates that 25% of the world's food crops are affected by mycotoxins during growth and storage (2). Various different types of health problems are linked to the ingestion of different types of mycotoxins by humans or animals (1). Many outbreaks related to food contamination by mycotoxins have occurred all over the world, which is a global concern as a health hazard. Summaries of the key characteristics of the main moulds and mycotoxins considered in this chapter can be found in the following tables.

Table (A): shows the environmental requirements for the moulds and yeasts growth and toxins production by key toxin producing moulds.

Moulds, growth, toxin production	**Temperature**			**a_w**			**Ph**		
	Min	**Opt**	**Max**	**Min**	**Opt**	**Max**	**Min**	**Opt**	**Max**
A. parasiticus & *A. flavus*									
Growth	6 °C (8)	35 °C (2)	54 °C (8)	0.81 (9)	0.95 (2)	1 (8)	2.1 (4)	4-7 (4)	11.2 (4)
Aflatoxin production	15 °C (4)	33 °C (2)	37 °C (4)	0.83 (8)	0.99 (2)	0.97 (8)	3 (4)	6 (4)	8 (18)
A. ochraceus									
Growth	8 °C (2)	30 °C (2)	37 °C (2)	0.78 (9)	0.95-0.99 (4)	>0.99 (4)	2.2 (4)	3-8 (4)	13 (4)
OTA production by *A. ochraceus*	12 °C (2)	25 °C-30 °C (2)	37 °C (2)	0.83-0.87 (2)	0.98 (2)	>0.99 (4)	-	-	-
F. graminearum									
Growth	<5 °C (27)	24 °C-26 °C (27)	<37 °C (27)	0.90 (27)	-	-	2.4 (27)	-	9.5-10.2 (27)
Deoxynivalenol production by *F. graminearum*	15 (33)		24 °C, 25 (8, 33)	0.95	-	0.995-	-	-	-
Zearalenone production by *F. graminearum*	-	25 °C-30 °C (2)	-	-		>0.98 (2)-	-	-	-
F. culmorum									
Growth	5 (33)	35 (33)		0.86 (33)					
Nivalenol production by *F. culmorum*	15 °C (33)	25 °C (33)		0.95 (33)		0.98 (33)			

Moulds, growth, toxin production	Temperature			a_w			Ph		
	Min	Opt	Max	Min	Opt	Max	Min	Opt	Max
T-2 toxin production by some *Fusarium* species	-	15 °C (8)	-	-	-	-	-	-	-
P. verrucosum									
Growth	0 °C (2)	20 °C (4)	35 °C (2)	0.79-0.83 (4)	0.95 (2)	>0.99 (4)	<2.1 (4)	6-7 (4)	>10 (4)
OTA production by *P. verrucosum*	5 °C-10 °C (2)	25 °C (2)	-	0.83-0.85 (2)	0.90-0.95 (2)	-	-	-	-
P. expansum									
Growth	-2 °C to -6 °C (6)	20 °C -25 °C (6)	30 °C (6)	<0.86 (6)	0.99 (6)	-	-	-	-
Patulin production by *P. expansum*	-	15 °C-2 0°C (6)	-	-	-	-	-	-	3.5 (7)
Yeasts growth (generally)	~0 °C (21)	-	~50 °C (21)	0.90-0.95 (21)	-	-	3 (21)	4.5-6.5 (21)	10 (21)

Key: - indicates no data

Table (B): Summarises and relates the mycotoxins, producing fungi, illnesses and affected crops information.

Mycotoxin	Major producing fungi	Affected crops	Illness
Aflatoxins: B_1 B_2 G_1 G_2	*A. flavus*, *A. parasiticus* (8) & *A. nomius* (13)	Cereal grains, peanuts, corn, cottonseed, figs, most tree nuts, milk, sorghum and walnuts (8)	Hepatocarcinoma (acute aflatoxicosis) Acute liver damage, cirrhosis, carcinogenic, teratogenic and immunosuppressive (18)
Ochratoxins: OTA	*A. ochraceus*, *P. verrucosum* (8) & *A. carbonarius* (13)	Cereals, barley, beans, coffee, feeds, maze, oats, rice, rye, wheat (8), cheese, meat products and dry foods (fish, fruit, nuts) (16)	Balkan nephropathy and chronic interstitial nephropathy, Testicular cancer (4) Kidney necrosis, teratogenic and immunosuppressive (18)
Patulin	*A. clavatus* (8) & *P. expansum* (13), *P. griseofulvum*, *P. roqueforti* var *carneum*, *Byssochlamys* and *Paecilomyces* (26)	Apple, apple juice, beans, wheat (8), apricots, grapes, peaches, pears, olives, cereals and low acid fruit juices (16)	Hepatotoxicity and teratogenicity affects (8). Increase the cell permeability and inhibits several enzymes in the cell (1)
Fumonisins B_1	*F. verticillioides*, *F. proliferatum* (4) & *F. subglutinans* (13)	Corn, sorghum and rice (8)	Oesophageal tumours, human neural tube defects (4), pulmonary oedema (pigs) (18)
Type 1 and Type 2 Trichothecenes – for example, T-2 and HT-2 toxins, and nivalenol and deoxynivalenol	*F. graminearum*, *F. equiseti*, *F. nivale*, *F. sporotrichioides* (4), *F. poae*, *F. chlamydosporum*, *F. acuminatum* (13), *F. culmorum*	Corn, cereals, feeds and hay (8)	Alimentary toxic aleukia; variable symptoms, leukaemia; depression of immune response (18) and inhibition of protein synthesis (16)
Zearalenone	*F. graminearum*, *F. equiseti* (4), *F. culmorum* & *F. crookwellense* (13)	Cereals, corn, feeds, rice (8)	Hyperoestrogenism (swine) (20), reproductive disorders (humans/animals) (3)

MYCOTOXINS

Toxins produced by foodborne fungi are termed mycotoxins; toxins produced by Basidiomycetes (e.g. mushrooms or toadstools) are not included in the mycotoxin group (3, 4). Mycotoxins are secondary metabolites produced by fungi and consist of relatively small, low weight molecules (1). They have four distinct types of toxicity - acute, chronic, mutagenic and teratogenic (5). Mycotoxins are produced only at certain stages of mould growth. Fungi produce mycotoxins under stressful conditions such as changes in temperature, moisture, aeration, or the presence of aggressive agents.

The action of mycotoxins in the human body is described as a mycotoxicosis (4). Symptoms of mycotoxicoses vary depending on the quantity of toxin consumed, the chemical structure of the toxin, the target site in the body, the host species affected, and the host status (1).

The problem of mycotoxin contamination is complex for a number of reasons, the difficulties encountered in detecting the toxin in the body, the different types produced, the fact that contamination might occur anywhere in the food chain, and that a food may contain more than one type of toxin (3). In addition, many mycotoxins are heat-resistant and are not easily inactivated by cooking and sterilising procedures used in food processing (6). Some mycotoxins may act synergistically, enhancing the effect of each toxin on the host body (7) e.g. co-occurrence of fumonisins and aflatoxins may cause enhanced liver carcinogenesis in humans (4). Therefore, the presence or absence of mould growth is not an indicator of the level of toxins present (7), although the isolation of toxigenic moulds should be treated as an index of the potential presence of mycotoxins, in much the same way that coliforms or Enterobacteriaceae can be used as indicators of poor sanitary practices, inadequate processing or post-process contamination, and *E. coli* as an index of faecal contamination.

Field crops are more susceptible to fungal attack if drought stressed, as a result of a lack of water, overcrowding, invasion by weeds, poor soil, insect

attack, or because some varieties are more susceptible to invasion by the fungus (8).

This chapter will cover the most common mycotoxins that are responsible for major epidemics in humans and animals in recent times.

Note: The literature on the subject of mycotoxin contamination can be confusing, as many fungal names have changed over the years, particularly within *Penicillium* and *Fusarium*; the attribution of a certain mycotoxin to a particular mould may have changed with time; take care with older references. Secondly, studies using pure cultures to grow mycotoxins in the laboratory have been fraught with problems when contaminated cultures may have been used inadvertently.

AFLATOXINS

Aflatoxins are highly potent carcinogens that were discovered in the early 1960s (4, 9, 10, 11). Aflatoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. Four major aflatoxins are known: B_1, B_2, G_1 and G_2. B and G indicate the blue and green fluorescent colours produced under ultra violet (UV) light, and 1 and 2 indicate the major and minor compounds respectively (4). When aflatoxins B_1 and B_2 are ingested by lactating cows, a proportion is hydroxylated in the liver and excreted in the cows' milk as aflatoxins M_1 and M_2; they have lower toxicity than B_1 and B_2 (3).

Sources

Aflatoxins B_1, B_2, G_1 and G_2 are commonly found in corn, peanuts, most tree nuts, dried fruits, figs, milk, oil-bearing seeds or spices and certain cereals (4, 6).

Illness Caused

Short-term acute symptoms are due to the ingestion of large quantities of toxins, whereas long-term chronic symptoms occur due to the ingestion of small quantities of toxin over a longer period. Short-term symptoms of aflatoxin intoxication include ataxia, tremor, elevated temperature, anorexia,

loss of appetite, weight loss, haemorrhage, bloody faeces and brown urine. Long-term effects include acute liver damage, liver cirrhosis, tumour indication and teratogenesis (3). Typical symptoms of aflatoxicoses include proliferation of the bile duct, necrosis, fatty infiltration of the liver, and hepatomas with generalised hepatic lesions (4).

OCHRATOXIN A (OTA)

Ochratoxin A (OTA) is produced mainly by *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium* (mainly *Penicillium verrucosum*) (8). It is a potent nephrotoxin and teratogen and is often found in association with citrinin, penicillic acid and other mycotoxins (7, 12).

Sources

Ochratoxin A is found in cereals, coffee, cocoa, dried fruit, spices, dried and smoked fish, soybeans, garbanzo beans, nuts and sometimes in grapes (6, 13). Another source of contamination is the consumption of food of animal origin that has been fed with ochratoxin A-contaminated feeds, e.g. pork (4).

Illness Caused

Due to its solubility in fat and slow rate of excretion from the host body, ochratoxin A accumulates in the fatty tissues (5). In humans, it is believed that ochratoxin A is carcinogenic (12) and might cause liver necrosis and kidney damage. It has been known to be fatal. High levels of consumption of toxin and a long exposure period can induce renal tumours and liver cancer (4). In test animals it is known to act as an immunosuppressor and a teratogen and can trigger mutagenicity in human cytochrome enzymes.

PATULIN

Patulin was first isolated in the 1940s from *Penicillium patulum* (1). Patulin is produced by some species of *Penicillium* mostly *Penicillium expansum*, *Penicillium roqueforti* var. *carneum* (also known as *Penicillium carneum*) and *Penicillium griseofulvum* (formerly known as *P. patulum*) (12), and also *Aspergillus* and *Byssochlamys* species (8).

Sources

Patulin is usually found in apples and apple-derived products, apricots, grapes, peaches, pears, olives, cereals and low acid fruit juices (12, 13).

Illness Caused

Patulin is hepatotoxic and teratogenic (4), and is very toxic to both prokaryotes and eukaryotes. It is believed to affect the cell membrane by increasing its permeability and can inhibit several enzymes in the cell *in vitro* (8). Human toxicity has not been confirmed (12), although based upon adverse effects due to patulin in animal studies, the FDA believes that humans may be at risk at some level of exposure to patulin (14).

FUMONISINS

Fumonisins were first described and characterised in the late 1980s (12). They are highly water soluble and are mainly produced by maize pathogens *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium subglutinans* (1, 13). Common fumonisin mycotoxins include: FB_1, FB_2, FB_3 (7) and FB_4.

Sources

Fumonsins are found mainly in corn (6).

Illness Caused

Fumonisins have a neurotoxic effect (including uncoordinated movements and blindness) (4), and can cause liver cancer in rats. Fumonisin B_1 can alter cardiovascular and hepatic function and elevate serum cholesterol. It can also affect immune function, cause kidney and liver damage, and can result in death (7). An epidemiological link between fumonisins and human neural tube defects has been reported (15). Fumonisin B_1 is classified as a class II carcinogen because of the possibility of it being a potent cancer trigger and promoter (4). It is believed that fumonisin B_1 is related to the occurrence of oesophageal cancer in China and South Africa (1).

TRICHOTHECENES

Trichothecenes are highly toxic metabolites produced by many species of *Fusarium* (3, 8), and include more than 200 different identified chemical structures (6, 12). They can be divided into Type A and Type B trichothecenes, with Type A being more toxic than Type B.

Type A trichothecenes include T-2, HT-2 and diacetoxyscirpenol. Type B trichothecenes include deoxynivalenol, nivalenol and 3- and 15-acetyldeoxynivalenol.

T-2 toxin and its derivative toxin

T-2 toxin was isolated in the mid-1960s (4) and is considered to be more toxic than HT-2 toxin. Major producers are *Fusarium acuminatum*, *Fusarium chlamydosporum*, *Fusarium equiseti*, *Fusarium poae* and *Fusarium sporotrichioides* (12, 16).

Diacetoxyscirpenol and monoacetylated derivatives

These are produced by *F. acuminatum*, *F. equiseti*, *F. poae* and *F. sporotrichioides* (12, 16).

Deoxynivalenol and its acetylated derivatives

These important mycotoxins are produced mostly by *Fusarium graminearum* and *Fusarium culmorum* (12).

Nivalenol and fusarenon-X

They are very similar to deoxynivalenol and its acetylated derivatives but are more toxic. Nivalenol is produced mostly by *F. poae*, *F. graminearum* and *Fusarium nivale* whereas fusarenon-X is produced by *F. graminearum* (12).

Sources

Deoxynivalenol is found mainly in wheat and barley, and sometimes in corn (17). Nivalenol is found in barley, wheat, wheat flour and rice (17). T-2 is found in corn (in field), corn products and feed (17). Diacetoxyscirpenol is found in grains, barley, corn, rye, safflower seeds, wheat and mixed feeds (1).

Illness Caused

Trichothecenes have many different toxic effects in humans and animals due to their structural diversity. Trichothecene mycotoxicoses are difficult to distinguish due to the multi-organ effect (gastrointestinal tract, hematopoietsis, nervous, immune, hepatobiliary and cardiovascular systems). Most of the major trichothecenes are cytotoxic and cause haemorrhage, oedema, and necrosis of skin tissues (4). Trichothecene toxicity also includes inhibition of the host protein synthesis (1). Symptoms of trichothecene poisoning are vomiting, diarrhoea, anorexia, nausea, abdominal pain, dizziness, headache, and gastrointestinal inflammation as rapid responses (18). Less immediate effects of trichothecenes include leucopenia, ataxia, haemorrhaging of muscular tissue and degeneration of nerve cells (3). Trichothecenes are known as feed refusal toxins due to loss of appetite being the first observed symptom (7).

T-2 toxin can cause alimentary toxic aleukia (ATA) which is considered to be the most important form of human food poisoning due to the ingestion of mouldy grains. Symptoms of ATA include fever, haemorrhagic rash,

bleeding from the nose, throat and gums, sepsis, and exhaustion of the bone marrow (3). T-2 toxin causes cellular damage in the bone marrow, intestines, spleen and lymph nodes, and is considered the most potent immunosuppressant of the mycotoxins (4).

Deoxynivalenol or vomitoxin can cause feed refusal 'anorexia' and emesis in humans/animals (4). Deoxynivalenol causes nausea, vomiting and diarrhoea when consumed in high doses, and causes weight loss and food refusal when consumed in low doses (1).

ZEARALENONE

Zearalenone, also called F-2, is mainly produced by *Fusarium* genera including *F. graminearum*, *F. culmorum* and *F. equiseti* (4, 8, 16). It can be produced in the field or during commodity storage (18).

Sources

Zearalenone is found in cereals, wheat, corn semolina and flour (6).

Illness Caused

Zearalenone is a phytoestrogen responsible for reproductive disorders in human and animals caused by its oestrogenic effect at high concentrations (7). This is due to the ability of zearalenone to mimic the body's production of oestrogen, causing feminisation in males, and interfering with the conception, ovulation and foetal development in females (17).

FOODBORNE MOULDS

Fungi can grow on crops in the field as well as in stored grain, resulting in rancidity, loss of nutrients, or damage to the germ layer reducing the quality and usability of grains, and colour, textural and odour changes. *Aspergillus*, *Penicillium* and *Fusarium* species are amongst the most common fungi associated with growth in, and damage to, food crops in the field, and in

store if poor storage conditions prevail after harvest, or previously dried commodities become rewetted (3). There is some variability in the growth data reported for many of the organisms included here.

Food mycology is a complex subject; many food spoilage moulds have sexual and asexual growth phases; both or either phase may be seen in a spoiled product. The nomenclature has changed considerably over the years; it is vital to make sure that any papers citing crops or products in which particular moulds are found use the up to date names. The same comment was noted previously with respect to mycotoxin production; for example, there have been numerous false reports of aflatoxins being produced by species other than the three already noted.

Some growth factors are much more important than others; water activity and temperature will determine which moulds grow and how fast growth will be, whereas pH has little effect, since most moulds are very tolerant of low pH.

There are a large number of other moulds that have been isolated from food ingredients, particularly cereals, oilseeds, and herbs and spices. These include *Eurotium*, *Cladosporium*, *Geotrichum*, *Mucor*, *Rhizopus*, *Moniliella*, *Paecilomyces*, *Wallemia*, *Byssochlamys*, *Talaromyces*, *Eupenicillium*, *Claviceps*, *Alternaria*, *Phoma*, *Phomopsis*, *Curvularia*, *Chaetomium*, *Xeromyces* and *Chrysosporium*. Some of these produce mycotoxins, to some of which legislative restrictions may apply (patulin from *Byssochlamys*, for example), others do not.

ALTERNARIA

Alternaria species are plant pathogens that can produce toxins in both pre- and post-harvest commodities. They are characterised by very large brown conidia with a characteristic ‘beak’ at the tip. The most common species is *Alternaria alternata*, others include *Alternaria tenuissima*, *Alternaria infectoria*, *Alternaria citri*, *Alternaria brassicicola* and *Alternaria brassicae*. *A. alternata* and *A. tenuissima* are pathogenic to a wide range of crops; the other species have more limited host ranges.

Types of Toxins Produced

Alternaria species produce tenuazonic acid, alternariol, alternariol monomethyl ester, altenuene and altertoxin I and II. These can inhibit protein synthesis, chelate metal ions and form nitrosamines (4). Tenuazonic acid can also be produced by *Phoma sorghina* and *Pyricularia oryzae* (4), and has been associated with onyalai, a haematological disease (12).

Source

A. alternata contaminates cereals, for example wheat, rice, sorghum, barley, nuts, e.g. peanuts, hazelnuts and pecans (16), in addition to various fruits and vegetables (17). For example, *A. alternata* has been isolated from tomatoes, where it can cause significant losses. Egg plants, peppers and bananas have all been noted to be capable of harbouring this mould. Other vegetables on which it can grow include cauliflowers, potatoes and melons.

Growth Requirements

Many species of *Alternaria* are found in temperate climates, and as such are tolerant of low temperatures. Very little data on growth requirements have been recorded for many species of *Alternaria*, apart from *A. alternata*. *A. alternata* can grow in conditions of very low oxygen (O_2) tensions (0.25%).

Temperature

A. alternata is able to grow from a minimum of between -5 °C to 6.5 °C, to a maximum of 36 °C, with an optimum around 25 °C.

Water activity

The reported minimum water activity (a_w) for growth is 0.88 at 25 °C. Maximum production of alternariols is at 0.98 a_w at 25 °C, and for tenuazonic acid, 0.90 a_w.

pH

A. alternata is able to grow from pH 2.7 - 8.0, with an optimum of 5.4.

ASPERGILLUS

The genus *Aspergillus* has been known for about 300 years, and its role in food spoilage is well-established. Mycotoxins produced by *Aspergillus flavus* (including aflatoxins and cyclopiazonic acid) were identified as the cause of 'turkey X disease' in the 1960s, when 100,000 turkey poults died in the UK. Other important mycotoxins from aspergilli include ochratoxin A and patulin. Some aspergilli have an ascomycete teleomorphic stage; for example, *Eurotium*, *Neosartorya*, and *Emericella*; some of these will be covered later. Many aspergilli are xerophilic, and present particular problems during commodity harvest, and during subsequent drying and storage.

About 30 species of *Aspergillus* or their teleomorphs are associated with food spoilage (16); the following will be dealt with below: *A. flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus ochraceus*, *Aspergillus candidus*, *Aspergillus restrictus*, *Aspergillus penicillioides*, *Aspergillus niger*, *Aspergillus carbonarius*, *Aspergillus fumigatus*, *Aspergillus clavatus*, and *Aspergillus versicolor* (12). Pseudonyms will be noted where these occur.

Types of Toxins Produced

The most important mycotoxins produced by *A. parasiticus* and *A. nomius* are aflatoxins B_1, B_2, G_1 and G_2, and B_1, B_2 and cyclopiazonic acid (CPA) by *A. flavus*.

A. ochraceus produces three toxins - A, B and C (3), with ochratoxin A (OTA) being the most common.

A. restrictus and *A. penicillioides* do not seem to produce any mycotoxins.

A. candidus produces a range of secondary metabolites, but only kojic acid has any noted biological activity.

Some isolates of *A. niger* can produce OTA. Many *A. carbonarius* isolates can produce OTA.

A. fumigatus can produce various tremorgenic toxins, including fumitremorgens, verruculogen and gliotoxin.

A. clavatus can produce patulin, tryptoquivalins, and cytochalasin E.

A. versicolor can produce sterigmatocystin, a precursor to aflatoxins, with a high proportion of isolates capable of producing the toxin.

Other important toxins produced by *Aspergillus* species include CPA, sterigmatocystin and patulin.

Source

A. flavus, *A. parasiticus* and *A. nomius* are three closely related fungi, which all produce aflatoxins, as noted previously. They produce spores of various shades of bright 'grassy' green. They are widely distributed in nature, apart from *A. nomius*; although this may be more a function of the shorter timescale during which it has been reported. They are mostly found in nuts and oilseeds, and more specifically, peanuts, corn (maize), cotton-seed and copra (coconut cake). Rice and various beans (mung, soy) are also sources. They are also common contaminants of spices, for example, peppercorns and chillies (16). Because of the importance of aflatoxins, many surveys have been conducted for the presence of *A. flavus*. As a consequence, it has been found in many different types of foods, partly due to its ubiquity, and partly because it is relatively easy to identify. All three species are particularly common in the tropics.

A. ochraceus produces yellow-brown (ochre) conidia. It is found in drying or decaying vegetation, seeds, nuts (including peanuts, pecans and betel nuts) and fruits (3). As with *A. flavus* it is commonly found in the tropics. Also found in smoked and salted dried fish, dried beans, biltong, dried fruit, and cereals, for example, rice, barley, corn and wheat (16). It is of major importance in green coffee, for which there are legislative limits for OTA. It is also noted on cheese and processed meat, for example, ambient stored salamis.

A. candidus is a moderate xerophile, producing white conidia. It is a very common contaminant of foods, particularly dried or intermediate moisture foods. It is commonly associated with stored cereals in temperate and tropical zones, including wheat, barley, maize and rice, and many types of nuts, including peanuts hazelnuts, walnuts and pecans. It is also associated with dried fish, processed meats including salamis, and dried beans (16).

A. restrictus and *A. penicillioides* are similar, both producing dull green conidia. They are very xerophilic, extremely slow growing fungi. They

probably occur more frequently than isolation indicates, due to relative slow growth rates and inconspicuous colonies. They are mostly isolated from dried cereals, for example, wheat, rice, maize, and dried beans, cured fish and spices, and are common from both tropical and temperate stored products (16).

A. niger and *A. carbonarius* are very similar, closely related moulds, both producing large, dark brown to black conidia. *A. niger* in particular is very commonly isolated from a large number of foods from warmer climates, for example the tropics, sub-tropics and the Mediterranean regions. Its dark spores lend protection against sunlight; it is commonly found on sun dried products, for example fish and grapes. It is found on fresh fruit causing post-harvest decay, and has been noted for causing severe losses in tomatoes, onions and garlic (16), and commonly found in nuts, cereals (especially maize) oilseeds, spices, cheese and meat products. *A. carbonarius* is associated with Mediterranean vine fruits.

A. fumigatus is a thermophile, being found particularly in products that have undergone heating or heat damage. For example, cocoa and coffee during fermentation, also noted from spices, oilseeds (particularly copra and soybeans), cereals (rice, wheat, barley), cured and processed meats, and less frequently on nuts. Because of its thermophilic nature, care should be taken if contamination with *A. fumigatus* is suspected, as it is a human pathogen under some circumstances.

A. clavatus is commonly associated with malting barley, in particular if temperature control is poor or spontaneous heating occurs (16). Its ability to grow at elevated temperatures means that it has been associated with 'barley maltsters' lung', an allergic condition caused by inhalation of fungal spores during the malting operation. It is also found in wheat and wheat flour, rice, and maize (16) and is more common in products from temperate than tropical zones.

A. versicolor is commonly found in a wide variety of foods. It is mostly isolated from stored cereals (wheat, maize, barley, oats and rice), oilseeds (soybeans, rapeseed and sunflower seeds) and nuts (peanuts, walnuts, hazelnuts, pistachios and pecans), green coffee beans, fermented and cured meats, biltong, dried fish, and dried spices (16).

Growth Requirements

A. flavus and *A. parasiticus* are similar in their growth requirements; they are moderately xerophilic, and do not produce toxins at the limits of their

growth. *A. ochraceus* is similar. *A. nomius* is closely related to *A. flavus*, except that it does not grow at low water activities (16).

Temperature

A. flavus grows in the temperature range of 10 - 12 °C minimum, to a maximum of 43 - 48 °C (3, 16) but optimal growth occurs between 33 - 35 °C (16, 19). Aflatoxins are produced over a temperature range of 13 - 15 °C minimum, up to 37 °C (3, 16), with maximum production at 33 °C (19). Overall patterns of growth in *A. parasiticus* are similar to *A. flavus* (3).

Growth of *A. ochraceus* occurs in the temperature range 8 - 37 °C, with optimum growth between 24 - 31 °C (19). Production of ochratoxins by *A. ochraceus* occurs in the temperature range of 12 - 37 °C, with optimum production occurring between 25 - 30 °C (19). *A. ochraceus* also produces penicillic acid in the temperature range of 10 - 31 °C (optimum at 16 °C) (3).

A. restrictus has a minimum growth temperature of 9 °C, an optimum of 30 °C and a maximum of 40 °C. Little data are recorded for *A. penicillioides*; it seems to grow at temperatures as low as 15 °C, with an upper temperature of about 37 °C (16).

A. niger has a minimum temperature range of 6 - 8 °C, an optimum of 35 - 37 °C and a maximum of 45 - 47 °C. Studies have shown that growth of *A. carbonarius* occurs at 32 - 35 °C for strains from a range of southern European countries, with no growth at <15 - >45 °C (19).

Growth data for *A. candidus* is very variable; growing at lower limits from 3 - 4 °C in some studies, up to 11 - 13 °C in others, with optima from 20 - 24 °C to 25 - 28 °C, or even 45 - 50 °C, and maxima from 40 - 42, 44 or 50 - 55 °C have been reported (16).

A. fumigatus is a highly thermophilic fungus, with a growth minimum of 12 °C, a maximum near to 55 °C, with an optimum from 40 - 42 °C.

Scant data for *A. clavatus* shows that it grows from 5 - 6 °C to 42 °C, with an optimum at 25 °C (16).

A. versicolor has a lower growth temperature of 9 °C, a maximum of 32 - 39 °C (35 °C mean), and an optimum of 27 °C.

Water activity

There is variable data for the minimum a_w for growth of *A. flavus*, minima are quoted from 0.78 at 33 °C to 0.84 at 25 °C (16), with 0.80 - 0.83 being more likely. The minimum for *A. parasiticus* is 0.81 (20). Aflatoxin

production occurs above 0.82 - 0.83 (4, 16), and is maximised at around 16 - 31 °C and 0.95 - 0.99 a_w. The minimum a_w for growth of *A. nomius* is 0.83 at 25 - 30 °C, and 0.81 at 37 °C (16).

Optimum growth of *A. ochraceus* occurs at a_w 0.95 - 0.99 (3), although it can grow in the range of a_w 0.78 - >0.99 (3, 20). Optimum production of OTA by *A. ochraceus* occurs at a_w of 0.98 with a minimum of 0.83 - 0.87 (19) and maximum of >0.99.

A. penicillioides has been reported to grow from a lower a_w of 0.73 - 0.76, and an optimum of 0.91 - 0.93. There is some evidence that it can grow down to a_w 0.68 on natural substrates (16); this can take up to 6 months, however. *A. restrictus* can grow to a_w 0.75.

A. niger is a xerophile able to grow down to a_w 0.77. *A. carbonarius* is probably similar. The optimum a_w for growth of *A. carbonarius* varies between 0.93 - 0.987 (19).

A. candidus has been reported to grow down to a_w 0.75, with an optimum of >0.98.

A. fumigatus has a lower a_w for growth of 0.82 at 40 °C. *A. clavatus* grows down to a_w 0.87 - 0.88, with patulin produced at a_w 0.99 but not 0.95. *A. versicolor* can grow to a_w 0.78 - 0.80.

pH

pH has little effect on the growth of *A. flavus*. Growth can occur over a wide range (2.1 – 11.2), although growth is slower at pH <3.5. Optimum aflatoxin production occurs at pH 6, with a 50% reduction at pH 4, and very little production at pH 3. Optimal growth for *A. parasiticus* occurs between pH 4 and 7. *A. parasiticus* fails to grow at pH 2.2.

Optimal growth for *A. ochraceus* occurs between pH 3 - 8 with a minimum pH for growth of 2.2 and a maximum pH of 13 (3).

No pH data exists for *A. candidus*, *A. clavatus*, *A. fumigatus*, *A. restrictus*, and *A. penicillioides*.

A. versicolor has a minimum pH of about 3.1 and a maximum above 10.2. *A. niger* can grow to pH 2.0; *A. carbonarius* is probably similar.

Preservatives

Sodium benzoate, even at a low concentration, can reduce the heat resistance of *A. flavus* especially at low pH. With *A. parasiticus*, sorbic acid is necessary at a level of 1,000 - 1,500 mg/kg to prevent growth at pH 5.0 - 5.5; acetic acid inhibits growth at a level of 8,000 mg/kg at pH 4.5.

Pimaricin (natamycin) inhibits growth of *A. ochraceus* at a concentration of 20 mg/kg. Sorbic acid and pimaricin both reduce the production of OTA and penicillic acid (3).

No preservative data exists for *A. candidus*, *A. clavatus*, *A. fumigatus*, *A. niger*, *A. carbonarius*, *A. restrictus*, *A. penicillioides* and *A. versicolor*.

BYSSOCHLAMYS

Byssochlamys is a soil born fungus almost exclusively associated with spoilage of fruit juice-based still drink products. This is due to the production of extremely heat-resistant ascospores, and the ability to grow at low oxygen tensions. Two species are of importance in this context: *Byssochlamys fulva* and *Byssochlamys nivea*.

Types of Toxins Produced

Patulin is produced by both fungi in fruit, but not in fruit juice after processing; i.e. any patulin in fruit juice will have occurred as a result of pre-process contamination. The minimum a_w for patulin production by *B. nivea* is 0.92 at 21 °C and 0.87 at 30 - 37 °C.

Source

B. fulva was first isolated from canned strawberries from Europe. Latterly, it has also been isolated from the USA and Australia, on strawberries, passion fruit juice, other berry fruits and apples, causing spoilage of tetrapack juices.

B. nivea is less common than *B. fulva*, occurring on apple products, fruit juices and bottled strawberries. It is more common in temperate areas, for example Europe, whereas *B. fulva* is found worldwide.

Growth Requirements

B. fulva can grow from >5 °C and > 37 °C. It is able to grow in fruit juices with from 20 - 80% carbon dioxide (CO_2) and less than 0.5% O_2, and very well from 20 - 60 CO_2. The D-value of ascospores are high; D_{90} from 1 - 12 minutes and a Z value of 6-7 °C.

B. nivea has similar growth relations to *B. fulva* under CO_2. Its temperature relations are probably similar to *B. fulva*. Thermal data indicate a D_{88} of 0.75 - 0.88 minutes, a Z value of 4.0 - 6.1 °C. *B. nivea* has an optimum a_w of 0.92 at 21 °C, 0.89 at 30 °C, and 0.87 at 37 °C.

CHAETOMIUM

Chaetomium is an ascomycete fungus that produces characteristic flask-shaped sexual structures called perithecia, easily visible to the naked eye on seeds, and in culture. There are many species of *Chaetomium*; most are associated with woody tissues, plant stems or paper, including paper sacks used for flour, since they are strong cellulose degrading fungi. Most species are less commonly found directly from foods, apart from *Chaetomium globosum*.

Types of Toxins Produced

Some species of *Chaetomium* can produce chaetoglobosins and chaetomin (21).

Source

C. globosum has been isolated at high rates from some from cereals, for example, wheat, barley, maize and rice, beans, including soybeans and mung beans, and nuts (walnuts, hazelnuts, cashews). Infection rates are as high as 32% from some commodities (16).

Growth Requirements

Some species are encountered in the tropics, others are temperate zone fungi; growth requirements reflect this difference. Ascospore germination can occur from 4 - 10 °C to 38 °C, with an optimum around 24 - 38 °C. *C. globosum* can grow to a_w 0.94 in soil. From limited studies, it appears that *C. globosum* can grow from pH 3.5 - 7.

CHRYSOSPORIUM

Chrysosporium species fall into three categories; they are either associated with hair, skin or nails (dermatophytes, some of these are pathogens), or soil, or foods. The former two categories normally require high water activities for growth, the latter are xerophiles. *Chrysosporium farinicola* (synonym *Ovularia farinicola*, teleomorph *Bettsia alvei*) and *Chrysosporium inops* are represented here

Types of Toxins Produced

No toxins are noted for either species.

Source

C. farinicola has been isolated from low water activity foods and commodities; prunes, sultanas, mixed dried fruit, chocolate, jelly crystals and coconut.

C. inops is associated with spice powders, dates, starch, jelly and hazelnuts.

Both are relatively rarely reported, but this may be due to the need to use low water activity media to isolate *Chrysosporium*, or it may not grow.

Growth Requirements

No water activity data exists for *C. farinicola*, but it is believed to grow similarly to *Chrysosporium fastidium* – a_w 0.69.

C. inops is able to grow at a_w 0.72, and has a temperature growth range of at least 20 °C minimum, and a maximum of at least 30 °C.

C. inops conidia have the characteristic of increasing heat resistance with age; D_{66} of 1.67 minutes after three weeks, and 5.49 minutes after spores were six weeks old. Some could survive heating at 66 °C for 1 hour. Both species can grow under low oxygen conditions.

CLADOSPORIUM

Cladosporium is an extremely common fungus that produces large quantities of easily dispersible spores. In this way it is a common surface contaminant of plant surfaces, and is often found on air plates. Some species are noted as plant pathogens. The spores are small and darkly pigmented, typical of fungi that use air as a dispersal mechanism, since dark pigmentation confers resistance to UV light. *Cladosporium* is easy to identify in culture, but individual species are harder to recognise. The organism produces large quantities of secondary conidia in culture with time; cross contamination of colonies within plates is common, if plates are disturbed.

Cladosporium cladosporioides and *Cladosporium herbarum* are the species most likely to be isolated from foods.

Types of Toxins Produced

No notable toxins are produced by *Cladosporium*.

Source

C. cladosporioides has been found in a very wide range of foods, particularly cereals (wheat, barley, rice, sorghum, and flours), nuts, dried fish, fresh and frozen meats, cheese, and other refrigerated foods. It has also been noted from some fresh fruits, including raspberries.

Growth Requirements

Some *Cladosporium* species are psychrophiles, and some are tolerant of low oxygen levels (0.25% oxygen).

C. cladosporioides is a psychrophile, able to grow at temperatures as low as -5 °C, with a maximum near to 32 °C. It can grow down to a_w 0.86 at 25 °C. *C. cladosporioides* is inhibited by 160 parts per million sorbic acid at pH 5.0.

C. herbarum is also a psychrophile; growth has been reported at -10 °C, with a maximum from 28 - 32 °C. It is reported to grow at a_w 0.88.

CURVULARIA

Curvularia is a plant pathogen that commonly invades cereal crops, particularly from semi-arid regions. There are a number of species; *Curvularia lunata* is the most common. Conidia are very characteristic – large, dark, with 3 - 4 transverse septa, often at an angle to each other, giving a distinctive curved shape to spores.

Types of Toxins Produced

There is no reliable data concerning mycotoxins.

Source

Curvularia lunata is mainly observed on cereal crops, as above, for example, sorghum, rice, barley, wheat, maize (16). It is extremely common on sorghum from India, Niger, Nigeria and Thailand (16, 34 - 37). Often present in a high percentage of samples, and at a high infection rate in samples. Also noted from fruit and vegetables, for example, tomatoes.

Growth Requirements

Limited growth data is available for *Curvularia lunata* – spores can germinate at a_w 0.86.

EMERICELLA

The genus *Emericella*, composed of 29 species, and represented here by *Emericella nidulans* (anamorph *Aspergillus nidulans*) is an ascosporic species that produces purple ascospores.

Types of Toxins Produced

E. nidulans produces the important mycotoxins sterigmatocystin and emestrin.

Source

E. nidulans is mainly isolated from cereals, (wheat, barley, rice maize and sorghum), flour, bread, beans, spices, chocolate and nuts.

Growth Requirements

E. nidulans has a growth range from 6 - 8 °C to 46 - 48 °C, although growth has been reported at 51 °C, with an optimum from 35 - 37 °C. It is able to grow to a_w 0.82 at 20 °C and 0.80 at 37 °C, and 0.90 at 15 °C. Ascospores are likely to be heat resistant, since it is a borderline thermophile.

EUPENICILLIUM

Eupenicillium species produce ascospores in enclosed cleistothecia. Ascospores are heat resistant, and *Eupenicillium* is therefore another genus that crops up from time to time causing spoilage of heat processed acid foods, for example fruit juices and other soft drinks. Thirty seven species are noted, *Eupenicillium cinnamopurpureum* is described as representative of the genus.

Types of Toxins Produced

No toxins are noted from *Eupenicillium*.

Sources

E. cinnamopurpureum has been isolated from cereals, flour, rice, dried beans (soy, red kidney and mung), and peanuts. As it is heat resistant, it can also be found in canned blueberries, and fruit juices.

Growth Requirements

The temperature range for growth is 4 - 6 °C, and a maximum of 35-38 °C.The minimum a_w for growth on salt media is 0.82, and on glycerol media, 0.78.

EUROTIUM

Eurotium species are amongst the most common fungi isolated from spoiled dried commodities and dried processed products. They are characterised by rapid growth over a range of lower water activities; they are highly xerophilic. There are about 20 species of *Eurotium*, with four being found commonly from foods; *Eurotium amstelodami* (anamorph *Aspergillus vitis*; synonym. *Aspergillus amstelodami*), *Eurotium chevalieri* (anamorph *Aspergillus chevalieri*), *Eurotium repens* (anamorph *Aspergillus reptans*, synonym. *Aspergillus repens*) and *Eurotium rubrum* (anamorph *Eurotium rubrobrunneus*, synonyms. *Aspergillus sejunctus*, *Aspergillus ruber*). They are distinguished from each other by their ascospore morphology.

Types of Toxins Produced

E. amstelodami can produce echinulins, and its cultures are toxic to chick embryos. *E. chevalieri* can produce echinulin and neoechinulin, feed refusal factors in swine.

E. repens does not seem to produce any mycotoxins. *E. rubrum* cultures seem to produce a toxic principle.

Sources

E. amstelodami is common across the world on many stored products. These include cereals - wheat (and flour, dough and bread), barley, rice, maize sorghum, oilseeds including soybeans and sunflower, nuts, including hazelnuts, peanuts and walnuts, dried salted fish and biltong; there are many more.

E. chevalieri has a similar host range to *E. amstelodami*, but is more commonly isolated than *E. amstelodami*, particularly from warmer zones. It is also common on processed and dried meats, chocolate, jam and cured fish.

E. repens again is a very commonly isolated species, with similar host range to *E. amstelodami*, and causes spoilage of cheese, meat products including salami, ham, biltong, prunes and strawberry puree. It is also used as a starter culture in the production of katsuobushi, a mould-fermented dried tuna product.

E. rubrum again is more common in warmer climates, and with a similar host range to *E. amstelodami*, also found from katsuobushi, the same as *E. repens*.

Growth Requirements

Eurotium grows very rapidly on a wide range of low water activity media, producing characteristic yellow to deep orange or red colonies with grey green sporing masses. This is caused by the production of large numbers of bright yellow cleistothecia together with clear or orange to red aerial hyphae, and as *Aspergillus*, anamorph.

Temperature

E. amstelodami can grow over the range >5 - 43 - 46 °C, with an optimum from 33 - 35 °C. With heating for 10 minutes in a pH 3.8 and a_w 0.98 medium, 80 - 85% survive at 60 °C, 1 - 3% survive 70 °C, and 0.2% survive 75 °C. It can tolerate 1% O_2, giving 50% growth. It can produce a chloroanisole taint, and ketonic rancidity in coconut. It is very proteolytic, and is resistant to propionic acid

E. chevalieri has a minimum temperature of > 5 °C, an optimum of 30 – 35 °C and a maximum from 40 - 43 °C. It is the most heat resistant *Eurotium* species, with heating of ascospores for 10 minutes in a pH 3.8 and a_w 0.98

medium, giving a survival rate of 18 - 25% at 70 °C, 0.5% at 80 °C. This calculates to a D_{80} of 3.3 minutes and a Z value of 12.8 °C. It can produce an isoprene taint, giving 'off' odours in bakery products.

E. repens can grow from a minimum of 4 - 5 °C, up to a maximum of 38 - 40 °C, with an optimum of 25 - 27 °C. Heating ascospores for 10 minutes in a pH 3.8 and a_w 0.98 medium gives a survival rate of 90% at 60 °C, 3% at 70 °C, and no survivors at 75 °C. Chloroanisole taint is produced.

E. rubrum has a growth temperature range of 5 - 40 °C, an optimum of 25 - 27 °C. Heating ascospores for 10 minutes in a pH 3.8 and a_w 0.98 medium gives a survival rate of 80 - 100% at 60 °C, 0.5% at 70 °C, with no survivors at 75 °C. Sorbate tolerance is developed after extended culture on sorbate media.

Water activity

E. amstelodami has a minimum a_w of 0.70 at 25 °C, spore germination takes 120 days at this a_w, other data suggests a minimum of 0.75, taking 100 days. The optimum is 0.90 - 0.96.

E. chevalieri has a minimum a_w of 0.71 at 33 °C, and 0.74 at 25 °C and pH 3.8, with an optimum of 0.94 - 0.95.

The minimum water activity for *E. repens* is 0.72 at a neutral pH and 20 and 25 °C. Other data suggests 0.74 - 0.75 at 25 °C, and a pH of 3.8 - 4.0. The optimum a_w is 0.95. It can possibly grow to a_w 0.69 on glucose-fructose media.

E. rubrum has a minimum water activity of 0.70 - 0.72 at 25 °C, 0.72 at 20 °C, or 0.73 at 25 °C, with an optimum of 0.94.

Preservatives

E. amstelodami is tolerant of sorbate, when successively culturing on increasing levels of sorbate, up to 0.07% sorbate at pH 5.5. *E. rubrum* can develop sorbate tolerance after extended culture on sorbate media.

FUSARIUM

Fusarium species can cause a variety of disorders, dependent upon the organism, and the toxin(s) it can produce. Examples of species are *Fusarium chlamydosporum*, *Fusarium culmorum*, *Fusarium solani*, *Fusarium equiseti*, *Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium poae*, *Fusarium semitectum*, *Fusarium subglutinans*, *Fusarium sporotrichioides* and *Fusarium verticillioides* (3).

Fusarium species are mainly plant pathogens and normally occur in association with plants and cultivated soils. *Fusarium* species are responsible for wilts, blights, root rots and cankers in legumes, coffee, pine trees, wheat, corn, carnations and grasses. Infection may occur in developing seeds, and in maturing fruits and vegetables. Typically able to grow only at higher water activities, damage is usually confined to pre-harvest, for cereals, or immediately post-harvest until drying is well under way and the water activity is below about 0.90. Vegetables can continue to be spoiled in store, due to their higher water activity. Fusaria can be divided broadly into temperate and tropical/subtropical types, the former mainly affecting cereals, the latter, tropical cereals and fruits and vegetables. Some temperate species are able to grow over the winter on snow-bound cereal crops.

Many species of *Fusarium* are able to produce potent mycotoxins, which, unlike many *Aspergillus* mycotoxins, have acute as well as chronic toxicity, and some only affect animals. Many of these seem to be associated with the pathogenesis mechanism in attacking plants. Some of these toxins are oestrogen mimics. Taxonomy is complex, and even today, several taxonomic schemes exist, rendering identification and naming a difficult subject for the inexperienced, as well as confusing which organism produces which toxins. Some have an ascomycete sexual stage in *Gibberella*. The scheme of Pitt and Hocking (16) will be followed here, with pseudonyms noted to aid literature searching.

Types of Toxins Produced

Fusarium mycotoxins include type A and B trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid (7).

F. chlamydosporum produces Type A trichothecenes including T-2 toxin, HT-2 toxin, monoacetoscirpenol, neosolaniol and iso-neosolaniol. It also produces zearalenone and chlamydosporol.

F. culmorum produces over 40 mycotoxins, with deoxynivalenol and derivatives, zearalenone, culmorin, sambucinol, chlamydosporol and moniliformin.

Mycotoxins produced by *F. equiseti* include Type A (diacetoxyscirpenol, T-2 and HT-2 toxin, neosolaniol), and Type B trichothecenes (nivalenol, deoxynivalenol, 15-acetyldeoxynivalenol, fusarenon-X) butenolide, zearalenone, culmorin, and chlamydosporol.

F. graminearum produces about 50 mycotoxins by including the important Type A toxins deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol and zearalenone. Minor toxins include sambucinol, culmorin and fusarins. Type B toxins including T-2 and HT-2 toxins have been reported from some isolates.

F. oxysporum produces moniliformin, zearalenone, nivalenol, sambutoxin and fusarenon-X.

F. poae mainly produces T-2 and HT-2 toxins, also nivalenol, deoxynivalenol, and diacetoxyscirpenol.

F. proliferatum produces fumonisins B_1, B_2 and B_3, and some produce moniliformin and fusaric acid.

The major mycotoxin produced by *F. semitectum* is zearalenone, and possibly diacetoxyscirpenol, nivalenol, fusarenon X and neosolaniol.

There is limited evidence for toxicity of *F. solani*, with furanoterpenoids, ipomeanols and ipomeanine being suspected.

F. sporotrichioides is a major producer of T-2 toxin; additionally, some isolates may produce butenolide, fusarenon-X, neosolaniol and nivalenol (3, 16, 21).

Moniliformin has been reported from *F. subglutinans*, also fusarin C, fumonisins (some reports) chlamydosporol and fusaric acid.

F. verticillioides produces fumonisin B_1 as the major mycotoxin, and fumonisin B_2. It may also produce moniliformin, fusaric acid, fusarin C and other fusarins.

Sources

F. chlamydosporum (synonym *Fusarium fusarioides*) is a soil borne fungus that grows in warmer climates. It is not regarded as aggressively pathogenic, but it has been isolated from some tropical cereals and nuts, for example, pecans, sorghum, and peanuts.

F. culmorum is found across the world as a soil-borne plant pathogen, mainly of cereals in temperate climates. It is commonly found on barley and

wheat, causing major problems as head blight of wheat, reducing yield and flour quality. It has also been noted on bananas, apples and pears.

F. equiseti is a widely distributed plant pathogen and soil saprophyte, from very cool zones to the tropics. It is commonly isolated from cereals, causing stem and root rots. Cereals affected include wheat, barley, rye, rice and maize. It has also been found on nuts, herbs, and vegetables (tomatoes, capsicums), and fruits including bananas. It has also caused spoilage of ultra heat treated (UHT) processed fruit juices, because of its ability to grow at low oxygen tensions (<1% oxygen).

F. graminearum (teleomorph *Gibberella zeae*) grows on temperate and subtropical cereals, including wheat, barley and maize. In barley, it is associated with 'gushing' of beer, and in wheat, head scab and crown rot, causing major losses where it occurs. In maize, it causes stalk and cob rots. Other crops include bananas where it causes crown rot, a major problem, sugar beet, sorghum and soybeans.

F. oxysporum is noted as a wilt pathogen of many crops, including cabbage, cucumbers, melons, tomatoes and peas. It also occurs in many cereals and nuts. It is one of the major causes of crown rot of bananas. It is found across the world. It is able to grow at low oxygen tensions; this ability, coupled with the production of heat-resistant chlamydospores, has allowed it to cause problems in ambient-stable heat processed fruit juices and ready to drink beverages.

F. poae occurs in temperate zones, mainly on cereals, including wheat, barley, oats, maize (cool zone) and soybeans.

F. proliferatum is closely related to *F. verticillioides*, similarly preferring to grow in warmer zones, and occurs mainly in maize, also in rice, sorghum and bananas.

F. semitectum (synonym. *Fusarium pallidoroseum*) is quite widespread in the tropics and sub-tropics, associated with maize and other cereals, including sorghum and rice, also fruits, including bananas (crown rot complex), citrus, also from temperate crops including tomatoes, oilseeds, beans and potatoes.

F. solani is a common soil-borne fungus, causing rots of potatoes, sweet potatoes, cassava and other root crops, also peanuts and other legumes, for example, peas and beans. Found in some cereals, including maize and rice, and fruits, for example bananas and guavas.

F. sporotrichioides is a commonly occurring species found in cereal crops, peanuts and soy beans (3).

F. subglutinans (synonyms. *F. moniliforme* var. *subglutinans*, *F. sacchari* var. *subglutinans*) has a similar host range to *F. verticillioides*, with

particular importance on maize, from Europe, North America, South East Asia. It also occurs on fruits, for example pineapples and bananas.

Fusarium verticillioides (synonym *F. moniliforme*) is commonly found in the humid temperate zone and the tropics, and is less common in cool temperate zones. It is a major problem in maize wherever it is grown, in which it causes stalk and cob rots. Also found in sorghum, rice, nuts, oilseeds, spices, oranges, yams, bananas (associated with crown rots) and biltong.

Growth Requirements

Very little information is available about *Fusarium* concerning the optimum conditions for growth and optimum toxin production requirements (3). Toxicological synergism has been reported among *Fusarium* mycotoxins (7).

Temperature

F. chlamydosporum has minimum, optimum and maximum temperatures for growth of 5 °C, 27 °C and 37 °C respectively.

F. culmorum has been reported to be psychrotrophic, growing from 0 °C, with an optimum at 21 - 25 °C and a maximum of only 31 °C (16, 21).

The optimum temperature for *F. equiseti* growth is 21 °C (11), with a minimum of -3 °C and maximum, depending on the isolate, of about 35 °C.

Optimum growth of *F. graminearum* occurs between the temperature range 24 - 26 °C. However, growth can occur at temperatures as low as <5 °C and as high as 37 °C. Optimum deoxynivalenol toxin and zearalenone production by *F. graminearum* occurs in temperatures of 24 °C (4), and 25 - 30 °C (19), respectively.

Optimum growth for *F. verticillioides* occurs from 22.5 - 27.5 °C but can range from 2.5 - 5 °C minimum, to 32 - 37 °C maximum.

F. oxysporum has an optimum temperature between 25-30 °C, a minimum of 5 °C, and a maximum around 37 °C.

The optimum temperature for growth of *F. poae* is 22.5 - 27.5 °C, with the minimum near 2.5 - 9 °C and a maximum near 32 - 33 °C.

Limited data exists for *F. proliferatum*; it is likely to be similar to *F. verticillioides*.

F. semitectum is able to grow from 3 - 37 °C, optimally at 25 °C.

F. solani grows optimally between 27-31 °C, with an upper limit around 37 °C.

Optimum growth for *F. sporotrichioides* occurs at 22.5 - 27.5 °C with a range of -2 °C to 35 °C (3). Optimum T-2 toxin production by some *Fusarium* species occurs at 15 °C (4).

F. subglutinans is likely to be similar to *F. verticillioides*.

Water activity

No data exists for water activity in relation to *F. chlamydosporum* and *F. semitectum*.

The minimum a_w for growth of *F. culmorum* is 0.87 at 20 - 25 °C and pH 6.5; at pH 4.0 growth does not occur below a_w of 0.90 (16).

The minimum a_w for growth of *F. equiseti* has been reported to be 0.92.

Optimum zearalenone production by *F. graminearum* occurs at a_w >0.98, but growth can occur at a_w of 0.90 (19).

Growth for *F. verticillioides* occurs in a_w range 0.87->0.99, with fumonisins B_1 and B_2 produced to 0.92.

F. oxysporum is able to grow to a_w 0.89, in extreme cases.

The minimum a_w for growth of *F. poae* is near 0.90 between 17-25 °C.

Limited data exists for *F. proliferatum*; it is likely to be similar to *F. verticillioides*.

F. solani can grow to a_w 0.90, after a prolonged period.

Growth for *F. sporotrichioides* ranges from a_w of 0.88 - >0.99 (3).

F. subglutinans is likely to be similar to *F. verticillioides*.

pH

No pH data exists for *F. chlamydosporum*, *F. verticillioides*, *F. poae*, *F. proliferatum*, *F. semitectum*, *F. Solani*.

F. culmorum can grow down to pH 4.0 at least.

Growth of *F. equiseti* occurs at pH 3.3 but not 2.4 and is rapid at pH 10.4 (16).

pH has very little effect on the growth of *F. graminearum*, with only a 5% variation in radial growth rates over the range pH 5 - 10 at 25 - 30 °C. Growth has been observed in the pH range of 2.4 - 10.2.

Optimum growth for *F. verticillioides* occurs at pH of 5.5 - 7.5 with a minimum pH of <2.5 and a maximum of >10.6.

The optimum pH for *F. oxysporum* is 7.7, and is able to grow in the range 2.2 - 9.0.

Optimum growth for *F. sporotrichioides* occurs at a pH of 5.5 - 9.5, with a minimum pH of <2.5 and a maximum of >10.6 (3).

F. subglutinans is likely to be similar to *F. verticillioides*.

GEOTRICHUM

(Teleomorph *Galactomyces geotrichum*, synonyms. *Oidium lactis*, *Oospora lactis*)

Geotrichum candidum is characterised by a yeast-like growth on agar, with individual hyphal cells becoming conidia with time. This has lead to the nickname 'machinery mould' since it is easily disseminated on factory machinery and processing lines.

Types of Toxins Produced

No toxins are noted for *Geotrichum*.

Sources

G. candidum is a post-harvest pathogen of citrus fruits, in which infection can lead to sour rots. It tends to occur on over-ripe fruit. It has also been isolated on tomatoes, capsicums, and vegetables, for example, carrots, potatoes, cucumbers and peas.

It has also been isolated from dairy products, for example, raw milk, and cheese. Conversely, it can be used in the manufacture of Brie, Camembert and some smear-ripened cheeses, for example, Limberger.

Growth Requirements

Capable of growth from 10 -35 – 38 °C, with an optimum from 25 - 30 °C. The minimum a_w for growth is 0.90. *Geotrichum* has a very low heat resistance – D_{52} of 30 - 40 minutes.

MONILIELLA

Moniliella is another example of a fungus whose mycelium is capable of breaking down to become spores. Growth is often yeast-like. It comprises two species: *Moniliella acetoabutans* and *Moniliella suaveolens*. *M. acetoabutans* is a noted problem in acid-preserved foods, due to an extreme tolerance of acetic acid.

Types of Toxins Produced

No toxins noted.

Sources

M. acetoabutans is found in fruit sauces, mayonnaise, vinegar and other acetic acid based preserves.

Growth Requirements

M. acetoabutans is able to grow in products with 4% acetic acid, and can survive in vinegar with an acid level of 10%.

MUCOR

Mucor is a very common genus, characterised by extremely fast growth on laboratory media. It produces large, moist sporing heads that are visible to the naked eye. Most species are saprophytic or weak pathogens, only

attaching to damaged crops or those that are past maturity. They are particularly common on fruits and vegetables. About 20 species are known to spoil foods; *Mucor hiemalis* and *Mucor plumbeus* are given as typical examples.

Types of Toxins Produced

No toxins are noted for either species.

Sources

M. hiemalis is associated with rotting of a variety of fruits and vegetables, also yoghurt, cheese, cereals (mainly as contaminants) wheat based foods, airline foods (Egypt), and confectionery.

M. plumbeus has been isolated from cheese, apple juice and cereals, including rice. It is a common contaminant of many foods.

Growth Requirements

M. hiemalis can grow from 0 - 5 to 37 °C.

M. plumbeus can grow from 4 - 5 to >25 and <37 °C, with an optimum from 24 - 25 °C. The lowest a_w for growth is 0.93. It can also grow in less than 1% oxygen.

PAECILOMYCES

Paecilomyces is characterised by fast growth on laboratory media across a range of temperatures, and comprises olive brown, copiously sporulated colonies giving cultures a dusty appearance. Many *Paecilomyces* species are insect pathogens, and are common soil fungi. One species is particularly common in foods; *Paecilomyces variotii*. One other species, *Paecilomyces fulvus*, is the anamorph of *B. fulva – P. variotii* is often confused with it.

Types of Toxins Produced

P. variotii does not seem to produce patulin, despite being closely related to the *B. fulva* anamorph.

Sources

P. variotii is a common soil contaminant, and is often found contaminating cereals, oilseeds, nuts, bread, meat products, spices, and commodities that have undergone heat damage.

Growth Requirements

The temperature range for *P. variotii* is from >5 - 45 - 48 °C, with an optimum at 35 - 40 °C, between which temperatures it grows very fast. It is a marginal xerophile, just growing at a_w 0.80. It is able to grow under low O_2 conditions in foods (16). *P. variotii* is sorbate resistant, converting sorbate to 1,3 pentadiene. It can also produce chloroanisoles from chlorophenols, giving 'mouldy' taints.

PENICILLIUM

Penicillium is a large genus containing 150 recognised species, of which 50 or more occur commonly. Many species of *Penicillium* are isolated from foods causing spoilage; in addition, some may produce bioactive compounds. Penicillin was first discovered in 1929, and since that time, *Penicillium* species have been investigated for other bioactive molecules with antibiotic properties. In turn, this led researchers to recognise citrinin and patulin as 'toxic antibiotics', later termed mycotoxins. Some toxins are produced by more than one species of *Penicillium*, and many species can produce more than one mycotoxin. For example, citrinin is produced by a number of *Penicillium* species and several Aspergilli (16). Toxicity due to *Penicillium* species varies - two groups of toxins that can be distinguished are those toxins that affect liver and kidney function, and neurotoxins, which cause sustained trembling in animals (3).

Penicillium taxonomy is a veritable minefield for the inexperienced, and is best left to those working in the field; whereas many Aspergilli can be crudely identified on the basis of colony morphology and colour, most Penicillia are some shade of bluish or grey-green to green, and to the untrained eye, microscopic features are similar and confusing. Broadly, *Penicillium* species can be divided into subgenera based on the degree of branching of the conidiophores: subgenus *Aspergilloides* has one branch point, *Furcatum* and *Biverticillium* two, and in subgenus *Penicillium*, three branch points; these latter are the most '*Penicillium*-like' when observed under the microscope, and indeed, they are the largest group. Pitt and Hocking (16) did much to simplify the taxonomy and provide a simple growth method which, together with some simple microscopy, greatly aids identification, and will be followed here. Two of the species detailed below (*Penicillium citrinum* and *Penicillium oxalicum*) are contained in subgenus *Furcatum*, the rest in subgenus *Penicillium*.

Important mycotoxins produced by *Penicillium* include ochratoxin A, patulin, citrinin and penitrem A (12). Some of the most important toxigenic species in foods are *Penicillium expansum*, *Penicillium citrinum*, *Penicillium crustosum* and *Penicillium verrucosum* (3).

A much larger number of *Penicillium* species are mainly associated with food spoilage. Those covered here include *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Penicillium digitatum*, *Penicillium griseofulvum*, *Penicillium italicum*, *Penicillium oxalicum* and *Penicillium viridicatum*; some of these produce mycotoxins.

Additionally, some species are associated with food fermentations, for example, *Penicillium roqueforti* and *Penicillium camemberti* with mould-ripened cheese, as well as causing spoilage in some cases. We will include data from the former species.

Types of Toxins Produced

Penicillium aurantiogriseum produces penicillic acid, and sometimes roquefortine C and verrucosidin.

The most common mycotoxin produced by *Penicillium chrysogenum* is roquefortine C, some produce cyclopiazonic acid, with very few being completely non-toxic. While not a toxin as such, *P. chrysogenum* is associated with tainting of shipping containers by conversion of chlorinated phenols used to preserve timber to chloroanisole, a potent tainting compound. *P. crustosum* is also noted for this.

P. citrinum produces citrinin, which is a significant renal toxin to monogastric domestic animals, it can also cause kidney damage after prolonged ingestion.

Most isolates of *P. crustosum* produce penitrem A, a potent neurotoxin, ingestion of which can lead to severe brain damage or death. It has been reported in field outbreaks involving sheep, cows, horses and dogs.

Penicillium digitatum does not produce any notable toxins.

P. expansum produces patulin, which produces neurological and gastrointestinal effects, and citrinin.

P. griseofulvum produces patulin, cyclopiazonic acid, roquefortine C and griseofulvin, the latter is an antifungal antibiotic.

Similarly with *P. digitatum*, *Penicillium italicum* does not seem to produce mycotoxins.

P. oxalicum produces secalonic acid D.

P. roqueforti produces PR toxin, roquefortine C and mycophenolic acid.

About 60% of *P. verrucosum* isolates produce ochratoxin A (3), with some also producing citrinin. *P. verrucosum* is the only *Penicillium* species to produce OTA.

Older papers suggested that *Penicillium viridicatum* could produce OTA, but these isolates have since been shown to be *P. verrucosum*. It has been shown to produce xanthomegnin and viomellein.

Sources

Penicillium is a common soil-borne saprophyte, and species are associated with many types of soil rots.

P. aurantiogriseum (synonym. *Penicillium puberulum*, *Penicillium cyclopium*, *Penicillium aurantiovirens*, *Penicillium martensii*, *Penicillium verrucosum* var. *cyclopium*) is an extremely common fungus, often associated with harvested and drying crops, including cereals and cereal products (wheat, flour, bran), nuts, including peanuts and hazelnuts, and spices. It causes ‘blue-eye’ corn and can also cause storage rot of fruits and vegetables.

P. chrysogenum (synonym. *Penicillium griseoroseum*, *Penicillium notatum*, *Penicillium melagrinum*) is another commonly found mould, at least as common as *P. aurantiogriseum*. Its fame comes from the ability to produce the antibiotic penicillin. It is very common from cereals (wheat, barley, maize, rice, and flours from cereals), vegetables, fruits, cheese, dried fish, nuts, spices, and snack foods.

P. citrinum can be found in almost every kind of food surveyed for fungi; common sources are milled grains and flour, and whole cereals, especially rice, wheat and corn. It is also isolated from nuts, including, peanuts, pistachios, pecans, hazelnuts and walnuts. Cured meats, coffee and cocoa beans have also provided isolates. In South East Asia, it is associated with about 6 - 7% of all Thai maize, 2 - 3% of all soybeans, and about 20% of all dried fish (16). It is especially noted from pre-harvest maize.

P. crustosum is a very common species found in foods and feeds, and causes spoilage of corn, processed meats, nuts, cheese and fruit juices. It can be a weak pathogen of citrus fruits and melons. It has also been isolated from cabbage, mung beans and peppers.

P. digitatum is widely distributed as a very strong pathogen of citrus fruits, particularly from warmer zones, producing a yellowish green growth on the surface of fruits. 'Nesting', where one infected fruit contaminates surrounding fruit packed in boxes, leading to a spreading of the rot, is common. Control is by using fungicide-treated waxed papers, and fungicide dips and sprays.

P. expansum can be found in fruits, specifically apple, apple products and pears (16, 22, 23), causing major losses if fruits are not treated with fungicides. It is also noted from tomatoes, strawberries, avocados, mangoes and grapes, and is less commonly isolated from stored commodities.

P. griseofulvum (synonym *Penicillium patulum*, *Penicillium urticae*) is commonly found in cereals, including wheat, barley, maize, nuts, including pecans, peanuts and pistachios, dried peas, meats, rapeseed, and health foods, generally from temperate zones.

P. italicum is a destructive pathogen of citrus fruits, producing a bluish grey rot. Control is similar to that for *P. digitatum*. It is rarely reported from other crops or fruits.

P. oxalicum is widespread in the tropics, growing on a wide variety of foods, from maize, rice, cowpeas and sorghum to peanuts, mung beans and spices. It is also noted from cassava, copra, barley, wheat, and fermented sausages.

P. roqueforti (synonym. *Penicillium carneum*, *Penicillium paneum*, *Penicillium roqueforti* var. *carneum*) is used in the production of Roquefort and other green veined cheeses. It is also a cheese spoilage organism. It can also cause spoilage of many other foods stored under refrigeration, since it can grow well at low temperatures. It can also cause spoilage in European rye bread, and some cereals, and more frequently meat and meat products, nuts and fresh vegetables.

P. verrucosum reported in temperate zones is associated with Scandinavian barley and is also isolated frequently from meat products in Germany and other European countries (3). There has been a lot of confusion between *P. verrucosum* and *P. viridicatum*, and early reports of mycotoxin production should be treated with caution unless the isolates are authenticated.

P. viridicatum has been reported from some cereals, though less frequently than *P. verrucosum*, fresh and dried fruits, soybeans, meat, spices, pasta, and nuts, although some may be misidentifications of *P. verrucosum*.

Growth Requirements

Penicillium species usually grow optimally at relatively low temperatures; they are ubiquitous in soil, cereal grains and other foods in temperate climates, and also in cool stores and refrigerated foods worldwide (3).

Whilst most moulds prefer oxygen at or near atmospheric levels, *P. roqueforti* is stimulated by high (15%) levels of carbon dioxide, and can tolerate oxygen levels of 1 - 2%. Some isolates can grow in conditions of 80% CO_2, 4.2% O_2, and 15.8% N_2. It can grow in O_2 levels around 0.5%.

Temperature

P. aurantiogriseum is very tolerant of cold conditions being able to grow at -2 °C, optimally at 23 °C, and a maximum at about 30 °C. Penicillic acid is produced optimally from 1 - 10 °C.

P. chrysogenum is a mesophilic fungus, growing from 4 - 37 °C, with an optimum of 23 °C.

Optimal growth for *P. citrinum* occurs between 26 - 30 °C with a minimum of 5 - 7 °C and a maximum of 37 - 40 °C. Optimal citrinin production occurs at 30 °C with a minimum of <15 °C and a maximum of 37 °C.

Optimal growth for *P. crustosum* occurs at 25 °C with a minimum of <-2 - 2 °C and a maximum of 30 °C. Optimal penitrem A production occurs between 20 - 26 °C with a minimum of <17 °C and a maximum of 30 °C (3).

P. digitatum grows from 6 - 7 °C to 37 °C.

Optimal growth for *P. expansum* occurs at 20 - 25 °C with a minimum range of -2 to -6 °C, and a maximum of 30 °C. Optimal patulin production occurs at 15 - 20 °C (22).

Growth for *P. griseofulvum* ranges from 4 - 35 °C with the optimum near 23 °C; patulin production occurs over the range 4 - 31 °C at 0.99 a_w and 8 - 31 °C at 0.95 a_w (16).

P. italicum is a psychrophile, able to grow between -3 °C and 32 - 34 °C, with the optimum from 22 - 24 °C. Others report 0 °C as the lowest temperature for growth.

P. oxalicum grows from 8 - >40 °C, growing optimally at 30 °C.

P. roqueforti is a psychrophile that grows vigorously at refrigeration temperatures but not above 35° C (16, 21).

Optimal growth for *P. verrucosum* occurs at 20 °C (3) with a minimum of 0 °C and a maximum of 31 °C. Optimal production of OTA by *P. verrucosum* occurs at 25 °C, with a minimum of 5 - 10 °C (19).

P. viridicatum is a psychrophile, able to grow as low as -2 °C, up to 36 °C, with an optimum at 23 °C.

Water activity

P. aurantiogriseum has been reported to grow at a_w 0.81.

P. chrysogenum is quite xerophilic, being able to grow down to a_w 0.78 - 0.81.

Optimum growth for *P. citrinum* occurs at a_w 0.98 - 0.99 with a minimum of 0.80 - 0.84 and a maximum of >0.99.

Maximum growth of *P. crustosum* occurs at a_w >0.99. Optimal penitrem A production occurs at a_w of 0.995 with a minimum a_w of 0.92 and a maximum a_w of 0.999 (3).

P. digitatum is not xerophilic, growing to a_w 0.90.

Optimal growth for *P. expansum* occurs at a_w of 0.99 with a minimum a_w of <0.86 (22).

The minimum a_w for germination of *P. griseofulvum* is 0.81 at 23 °C and 0.83 at 16 °C or 30 °C (16) and patulin is produced by the fungi down to a_w of 0.88.

P. italicum is able to grow to a_w 0.87.

The lowest a_w for *P. oxalicum* growth is 0.86.

The minimum a_w for growth of *P. roqueforti* is 0.83 (21).

Minimum a_w for growth of *P. verrucosum* is 0.80. Optimum production of OTA occurs at a_w 0.90 - 0.95 with a minimum a_w 0.83 - 0.85 (19).

P. viridicatum can grow as low as a_w 0.80 - 0.81, it is therefore slightly xerophilic.

pH

No data exists for *P. oxalicum*, *P. aurantiogriseum*, *P. chrysogenum*, *P. digitatum*, *P. griseofulvum*, or *P. viridicatum*.

Optimum growth of *P. citrinum* occurs at pH 5.0 - 7.0 with a minimum pH of <2.2 and a maximum of >9.7.

Optimum growth of *P. crustosum* occurs at pH 4 - 9 with a minimum of <2.2 and a maximum of >10 (3).

Patulin production by *P. expansum* is optimal at low pH level (less than 3.5); this may be due to patulin's instability at higher pH. Growth of *P. expansum* can occur at a relatively high pH, as they can reduce the pH level of the medium while growing until the medium reaches the right pH level for patulin production (23).

P. italicum can grow between pH 1.6 – 9.8.

P. roqueforti can grow from pH 3 - 10.

Optimum growth of *P. verrucosum* occurs at pH 6 - 7 with a minimum pH of <2.1 and a maximum of >10.

Preservatives

The minimum inhibitory concentration (MIC) of sorbic acid for *P. chrysogenum* is 1 - 2 mmol/l, from pH 4 - 6.

The MIC of sorbic acid to prevent the growth of *P. crustosum* is 2,000 mg/kg at 4 °C, and 6,000 mg/kg at 25 °C. *P. crustosum* is noted as able to degrade sorbic acid to 1,3 pentadiene, which can lead to off-odours, and an obvious loss of effective preservative.

P. digitatum is inhibited by 0.02 - 0.025% sorbic acid at pH 4.7.

For *P. verrucosum*, maximum inhibition of OTA production occurs at an MIC of 1,500 mg/kg, using sorbic acid at 20 °C. The optimum water activity to inhibit OTA production by *P. verrucosum* at 1,500 mg/kg of sorbic acid level is 0.95 - 0.99 (3).

PHOMA

Phoma species are characterised by the production of single celled, asexual conidia within flask-shaped pycnidia, which are superficially like perithecia. There are a large number of species of *Phoma*, most of which occur in temperate zones, with a few in the tropics. *Phoma* species are relatively easy to identify to genus level, much harder to species level; *Phoma sorghina* is easier because of characteristic growth on laboratory media. It is characterised here because of its common occurrence on sorghum, and the ability to produce mycotoxins.

Types of Toxins Produced

Tenuazonic acid is produced by *P. sorghina* (4), and has been associated with onyalai, a haematological disease (12).

Sources

P. sorghina has been isolated on grains, particularly sorghum and rice, also some fruits, for example, bananas.

Growth Requirements

There is little data on growth requirements for *P. sorghina*, but it can grow between 5 - 37 °C (16).

RHIZOPUS

Like *Mucor*, *Rhizopus* is a fast growing genus, and a member of the Zygomycete (class/division). *Rhizopus* species are very common contaminants of crops and processed foods, and very commonly contaminate laboratory cultures over growing other colonies.

Some species of *Rhizopus* (*Rhizopus oligosporus*) are used in food fermentations in South East Asia and Africa. *Rhizopus oryzae* (synonym

Rhizopus arrhizus) and *Rhizopus stolonifer* (synonym *Rhizopus nigricans*) are those most commonly associated with foods.

Types of Toxins Produced

Some conflicting evidence seems to suggest *R. stolonifer* and *R. oryzae* cultures are toxic to ducklings, but the toxins are unknown.

Sources

R. stolonifer is the most likely species to be isolated from foods, causing soft rots of fruits and vegetables, from temperate to tropical zones. Rots typically occur post-harvest, or on produce once in store.

R. oryzae is also found on fruit and vegetables, but is more common in the tropics than *R. stolonifer* – it grows faster at higher temperatures. It is also found on peanuts, and as a surface contaminant of many tropical cereals, beans, oilseeds and nuts.

Growth Requirements

R. oryzae grows from 4 - 5 to 35 - 37 °C, optimum ~25 °C. Spores germinate down to a_w 0.84 at 25 °C. It is capable of very fast growth under optimum conditions of 25 °C and a_w 0.99 achieving 2 mm/hour radial growth.

R. oryzae grows from 7 to 42 – 45 °C, with an optimum of 37 °C. Spores can germinate down to a_w 0.88. The radial growth rate at the optimum temperature and a_w is 1.6 mm/hour.

TALAROMYCES

Talaromyces is characterised in some species by the production of heat resistant ascospores, and morphologically has similarities with *Byssochlamys*. The asexual stage is *Penicillium*–like, as with *Byssochlamys* and *Paecilomyces*. There are about 25 species of *Talaromyces*; *Talaromyces flavus* and *Talaromyces macrosporus* are described. *T. flavus* is often isolated from pasteurised fruit juices and juice based drinks.

Types of Toxins Produced

T. flavus is not known to produce any mycotoxins, *T. macrosporus* can produce duclaxin.

Sources

T. flavus is a common soil contaminant isolated from sub-tropical and tropical regions (16). It is common from cereals.

T. macrosporus is frequently found in heat processed juices causing spoilage; it is rare from other sources.

Growth Requirements

T. flavus has a growth minimum of >5 °C to >37 °C, with an optimum from 25 - 30 °C. It has lower heat resistance than *T. macrosporus*.

T. macrosporus can grow from 5 °C to >37 °C, with an optimum from 25 - 30 °C. Ascospores are very heat resistant; with a D_{80} of 190 minutes, $D_{88.7}$ of 22 minutes, $D_{90.2}$ of 7 minutes, D_{91} of 2.9 - 5.4 minutes and Z value of 5.2 - 10.3 minutes.

TRICHODERMA

Trichoderma species are common contaminants of crops, often noted in vegetables. Many species are pathogens of other fungi; some have been used as biological control agents, for example, *Trichoderma harzianum* and *Trichoderma viride* (31, 32). They can also destroy laboratory cultures if they occur as contaminants. *T. harzianum* is the most common species from foods.

Types of Toxins Produced

No toxins are noted, but *Trichoderma* species are aggressively pathogenic to other fungi. *Trichoderma* species produce a range of pungent volatile compounds.

Sources

T. harzianum is a common soil contaminant; it is often associated with rotting wood. It has been isolated from cassava, apples, cereals, nuts and beans, though in many cases that may have been in association with other fungi that it was parasitizing.

Growth Requirements

T. harzianum has a growth range from 5 - 36 °C, with an optimum at 30 °C. The minimum a_w is 0.91 at 25 °C.

WALLEMIA

Wallemia is represented by one species, *Wallemia sebi*, an extremely xerophilic mould. It is characterised by the production of large quantities of small round conidia from very small, dull chocolate brown colonies.

Types of Toxins Produced

W. sebi produces walleminol A&B, which have toxicity to brine shrimps and rat liver cells.

Sources

W. sebi is associated with a wide range of foods, from cereals, spices, nuts and dried fruit to dried and salted fish, where contamination causes discrete brown spots; ‘dun’ mould. It has also been isolated from jam, condensed milk, jelly, and fruit cakes, where it may grow unobtrusively on the dried fruit, spreading out into the cake. It is also found on chocolate icing on cakes.

Growth Requirements

W. sebi is able to grow between >5 and <37 °C. It can grow over a a_w range from 0.997 - 0.69 at 25 °C, which is very unusual – xerophiles do not usually grow at very high water activity. *W. sebi* tolerates salt reasonably well; it is able to grow down to a_w 0.75 - 0.80 with NaCl, at pH 6.5 and 25 °C. Spores germinate in 1 - 2 days at a_w 0.92 - 0.997, and 5 days at a_w 0.85. Spores are very heat sensitive; with $D_{57.5}$ of 1 minute, and a Z value of 8.1 °C.

XEROMYCES

Xeromyces has the distinction of being the most xerophilic micro-organism yet discovered, able to grow to a_w 0.61. It is rarely isolated, probably due to the requirement of a very low a_w to isolate it effectively. *Xeromyces bisporus* is the sole representative of the genus. It is an ascosporic fungus, producing very heat-resistant ascospores, and less heat-resistant conidia.

Types of Toxins Produced

X. bisporus is not noted to produce any mycotoxins.

Sources

Because of slow growth on more normal a_w media substrates, it is commonly overlooked. It is seen primarily on long shelf-life products, for example, liquorice, dried prunes, tobacco, currants, and other dried fruit, chocolate, and other confectionery, cakes, cookies and honey.

Growth Requirements

X. bisporus cannot grow at a_w >0.96; on glucose/fructose media it can grow to a_w 0.61, taking 120 days for spores to germinate. The optimum a_w is 0.85, and it can still grow rapidly at a_w 0.75. Conidial production occurs at a_w 0.66 after 80 days. Ascospores can be produced as low as a_w 0.67. It is less

tolerant of low water activity where salt is used to moderate the water activity. It is very tolerant of CO_2 - it can grow in 90% CO_2 and 1% O_2.

Heat resistance varies; 0.1% of ascospores survive at 80 °C for 10 minutes. At pH 5.4, a_w 0.90 and 90 °C, it takes more than 2 minutes to kill 2 x 10^3 ascospores, 4 minutes at 85 °C, and 9 minutes at 80 °C. D-values calculate as $D_{82.2}$ for 2.3 minutes and a Z value of 16.0 °C.

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OTHER SPOILAGE MOULDS

Claviceps spp. can produce ergot alkaloids on cereals. Outbreaks of ergotism were the earliest recognised human mycotoxicoses and occur occasionally. Major ergot alkaloid producer species are *Claviceps purpurea* (17) and *Claviceps paspali* (12). *Phomopsis* can infect lupin plants and lupin seeds, and produce lupinosis toxin (phomopsin). Phomopsin is a hepatotoxin that affects sheep health after grazing on lupins. *Monascus* ruber is used commercially to produce 'red rice'; there is some evidence that it can produce the mycotoxin citrinin (12).

FOOD SPOILAGE YEASTS

INTRODUCTION

Yeasts are single-celled fungi that are capable of asexual vegetative reproduction via budding or fission. In addition, some food spoilage yeasts also have a sexual reproductive mechanism by which ascospores are produced. They are capable of rapid growth and often produce off-flavours, acid and gas, making them common food spoilage organisms of many foods and beverages (1, 3). Their single-celled growth mechanism makes them better suited to growth in liquid beverages. Many yeast species can also spoil the surface of fruits and vegetables (1).

Yeast classification can be very complicated. It is based primarily on the type and appearance of sexual spores that they produce - either ascospores or basidiospores. A third group, the imperfect yeasts, do not as yet produce a sexual state (2).

Classification to species level relies on biochemical tests (fermentation and assimilation) and on morphological appearance, usually of the sexual stage of the yeast. Further complications occur with yeast classification as a result of the ability to reproduce both sexually and asexually. In nature and in the laboratory, yeasts usually reproduce asexually, producing an anamorphic state. When they reproduce sexually they produce a teleomorphic state. This has often led to 2 or more names being applied to the same organism, one for the anamorph and another for the teleomorph.

As the science behind the identification of yeasts progresses, especially with the development of DNA and RNA analysis, many of the anamorph - teleomorph connections have been identified and only 1 name (the teleomorph) is used, however this is not the case for all yeasts and the anamorphic name becomes an associated synonym. Development of genetic techniques has also led to taxonomic rearrangements and as a result numerous synonyms are attributed to some yeast species (1, 2). Lists of synonyms for each organism are not included in this chapter, unless relevant; only the species name commonly used in food microbiology.

Yeasts have an important role in the production of many fermented foods, for example, bread, beer, wine, vinegar and cheese. However, some of the

same species that are used in fermentations can also be spoilage agents if they are allowed to contaminate and grow in food products.

Yeasts typically spoil high acid, low pH, high sugar (>10%), high salt (>5%) or weak organic acid (sorbic, acetic, benzoic acid) preserved products. Therefore fruit and fruit-based products, sugar syrups, alcoholic or carbonated beverages, salad dressings and other acid sauces, dairy products, and fermented foods are often associated with yeast spoilage. Due to the wide variety of foods spoilt, yeasts have enormous commercial and economic significance. Management of spoilage by yeasts is primarily achieved via effective quality control (3).

About 10 yeast species are thought to be responsible for the majority of food and beverage spoilage. Most yeasts involved in soft drinks spoilage are categorised as either 'opportunistic spoilage' species or 'spoilage yeast' due to poor factory hygiene (5). Davenport (6, 7, 8) divided yeasts that can cause spoilage in soft drink factories into four groups:

- Group 1 are spoilage organisms that are well adapted to growth in soft drinks, and are able to cause spoilage with very low cell numbers (as few as one cell per container). Characteristics of Group 1 yeasts include osmotolerance, they are aggressively fermentative, preservative resistant (particularly weak organic acids), and a have requirement for vitamins. *Zygosaccharomyces bailii* is a typical example of this group, and this group is one of Pitt and Hocking's 10 key spoilage yeasts (1).
- Group 2 organisms are described as spoilage or hygiene organisms. They are able to cause spoilage of soft drinks, but only if something goes wrong during manufacturing, for example, the preservative is added at too low a level or is absent, there is ingress of oxygen, there is a pasteurisation failure, or there are poor standards of hygiene. These are common contaminants in factories, but are severely restricted if good hygiene practices are followed.
- Group 3 organisms are indicators of poor hygiene standards; they do not grow in soft drinks, even if present at high numbers. These are typical of the yeasts found in many factories. The higher the count, the worse the hygiene state of the factory.
- Group 4 yeasts are known as aliens, those out of their normal environment. An example would be *Kluyveromyces lactis*, a dairy spoilage yeast.

Although yeasts can cause food spoilage, they are not known to cause food poisoning in the strict sense of the term. Typical effects of yeast growth in foods and beverages include production of acid, or acid and gas, visible growth leading to cloudiness, and changes to the product texture, flavour, odour or colour including the production of taints or 'off' flavours. It is estimated that fermentative yeasts cause 5% of visible spoilage in foods and beverages, whereas most of the less obvious spoilage is caused by opportunistic spoilage yeasts, due to poor hygiene practices. Ingestion of beverages spoiled by *Saccharomyces* and *Zygosaccharomyces* yeasts can cause gastrointestinal disorders which might be as a result of the yeasts' metabolites (3).

Generally speaking, some yeasts and facultative anaerobes some are fermentative, Even film forming yeasts have fermentative abilities. Some species are aerobic only and this is stated where appropriate in this chapter.

Where an anamorphic version of a yeast strain is mentioned here that has a teleomorphic counterpart also included in this section, the information will be included under the relevant teleomorph section only.

AUREOBASIDIUM

Aureobasidium pullulans (teleomorph *Discosphaerina fulvida*) is a 'yeast-like mould'. Microscopically it produces fungal hyphae as well as budding yeast-like cells. These yeast-like cells are actually conidia (1). It is a Davenport class 3 organism and therefore its presence in food is an indicator of poor factory hygiene. It is often used as an indicator of air quality as it can be found contained in dust (5). *A. pullulans* is an opportunistic human pathogen causing pneumonia and asthma in sensitised individuals.

Sources

A. pullulans is ubiquitous in many moist or decaying environments; it has been isolated from a wide range of food types but is not often the cause of spoilage. It has been isolated from frozen fruit pies (cherry, blueberry, apple), meat (causing black spot), cheese, cabbage and other brassica, asparagus, green beans, cucumber, strawberries, grapes, apples, pears, citrus fruits and citrus fruit products, seafood and fish, olives, barley, oats, wheat and wheat flour and nuts (1, 2, 10, 19). It is also found in fresh water (2).

Growth Requirements

Temperature

Growth of *A. pullulans* occurs between 2 – 35 °C with an optimum of 25 °C (1). Some strains may be capable of growth at -8 °C (19). This ability to grow at low temperatures is confirmed by the isolation of *A. pullulans* from frozen foods although spoilage does not occur at these temperatures (17).

The heat resistance of *A. pullulans* is very low (1).

pH

A. pullulans displays mycelial growth under conditions of low pH but yeast-like growth at higher pH (6.0 – 6.5). Production of the extra-cellular polysaccharide pullulan occurs down to pH 2.0 (21).

Water activity

Growth of *A. pullulans* below water activity (a_w) of 0.90 is not thought to occur (1).

Preservatives

No data is available relating to the effects of preservatives on *A. pullulans*. The organism does produce its own antifungal compound, Aureobasidin A (AbA) which is effective in the prevention of post-harvest disease of fruits by pathogenic fungi (20).

CANDIDA

The family Candidaceae has formally been used as a catch all classification for any organism that can't be categorised anywhere else. It contains the anamorphic versions of many ascospore producing yeasts. It is a very large heterogeneous family containing approximately 200 species, some with and some without teleomorphic counterparts (2). It should be noted that the genus *Torulopsis* was merged with *Candida* in 1978 although there is still

some dispute as to whether it should be a separate genus. Key species involved in food spoilage are *Candida parapsilosis*, *Candida holmii* (synonym *Torulopsis holmii*, teleomorph *Saccharomyes exiguus*), *Candida krusei* (teleomorph *Issatchenkia orientalis*) and *Candida tropicalis*. *C. parapsilosis* is a Davenport class 2 organism, and *C. tropicalis* a class 3 organism (5).

C. tropicalis is also a human pathogen and has been reported as causing yeast infections, primarily in immunocompromised individuals, following consumption of *C. tropicalis* containing food. *C. parapsilosis* is also pathogenic to man (1).

Sources

C. parapsilosis has been isolated from butter, cheese, salad dressings and yoghurt (it has lipolytic properties), also from raw meat, processed meats, fish and from fruits, fruit products, pickles, soft drinks, alcoholic beers and wines, bread, carrots and corn (1, 2, 5, 8). *C. tropicalis* has been isolated from processed meats, cheese, yoghurt, citrus and tropical fruits, apple juice, soft drinks, rice, corn, lager, wine, bread flour and dough and fermented and acid preserved foods e.g. brine, cocoa (2, 8).

Growth Requirements

Temperature

C. parapsilosis grows between 8 and 42 °C with an optimum of 35 °C (1).

D-values for *C. parapsilosis* are 2.2 minutes at 62 °C.

pH

C. parapsilosis also shows a high degree of resistance to low pH (5). It is also alkaline-tolerant and can grow at pH 10 – 10.5 (12). *C. tropicalis* is also reported to be alkanotrophic.

Water activity

C. parapsilosis has been reported to be resistant to as much as 20% salt.

Preservatives

C. parapsilosis also shows a high degree of resistance to preservatives and has been shown to grow in the presence of 10 - 20 g/l sorbic acid at pH 4.0 (2).

CRYPTOCOCCUS

Cryptococcus are a large heterogeneous genera of imperfect fungi containing the species *Cryptococcus albidus* and *Cryptococcus laurentii*, these species being the most often associated with foods. Both of these strains are anamorphic and they reproduce by budding. *Cryptococcus* spp. are strictly non-fermentative aerobes (2). Both species are Davenport class 3 yeasts (5).

Cryptococcus neoformans, another member of the *Cryptococcus* genera, is an opportunistic human pathogen causing cryptococcosis and fungal meningitis.

Sources

Both *Cry. albidus* and *Cry. laurentii* are frequently isolated from soil from forest and tundra. *Cry. albidus* is also associated with grass. They are both commonly found in freshwater; they can be airborne and colonise the leaves and surfaces of plants. Both species have been isolated from wheat and rye, but not from flour, fermented or cured meat, pear, grape, strawberries, tropical fruits, citrus fruit, corn, cabbage, chilled potato and pasta salads, pork, beef and lamb (2, 5).

In addition *Cry. albidus* has been isolated from fish and shellfish, apple, cherries, carrot, milk, cheese and sugar syrups (2, 8).

Cry. laurentii has also been isolated from chicken, biltong, fresh and frozen oysters, fresh-cut salads, cottage cheese, butter (*Cry. laurentii* is lypolytic) and wine (2).

Growth Requirements

Temperature

Cryptococcus spp. are psychrotrophic with may strains capable of growth from 0 – 25 °C (2).

Cry. laurentii made up 90% of the yeast population of wrapped loin of lamb stored at -5 °C (2, 5, 19). Growth of *Cry. laurentii* (although limited) has been reported at -7.5 °C but not at -10 °C (18).

pH

Cry. laurentii is alkali-tolerant and can grow at pH >8. Both *Cry. albidus* and *Cry. laurentii* have been isolated from low-pH products (2).

Water activity

Both *Cry. albidus* and *Cry. laurentii* have been isolated from low-water activity products (2). *Cry. laurentii* can grow in the presence of high concentrations of salt.

Preservatives

Cryptococcus spp. are sensitive to sulphite preservatives (5).

An irradiation dose of 10 kGy is required to prevent the growth of *Cry. albidus* (2).

Some strains of *Cry. laurentii* have been shown to produce an antibacterial compound (5).

Some strains of *Cryptococcus* have been shown, *in vitro*, to be inhibited by garlic when diluted 1:1024 (22).

DEBARYOMYCES

Debaryomyces hansenii (anamorph *Candida famata*) is a fermentative yeast (although often weakly) with lypolytic and proteolytic properties. It is a class 2 Davenport soft drink spoilage organism (5). *D. hansenii* is one of the

most common foodborne yeasts and its psychrotrophic and halophilic/osmophilic nature make it a serious spoilage organism in many foods.

C. famata is an opportunistic human pathogen, affecting the immunocompromised (8).

Sources

D. hansenii is often isolated from sea water, seafood (23) and salt brines used for olives, bacon, hams or cheese, from soy sauce, fermented or dry cured meats and salamis (producing a surface slime), fresh meat including lamb, pork, poultry, snails and beef, orange (and other fruit) juice, yoghurt, butter, raw milk, cheese, mayonnaise and dressings, olives, mushrooms, berries, grapes, apples, pears, citrus and tropical fruits, high sugar products, cocoa, barley, corn, wine and beer (1, 2, 5, 10).

Growth Requirements

Temperature

D. hansenii has an optimum growth range of 2 - 33 °C with an optimum of 24 - 27 °C in 10% sucrose, rising to 27 - 30 °C in 60% sucrose. It is rare for *D. hansenii* to grow at 37 °C, but its maximum growth temperature increases with increased amounts of glucose with a maximum of 38 °C at 60% sucrose (1).

Twenty minutes at 60 °C is sufficient to kill 10^5 vegetative cells per millilitre of *D. hansenii* (1).

pH

One strain of *D. hansenii* has been reported as capable of growth at pH 2.0 when an inorganic buffer is used, all other strains used in the study grew at pH 2.5 when citrate-phosphate buffer was used (25). *D. hansenii* is able to tolerate high salt levels at pH 3.0 (2).

Water activity

D. hansenii is able to grow in foods with a salt concentration as high as 24% (a_w of 0.84). Growth of the anamorphic form has been recorded at a_w of 0.65 (1, 8, 10).

Preservatives

D. hansenii is inhibited by 250 mg/l benzoic acid at pH 3.0 and 500 mg/l at pH 5.0. 250 mg/l sorbic acid is sufficient for inhibition at pH 3.0 and 5.0 (24). 200 µg/l of acidified nitrite gives pronounced growth inhibition of *D. hansenii* at pH 4.5 but not at pH 5.5 (25).

An irradiation dose of 7.5 kGy is required to prevent the growth of *D. hansenii* (2).

Vanillin at a concentration of 0.2% is effective against the growth of *D. hansenii* in apple puree, pH 3.5, a_w 0.95 for 40 days at 27 °C (2).

DEKKERA

Dekkera (*Brettanomyces*) spp. produce off-flavours in spoiled beverages via the production of various volatile compounds. Phenolic compounds are produced by the organism during wine production. Spoilage can also be as a result of acetic acid production, surface films or turbidity in liquid samples and swelling from gas production (to high enough pressure to explode bottles (3)). Unusually fermentation is triggered in this genus by the presence of oxygen and the genus is resistant to high levels of dissolved carbon dioxide: up to 4.45 volumes (5, 26).

Dekkera bruxellensis (anamorph *Brettanomyces bruxellensis*) and *Dekkera anomala* (anamorph *Brettanomyces anomalus*) are the only 2 recognised species within the *Dekkera* genus. It is still unclear as to whether *Brettanomyces naardenesis*, another important spoilage organism, only occurs in the imperfect form and has no teleomorph, or if the synonym *Dekkera naardenesis* is in fact the sexual stage of this organism (2, 26).

Dekkera bruxellensis, *anomala* and *naardenesis* are all Davenport class 1 soft drink spoilage organisms (5)

Sources

Dekkera spp. are mostly associated with fermented foods but are not commonly the cause of spoilage of these foods and appear to only occur in large numbers when other micro-organism species have been inhibited, which may be due to an exceptional resistance to minimal nutrient availability (26).

D. bruxellensis is almost exclusively isolated from alcoholic drinks such as beer, cider, sherry and wine (although never from grapes) and from soft drinks, but has also been isolated from cheese and olives (1, 2, 5, 26).

D. anomala has been isolated from beer, grape must, wine (including sparkling wine), sherry, dry ginger ale, cola, cheese, and brewery equipment (2, 5, 26). Note, the isolation from wine may have been *D. bruxellensis*; the two species are very difficult to differentiate between (26).

D. naardenesis has been found in carbonated drinks: lemonade, soda and tonic water, cheese (26).

Dekkera/Brettanomyces spp. have also been isolated from spoiled fruit yoghurt and sour dough (1, 10) and can be isolated from the air in fruit orchards (26).

Growth Requirements

Temperature

No data is available regarding the growth temperatures of *Dekkera/Brettanomyces* spp. however, enzyme activity is reported as occurring between 15 – 30 °C (28).

Brettanomyces spp. are reported as being capable of surviving heating for 10 minutes at 60 °C but not 20 minutes (1). However, another study reports D_{55} values of 0.3-0.4 minutes and a Z value of 4.4-5.8 °C for *D. bruxellensis* and D_{55} values of 0.2 minutes and a Z value of 4.5 °C for *D. anomala* (30).

No specific data is available for the heat resistance of spores of *Dekkers* spp. however it is unlikely that the organism would sporulate in wine (30).

pH

D. bruxellensis has been reported to grow at pH 1.8 in a hydrochloric acid-acidified medium and pH 2.3 in a citric acid-acidified medium (1, 10).

Species of *Dekkera* do not grow above pH 8.0 (2).

Water activity

No specific water activity data is available but *D. bruxellensis* is capable of growth in 35% glucose and 45% sucrose solutions (26).

Preservatives

D. bruxellensis is reported to be resistant to 75 mg/l sodium dioxide, 950 mg/l sorbic acid, 100 - 200 ppm benzoic acid, and is capable of growth in 15.5% ethanol at pH 3.5. Some strains may be sensitive to sodium dioxide at 20 - 50 mg/l (26, 28, 29).

D. anomala is reported to be resistant to 8% ethanol (29).

Ninety percent of a population of *D. bruxellensis* can be inhibited by 200 μg/l of a commercial β-glucanase preparation (27).

HANSENIASPORA

Hanseniaspora uvarum (anamorph *Kloeckera apiculata*) is a strongly fermentative yeast that produces numerous extracellular compounds in addition to ethanol including acetic acid and ethyl acetate, a vinegary off-flavour in wine. It also produces a haze in liquid products (1, 2, 5). *H. uvarum* is a Davenport class 2 soft drink spoilage organism (5). *H. uvarum* is an opportunistic human pathogen (3).

Sources

H. uvarum can be isolated from soil, grapes, apples, coffee, malting barley, beer, cider, salami, figs, tomatoes, canned black cherries, plums, peaches, pears, apricots, strawberries, currants, citrus fruits and juices, other fruit juices and syrup and soft drinks (1, 2, 5).

Growth Requirements

Temperature

The minimum growth temperature for *H. uvarum* is reported as 8 °C and the maximum approximately 40 °C (1). A greater amount of ethanol and cell growth is seen at 10 °C compared to 25 °C in apple juice fermentations (31).

A 5 log reduction in cells can be achieved via heating for 30 seconds at 60 °C (1).

pH

H. uvarum has been reported as showing no growth at pH 8.0. It has been shown to be capable of growth at pH 1.5 – 2.0 (2, 24).

Water activity

Reported minimum water activity for growth of *H. uvarum* are; 0.90 in the presence of glucose or sucrose, 0.93 in fructose and 0.95 in sodium chloride, making *H. uvarum* a not especially osmotolerant yeast (2). *H. uvarum* is also reported as being capable of growth in the presence of 12.5% sodium chloride at pH 2 – 3, and 50% sucrose between pH 2 -7 (24).

Preservatives

The minimum inhibitory concentration of benzoic acid for the inhibition of *K. apiculata* is reported to between 188 – 244 mg/l at pH 3.5 and 25 °C and sorbic acid 157 mg/l. Levels of sulphur dioxide at 100 mg/l have been reported to be tolerated by *K. apiculata* in red wine (1, 13). Another study reports growth of *K. apiculata* in the presence of 750 mg/l benzoic acid at pH 5.0 and 1,200 mg/l at pH 7.0, Sorbic acid did not inhibit growth at a level of 250 mg/l at pH 5.0 or 1,200 mg/l at pH 7.0. Benzoic or sorbic acid at 250 mg/l were sufficient to inhibit growth at pH 3.0 however (24).

H. uvarum is considered to be ethanol sensitive, but is capable of growth in ethanol at 9% and can survive at 12.5% at 15 °C; sensitivity is increased at 10 and 30 °C (1).

ISSATCHENKIA

Issatchenkia orientalis (anamorph *Candida krusei*) is a widespread organism which produces a surface film on spoiled products. It is a highly fermentative species and the surface film is often supported by or contains gas bubbles. It is a Davenport class 2 yeast - one that is present in the manufacturing facility and causes opportunistic spoilage following a manufacturing error. (5)

Sources

Iss. orientalis has been isolated from fresh water, fruits and fruit products including juice concentrates and soft drinks, cocoa beans, olives, pickles, figs, tomato sauce, mayonnaise, yoghurts, cheese, corn, malt, beer and wine (2, 5, 12).

Growth Requirements

Temperature

The minimum growth temperature for *Iss. orientalis* is 7 °C and the maximum approximately 47 °C (1, 2).

Iss. orientalis is relatively heat resistant with a D_{56} of 30 minutes but a D_{65} of <2 minutes.

pH

Growth of *Iss. orientalis* (*C. krusei*) has been shown at pH 1.3 when the media was acidified with hydrochloric acid (1, 2)

Water activity

Iss. orientalis has been isolated from salt brines (5) and is able to grow in very concentrated fruit juices (2).

Preservatives

Iss. orientalis (*C. krusei*) is preservative-resistant and has been reported to grow in the presence of 335 ppm sorbic acid, 360 ppm benzoic acid and 30 ppm free sulphur dioxide at pH 3.5 (1). It is capable of developing an increased resistance to preservatives when grown in their presence at sub-inhibitory concentrations (13). It is also resistant to ethanol and to 1.5% acetic acid (5, 14).

An irradiation dose of 5.5 kGy is required to prevent the growth of *Iss. orientalis* (2). Additionally natamycin has been shown to be effective against *Iss. orientalis* in wine at a level of 1.25 mg/l (15).

KLUYVEROMYCES

The genus *Kluyveromyces* currently contains six species; *Kluyveromyces lactis* (anamorph *Candida sphaerica*) and *Kluyveromyces marxianus* (anamorph *Candida kefyr*) will be the species discussed here. It is believed that *K. lactis* has been reclassified as *K. marxianus* (10). *Kluyveromyces* are capable of assimilating and fermenting lactose and therefore of fermenting diary products, they are also able to assimilate lactic acid and hydrolyse casein (2, 5). *K. marxianus* is an opportunistic human pathogen (3).

Sources

K. marxianus is frequently isolated from sugar cane, figs (dried), molasses, cheese and yoghurt (causing blowing of packages and 'off' flavours), kefyr grains, raw milk, ham, beer and wine, soft drinks and fruit juices, dough and bakers yeast. It has also been found on cherries, plums, currants and onion (2, 5, 8).

K. lactis has been isolated from cream, cheese and yoghurt (causing blowing of packages and 'off' flavours), raw milk and cocoa (2, 5).

Growth Requirements

Temperature

K. lactis has been reported to grow at 47 °C (2).

K. marxianus is relatively thermotolerant and is capable of growth at 48 °C, with a few strains able to grow at 52 °C. *K. marxianus* is tolerant of 55 °C (2). The organism is inactivated by temperatures used for milk pasteurisation.

As dairy spoilage organisms, *Kluyveromyces* spp are typically capable of growth at low temperatures, *K. marxianus* grows well at 6 - 10 °C (5).

pH

K. marxianus is able to grow at pH 8.0 but is inhibited at pH 3.0 in an inorganic buffer, growth at pH 2.5 does occur in citrate-phosphate buffer (24).

Water activity

No specific data are available relating to minimum water activity for the growth of *Kluyveromyces* spp., but as dairy spoilage organisms, *Kluyveromyces* are typically salt-tolerant (2, 5), however in laboratory trials they do not grow in the presence of 10% sodium chloride (25). *K. marxianus* is frequently isolated from low water activity foods (such as syrups and concentrates) and is moderately xerotolerant and capable of growth in 50% sucrose (2, 24).

Preservatives

K. marxianus is able to grow in the presence of 500 mg/l benzoic acid at pH 5.0 and 1,200 mg/l at pH 7.0. Sorbic acid did not inhibit growth at a level of 250 mg/l at pH 5.0 or 1,200 mg/l at pH 7.0. Benzoic or sorbic acid at 250 mg/l was sufficient to inhibit growth at pH 3.0 however (24). *K. marxianus* has a minimum inhibitory concentration of ethanol between 8.6 – 9.4% (2).

PICHIA

The genus *Pichia* is a large and heterogeneous group containing more than 100 species. The 2 species included in this chapter are *Pichia anomala* (anamorph *Candida pelliculosa*) and *Pichia membranaefaciens* (anamorph *Candida valida*), which is the type species. An additional species *Pichia guilliermondii* and several other *Pichia* spp. have a role in food spoilage but are not covered by this chapter. *Pichia* spp. are film-forming yeasts. *P. membranefaciens* and *P. anomala* are Davenport class 2 soft drink spoilage organisms (5). Both species are opportunistic human pathogens (3).

Sources

P. membranaefaciens is commonly associated with salt brines used with olives and cheese, in acetic acid-preserved foods such as onions, gherkins, beetroot and sauerkraut and tomato sauce. It has also been isolated from mayonnaise-based salads, fermented milk products, yoghurts, beer (producing film and excessive esters), wine, cider, minced beef, poultry, sausages, fish and shrimp, cereal grains, flour, cocoa, molasses, sugar syrups, honey, fresh-cut salads, grapes, figs, tropical fruits, citrus fruits and citrus fruit products and soft drinks (1, 2, 10).

P. anomala is responsible for the spoilage of yoghurt, bread, cheese, soft drinks, beer and wine. It has also been associated with apples, cherries, plums, citrus fruits, tropical fruits, corn, cut salads, pickles, olives, sausage, ham, fish and shellfish, cocoa, coffee, jam, confectionery and sugar syrups (2, 5).

Both yeasts have been responsible for the spoilage of acetic acid preserved dressing and mayonnaise (1).

Growth Requirements

P. membranaefaciens and *P. anomala* are fast-growing yeasts under aerobic conditions, they form surface films that can then break off and form deposits in the product as well as produce gas and ‘off’ flavours (5). Both species have lipase activity (2).

Temperature

P. membranaefaciens does not usually grow at 37 °C and its maximum is between 32 – 37 °C (1, 5); its minimum growth temperature is 5 °C (1). *P. membranaefaciens* grows well between 6 and 10 °C (5).

The maximum growth temperature for *P. anomala* is between 35 and 37 °C (2).

Vegetative cells of *P. membranaefaciens* are heat sensitive, having a D_{60} of <2 minutes (1).

D_{60} values for *P. anomala* (*C. pelliculosa*) are 4.90 minutes in pineapple juice, 3.70 minutes in passion fruit nectar, 3.62 minutes in guava juice and 1.81 minutes in tartaric buffer solution (33).

pH

Growth of *P. membranaefaciens* occurs at pH 1.9 in hydrochloric acid-acidified media and 2.1 - 2.2 in media acidified with organic acids (1, 13). No growth occurs at pH 8.0 (2). Neither species is able to grow at pH 2.0 in the absence of salt (24).

P. anomala has a minimum pH for growth of approximately pH 2.5 without the presence of salt, pH 2.0 with salt; it is capable of growth at pH 8.0 but not at pH 9.0 (24).

Water activity

Growth, albeit weak, of *P. membranaefaciens* has been reported at a_w 0.90, the equivalent to 15.2% sodium chloride (1) or 0.94 (2). It is able to grow to 0.90 in glucose and sucrose and 0.92 in fructose (2).

P. anomala grows in 15% sodium chloride and 70% sucrose (2. 24)

Preservatives

P. membranaefaciens is considered to be preservative-resistant (5) and has been reported to grow in the presence of 3% acetic acid (13, 32), 750 mg/kg benzoic acid at pH 5.0, 1,500 mg/kg sodium benzoate at pH 4.0 and 3,000 mg/kg benzoate at pH 4.5 (1, 24). Some strains are reported as capable of growth in 10 – 20 g/l sorbate at pH 4.0 (2), 250 mg/l sorbic acid at pH 5.0 and 1,200 mg/l at pH 7.0 (24).

P. anomala has a minimum inhibitory concentration of ethanol of between 10.0 – 10.9% (2). It is inhibited by 250 mg/l benzoic acid at pH 3.0 but can grow in the presence of 500 mg/l at pH 5.0 and 1,200 mg/l at pH 7.0 (24). Resistance to sorbic acid is >250 mg/l at pH 5.0 and >1,200 mg/l at pH 7.0 (24).

RHODOTORULA

Rhodotorula glutinis (teleomorph *Rhodospridium toruloides*) and *Rhodotorula mucilaginosa* are two closely-related species of the *Rhodutorula* genus that are frequently found in food, they are strictly aerobic and do not ferment. Both species produce extracellular lipases and proteases (1). Although spoilage caused by these organisms is rare, their ability to grow at low temperatures, low pH and in the presence of salt, combined with their lipolytic activity makes them organisms of concern. Both species are Davenport class 3 soft drink spoilage organisms (5). *Rhodutorula* spp. are opportunistic human pathogens (3).

Sources

Rhodutorula spp. are spoilage organisms of yoghurts, cream, butter, cheese, apple sauce and pie filling, jam, strawberries, mayonnaise and dressings. They are commonly associated with honey, soil, fresh water, fresh fruit including grapes, apples, pears, cherries and vegetables, processed vegetables, cereals, flours, dough, malting barley, olives, citrus fruits and fruit juice concentrates, seafood including fresh and frozen oysters, fresh (lamb, beef, pork, chicken) and processed meat (British sausages, fermented sausage, dry cured hams), raw milk, cocoa, coffee, beer and wine (1, 2, 5).

Growth Requirements

Temperature

Rh. mucilaginosa has a minimum growth temperature between 0.5 - 5 °C and a maximum near 35 °C. It may be capable of growth below 0 °C (1).

Heat resistance of *Rhodutorula* spp. may be high for a non-spore-forming yeast with some strains of *Rh. mucilaginosa* and *Rh. glutinis* surviving 62.5 °C for 10 minutes (10^5 cell/ml initial concentration) (1). Another study reports a D_{60} for *Rh. mucilaginosa* of 0.158 minutes at pH 4 (10, 35), 0.159 minutes at pH 3.5 and 0.120 minutes at pH 3.0 (34).

pH

pH 2.2 is the minimum pH for growth of *Rh. mucilaginosa* (1). *Rhodutorula* spp. are alkali-tolerant and capable of growth above pH 8.0 (2).

Water activity

The minimum water activity for growth for growth of *Rh. mucilaginosa* is reported to be 0.92 (1) in fructose and 0.90 in glucose, sucrose or sodium chloride (2).

Preservatives

Growth of *Rh. mucilaginosa* is inhibited by >100 mg/kg benzoic acid or sorbic acid at pH 4.0 or less (1).

Rh. glutinis is able to assimilate nitrate for use as a nitrogen source (1), which also makes it a possible soft drink spoilage organism (5).

The red pigmentation in *Rh. mucilaginosa* and *Rh. glutinis* provides protection against UV light (2).

SACCHAROMYCES

Saccharomyces cerevisae is strongly fermentative; it commonly occurs in foods and is one of the most frequently reported yeasts isolated from food and drink (16). It is a Davenport class 2 organism (5).

Saccharomyces exiguus (teleomorph *Candida holmii*) resembles *S. cerevisae* in terms of its spoilage characteristics, i.e. it is fast-growing and vigorously fermentative. It is a Davenport class 1 organism (5).

Sources

Saccharomyces cerevisae is of course key in the production of bread and alcoholic drinks, it has however also been associated with spoilage and has been isolated from fruits (although rarely from the surface) and fruit nectar, soft drinks including fruit juice and concentrates, sports drinks and water containing aloe vera, honey, fermented milk products (although rare), cheese, meat products (occasionally), bakery products, beer, cider and wine and cocoa (1, 16). Insects and unwashed bottles are thought to be vectors of *S. cerevisae* (5).

S. exiguus has been associated with the spoilage of soft drinks, fruit juices, carbonated beverages and cordials, mayonnaise (vinegar and salt-preserved), coleslaw, cheese, salads, fermented or pickled vegetables and occasionally from meat products (2, 5, 16).

Growth Requirements

Temperature

S. cerevisae has an optimum growth temperature of 33 - 35 °C. Its reported minimum growth temperature is 4 °C in 10% glucose and 13 °C in 50% glucose. Maximum temperatures for growth are 38 - 39 °C, but a few strains have been reported to be capable of growth at 41 - 43 °C (1, 16).

S. exiguus will not grow above 37 °C (5) and has shown variable growth at 35 °C. It will grow at 4 °C (16).

S. cerevisae is one of the most heat resistant yeasts studied thus far and thermal inactivation of vegetative cells of *S. cerevisae* requires a D_{60} of 0.1 - 0.3 minutes and greater than 1 minute for some strains. Ascospores of *S. cerevisae* are considerably more heat resistant and require 5.1 - 17.5 minutes at 60 °C. D values are increased with decreasing water activity. (1, 16).

pH

Saccharomyces spp. generally show strong growth between pH 3.0 - 7.0. Minima and maxima for growth are typically pH 1.5 – 2.5 and 8.0 – 8.5 (16). *S. cerevisae* is capable of growth at pH 1.6 when the acidulant is hydrochloric acid (1, 13).

S. exiguus has shown growth in extremely acidic environments (5).

Water activity

Saccharomyces spp. are neither osmo- nor xerotolerant and do not grow at water activities of less than 0.85 - 0.88 (16). The lowest reported water activity at which *S. cerevisae* is capable of growth is 0.89 a_w (1, 16). However, some strains have been reported to grow in 55 – 65% sugar solutions (2).

S. exiguus does not grow at glucose levels of 50% but some strains are capable of growth in 10% sodium chloride (16).

Preservatives

Some strains of *S. cerevisae* are resistant to 100 – 600 ppm, 10 ppm acetic acid, 75 – 100 ppm sulphur dioxide and 200 – 600 ppm sorbic acid at pH 3.5. Organic acid resistance is decreased with increasing sugar content (16). It is tolerant of up to 20% ethanol (16). High hydrostatic pressure and several plant extracts, e.g. onion, essential oil and vanillin have been shown to be effective in the inactivation of *S. cerevisae* (2, 16). An irradiation dose of 18 kGy is required to prevent the growth of *S. cerevisae* (2).

S. exiguus has been shown to be resistant to sorbic, benzoic and acetic acid but variations are seen in this resistance between strains. Some strains can grow in the presence of 800 ppm benzoic and 500 ppm sorbic acid at pH 4 (5).

SCHIZOSACCHAROMYCES

Schizosaccharomyces pombe is an uncommon cause of food spoilage however, its xerophilic nature, its resistance to preservatives and its ability to grow at 37 °C gives it the potential to cause spoilage in warm climates. It shows unusually slow growth with a 4-hour doubling time, can ferment malic acid and produces hydrogen sulphide ‘off’ flavours if sulphite is present. It is a Davenport class 1 organism (1, 5).

Sources

Schizo. pombe has been isolated from fruit juices and fruit juice-containing soft drinks, fruit concentrates, raisins, dried dates, cane sugar, sugar syrups, cocoa, cider, beer and wine (1, 5).

Growth Requirements

Temperature

Schizo. pombe grows as vigorously at 37 °C as at 25 °C (1).

D-values of D_{65} for 0.21 - 1.48 minutes have been reported dependent upon the type of sugar present (1).

pH

No pH data could be found for *Schizo. pombe* however, as it is a spoilage yeast of wine and carries out the malolactic fermentation that reduces the wine's acidity it is likely to be capable of growth at pH >3.5.

Water activity

Schizo. pombe is known to be xerophilic and can grow in 50% glucose, but no data for growth limits is available (1, 8).

Preservatives

Schizo. pombe is resistant to 120 ppm sulphur dioxide at pH 3.5 and up to 600 ppm benzoic acid (1). Minimum inhibitory concentrations of ethanol are 11.3 - 13.7% (2).

ZYGOSACCHAROMYCES

Zygosaccharomyces contains the most significant spoilage yeast species in the food and drink industry (specifically, *Zygosaccharomyces bailii*,

Zygosaccharomyces rouxii, *Zygosaccharomyces lentus* and *Zygosaccharomyces bisporus*). They are osmotolerant (*Z. rouxii*), resistant to weak acid preservatives (*Z. bailii*), and are able to ferment hexose sugars such as glucose and fructose (5). Spoilage caused by *Zygosaccharomyces* species can cause a serious physical injury, for example, exploding glass bottles due to the gas pressure generated as a result of yeast growth and sugar fermentation (5, 11). As fermentative yeasts, *Zygosaccharomyces* spp. are facultative anaerobes, and are able to grow under low oxygen tension in beverages (9). *Zygosaccharomyces* yeasts are a class 1 Davenport spoilage organism.

Sources

Foods preferred by *Zygosaccharomyces* yeasts for colonisation tend to be acidic, with high concentrations of fermentable sugars. *Zygosaccharomyces lentus* is found in dairy products, meat products, fruit and fruit products, bread, baking products, wine, chocolate, coffee and soy products, with breweries also being a recognised source (5). *Z. rouxii* has been found in confectionery products, jam, jellies, fruit concentrates, syrups, and oriental fermented foods such as soy sauce. *Zygosaccharomyces mellis* is found in honey (9).

Growth Requirements

Spoilage with *Z. bailii* can occur from an initial inoculum of just 1 yeast cell per litre of product.

Temperature

Z. bailii has an optimum growth range of 30 - 32 °C, minimum growth temperatures have been reported as 6.5 °C and maxima of 40 °C (1). *Z. lentus* can grow at low temperatures, 4 °C, and hence it is considered a potential spoilage organism in chilled food products (5). No *Zygosaccharomyces* species is reported to grow at 45 °C.

The heat resistance of *Z. bailii* vegetative cells have been reported as D_{60} of 0.1-0.3 minutes. Spore D_{60} values are 8 - 14 minutes. *Z. rouxii* has a D_{60} of <0.1 minutes at 0.94 water activity (a_w), but 10 minutes at 0.85 a_w (1).

pH

Z. bailii can grow in a minimum pH of 1.8 (10) and *Z. rouxii* has been reported to grow between pH 1.8 - 8.0 (11).

Water activity

Z. bailii has a reported minimum a_w of 0.80 at 25 °C (5). *Z. rouxii* is the most osmotolerant yeast known i.e. it can grow in foods with a high sugar concentration, with a water activity as low as 0.62. Its optimum a_w value for growth is 0.95 (9).

Preservatives

Weak organic acids, for example, acetic acid, sorbic acid, and benzoic acid, are effective preservatives for most *Zygosaccharomyces*. *Z. bailii* is a major nuisance, however, particularly in beverage factories, because it is resistant to acetic acid, ethanol, sorbic acid, benzoic acid and high sugar concentrates (4). *Z. bailii* has been isolated from spoiled products with preservative levels well in excess of those permitted in soft drinks in the EU.

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HACCP

Introduction

The Hazard Analysis Critical Control Point (HACCP) system is a structured, systematic approach to ensuring food safety. HACCP provides a means to identify and assess potential hazards in food production and establish preventive control procedures for those hazards. A critical control point (CCP) is identified for each significant hazard, where effective control measures can be defined, applied and monitored. The emphasis on prevention of hazards reduces reliance on traditional inspection and quality control procedures, and end-product testing. A properly applied HACCP system is now internationally recognised as an effective means of ensuring food safety.

In addition, HACCP utilising all seven principles to an appropriate level is now a requirement for all food businesses in the UK and Europe, as per the new EU food hygiene legislation (see legislation chapter in this book). This ensures that small businesses are able to design a HACCP system that is appropriate for the type of business.

The HACCP concept can be applied to new or existing products and processes, and throughout the food chain from primary production to consumption. It is compatible with existing standards for quality management systems such as the ISO 9000 series and the British Retail Consortium (BRC) Global Standard for Food Safety, furthermore, HACCP procedures can be fully integrated into such systems. The new ISO 22000 series incorporates safety and quality into one international standard.

The application of HACCP at all stages of the food supply chain is being actively encouraged, and is increasingly required, worldwide. For example, the Codex Alimentarius advises that "the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety".

Definitions

HACCP - A logical and scientifically based system, which identifies, assesses and controls hazards that are significant for food safety.

Control measure - An action or activity that can be used to prevent, eliminate, or reduce a food safety hazard to an acceptable level.

Corrective action - An action to be taken when loss of control at a CCP is indicated by monitoring.

Critical Control Point (CCP) - A step in the food chain at which control can be applied, and is essential to prevent, eliminate, or reduce a food safety hazard to an acceptable level.

Critical limit - A predetermined value for a control measure marking the division between acceptability and unacceptability.

Hazard - A biological, chemical or physical agent in, or property of, food that has the potential to adversely affect the health of the consumer.

Hazard analysis - The process of collecting and assessing information on hazards and the conditions leading to their presence, to determine which are significant for food safety and should be addressed by the HACCP system.

Monitoring - Conducting a planned sequence of observations or measurements to assess whether a CCP is under control.

Step - A raw material, location, procedure, operation or stage in the food production process.

Verification - The application of supplementary information, in addition to monitoring, to determine the effectiveness of the HACCP system.

Application of the HACCP System

The HACCP system consists of the following seven basic principles:

1. Conduct a hazard analysis
2. Identify the Critical Control Points (CCPs)
3. Establish critical limit(s)
4. Establish a system to monitor control of the CCP

5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control
6. Establish procedures for verification to confirm that the HACCP system is working effectively
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

It is recommended by the Codex Alimentarius that the practical application of the HACCP principles be approached according to the steps described in the logic sequence (Fig. 1). The decision tree (Fig. 2) should be used to determine the CCPs.

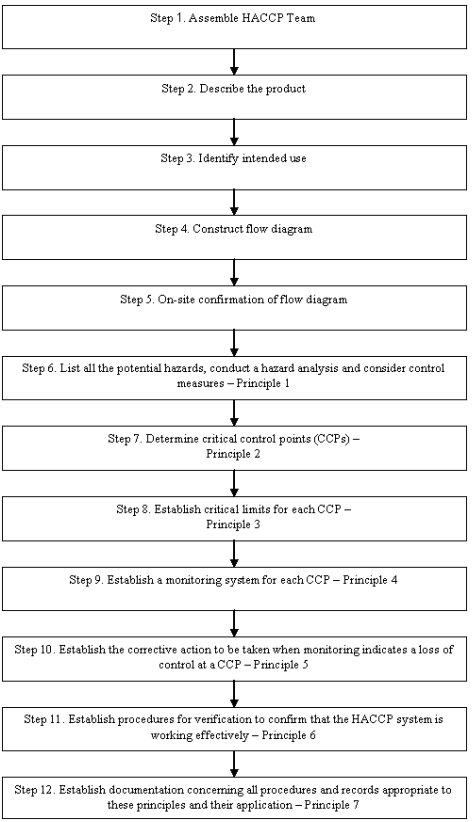
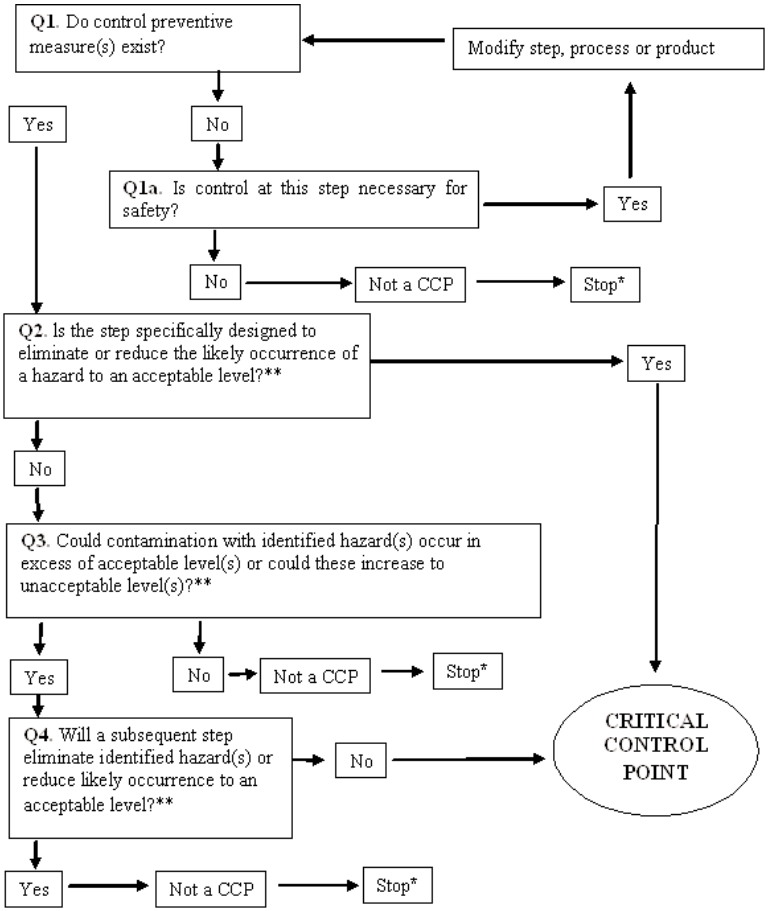


Fig. 1. Logic sequence for application of HACCP

Process Step Decision Tree



* Proceed to the next identified hazard in the described process
** Acceptable and unacceptable levels need to be defined within the overall objectives in identifying the CCPs of the HACCP plan.

Fig.2. CCP Decision Tree (Adapted from Codex Alimentarius Commission, 1997)

The stages of a HACCP study

1. Assemble HACCP team

An effective HACCP plan is best carried out as a multidisciplinary team exercise to ensure that the appropriate product-specific expertise is available. The team should include members familiar with all aspects of the production process as well as specialists with expertise in particular areas such as microbiology or engineering. If expert advice is not available on-site, it may be obtained from external sources.

The scope of the plan should be determined by defining the extent of the production process to be considered and the categories of hazard to be addressed (e.g. biological, chemical and/or physical).

2. Describe the product

It is important to have a complete understanding of the product, which should be described in detail. The description should include information such as composition, physical and chemical structure (including a_w, pH, etc.), processing conditions (e.g. heat treatment, freezing, smoking etc.), packaging, shelf-life, storage and distribution conditions, and instructions for use.

3. Identify intended use

The intended use should be based on the expected uses of the product by the end-user or consumer (e.g. is a cooking process required?). It is also important to identify the consumer target groups. Vulnerable groups of the population, such as children or the elderly, may need to be considered specifically.

4. Construct flow diagram

The flow diagram should be constructed by the HACCP team and should contain sufficient technical data for the study to progress. It should provide an accurate representation of all steps in the production process from raw materials to the end product. It may include details of the factory and equipment layout, ingredient specifications, features of equipment design, time/temperature data, cleaning and hygiene procedures, and storage conditions.

5. On-site confirmation of the flow diagram

The HACCP team should confirm that the flow diagram matches the process that is actually carried out. The operation should be observed at all stages and any discrepancies between the flow diagram and normal practice must be recorded and the diagram amended accordingly. It is also important to include observation of production outside normal working hours, such as night shifts. It is essential that the diagram is accurate, because the hazard analysis and decisions regarding CCPs are based on these data.

6. List all the potential hazards, conduct a hazard analysis and consider control measures (Principle 1)

The HACCP team should list all hazards that may reasonably be expected to occur at each step in the production process.

The team should then conduct a hazard analysis to identify which hazards are of such a nature that their elimination or reduction to an acceptable level is essential to the production of safe food.

The analysis is likely to include consideration of:

- The likely occurrence of hazards and the severity of their adverse health effects;
- The qualitative and/or quantitative evaluation of the presence of hazards;
- Survival or multiplication of pathogenic micro-organisms;
- Production or persistence of toxins.

The HACCP team should then determine what control measures exist that can be applied for each hazard.

Some hazards may require more than one control measure for adequate control and a single control measure may act to control more than one hazard.

Note: it is important at this stage that no attempt is made to identify CCPs, since this may interfere with the analysis.

7. Determine critical control points (CCPs) (Principle 2)

The determination of CCPs in the HACCP system is facilitated by using a decision tree (Fig. 2) to provide a logical, structured approach to decision making. However, application of the decision tree should be flexible and its use may not always be appropriate. It is also essential that the HACCP team has access to sufficient technical data to determine the CCPs effectively.

If a realistic hazard has been identified at a step where control is required for safety, but for which no control exists at that step or any other, then the process must be modified to include a control measure.

8. Establish critical limits for each CCP (Principle 3)

Where possible, critical limits should be specified and validated for each CCP. More than one critical limit may be defined for a single step. For example, it is usually necessary to specify both time and temperature for a thermal process. Criteria used to set critical limits must be measurable and may include physical, chemical, biological or sensory parameters.

9. Establish a monitoring system for each CCP (Principle 4)

Monitoring involves planned measurement or observation of a CCP relative to its critical limits. Monitoring procedures must be able to detect loss of control of the CCP and should provide this information with sufficient speed to allow adjustments to be made to the control of the process before the critical limits are violated. Monitoring should either be continuous, or carried out sufficiently frequently to ensure control at the CCP. Therefore, physical and chemical on-line measurements are usually preferred to lengthy microbiological testing. However, certain rapid methods such as ATP assay by bioluminescence may be useful for assessment of adequate cleaning, which could be a critical limit for some CCPs.

Persons engaged in monitoring activities must have sufficient knowledge, training and authority to act effectively on the basis of the data collected. These data should also be properly recorded.

10. Establish the corrective action to be taken when monitoring indicates a loss of control at a CCP (Principle 5)

For each CCP in the HACCP plan, there must be specified corrective actions to be applied if the CCP is not under control. If monitoring indicates a

deviation from the critical limit for a CCP, action must be taken that will bring it back under control. Actions taken should include proper isolation and disposition of the affected product and all corrective actions should be properly recorded.

11. Establish procedures for verification to confirm that the HACCP system is working effectively (Principle 6)

Verification and auditing methods, procedures and tests should be used frequently to determine whether the HACCP system is effective. These may include random sampling and analysis, including microbiological testing. Although microbiological analysis is generally too slow for monitoring purposes, it can be of great value in verification, since many of the identified hazards are likely to be microbiological.

In addition, reviews of HACCP records are important for verification purposes. These should confirm that CCPs are under control and should indicate the nature of any deviations and the actions that were taken in each case. It may also be useful to review customer returns and complaints regularly.

12. Establish documentation concerning all procedures and records appropriate to these principles and their application (Principle 7)

Efficient and accurate record keeping is an essential element of a HACCP system. The procedures in the HACCP system should be documented.

Examples of documented procedures include:

- The hazard analysis
- Determination of CCPs
- Determination of critical limits

Examples of recorded data include:

- Results of monitoring procedures
- Deviations from critical limits and corrective actions

The degree of documentation required will depend partly on the size and complexity of the operation, but it is unlikely to be possible to demonstrate that an effective HACCP system is present without adequate documentation and records.

Implementation and Review of the HACCP Plan

The completed plan can only be implemented successfully with the full support and co-operation of management and workforce. Adequate training is essential and the responsibilities and tasks of the operating personnel at each CCP must be clearly defined.

Finally, it is essential that the HACCP plan be reviewed following any changes to the process, including changes to raw materials, processing conditions or equipment, packaging, cleaning procedures and any other factor that may have an effect on product safety. Even a small alteration to product or process may invalidate the HACCP plan and introduce potential hazards. Therefore the implications of any changes to the overall HACCP system must be fully considered and documented and adjustments made to the procedures as necessary.

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EC FOOD HYGIENE LEGISLATION

General Principles

Introduction

Hygiene is an important aspect of food safety, and therefore regulation of food hygiene plays an important part in most countries' food legislation. Hygiene is applied to all parts of the production process, from sourcing of raw materials and structural requirements in the factory or processing facility, personnel hygiene, processing and production of food products, final product specifications (that may include microbiological criteria), transport and delivery vehicle requirements, through to storage conditions at the point of final sale. Over the years microbiological standards have been used as one means of trying to ensure the microbiological quality and safety of raw materials and food products, especially where climatic conditions are warmer, and a number of such standards have been prescribed in food legislation in many countries. However, the application of such standards cannot in themselves guarantee a safe and quality product and use of microbiological standards in end-product testing alone is no substitute for good hygienic practices during production. Preventative measures within the implementation of good hygienic practices is considered more effective. With this in mind, emphasis in recent years has been on the concepts of Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point (HACCP) systems, within which microbiological criteria can play a part.

The key aim of European food legislation is to ensure that safe and quality food products can be freely traded within the European Union while maintaining the confidence of consumers in their purchases. Regulation (EC) 178/2002 of the European Parliament and of the Council of 28 January 2002, as amended, laying down the General Principles and Requirements of Food Law, establishing the European Food Safety Authority and laying

down protection in matters of food safety (*Official Journal of the European Communities*, **45** (L31) 1-24) lays down food safety requirements, establishes measures needed to ensure unsafe food is not put on the market, and ensures that systems are in place to identify and respond to food safety problems. Food business operators have an obligation to withdraw unsafe food from the market. Ensuring food safety means all aspects of the production chain need to be taken into account; general requirements for safe food to be placed on the market are also necessary to ensure the effective functioning of the internal market within the EU. It is necessary to be able to trace a food or food ingredient so that a comprehensive system of traceability has been set up. The food business operator is best placed to ensure the supply of his product(s), so has the primary responsibility for ensuring food safety. This Regulation provides the basis for ensuring a high level of consumer protection, human health and consumer interest, while also taking into account the wide diversity of food supply in Europe.

As public health and safety is a key issue for the EC, there have been a number of EC Directives concerning food hygiene over the years in important areas such as the dairy and meat sectors. This piecemeal approach led to inconsistencies across sectors and following a complete review of the hygiene sector, completely revised food hygiene legislation is now in place at a European level.

Full copies of the EC regulations and directives referred to can be accessed via www.eurlex.com and www.ec.europa.eu.

Framework of current EU food hygiene legislation

The revised food hygiene legislation at European level covers the following key points:

- Modernisation, consolidation and simplification of previous EU food hygiene legislation;
- Application of effective and proportionate controls throughout the food chain, from primary production to sale or supply to the final consumer (the so-called 'farm to fork' approach);
- Focus on the controls needed to ensure public health;
- Clarification that it is the primary responsibility of food business operators to ensure food is safely produced.

There are three key regulations in the so-called hygiene package. These are Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004, as amended, on the hygiene of foodstuffs (*Official Journal of the European Communities* 2004, **47** (L139) 1-54), Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004, as amended, laying down specific hygiene rules for foods of animal origin (*Official Journal of the European Communities* 2004, **47** (L139) 55-205) and Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004, as amended, laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption (*Official Journal of the European Communities* 2004, **47** (L139) 206-320). The first of these concerns all food business operators; due to the nature of products of animal origin, specific additional requirements are detailed in the second of these for food businesses handling products of animal origin.

Primary producers such as farmers and growers are now included in the scope of the food hygiene legislation, in many cases for the first time. Depending on the type of business, food businesses need to be registered with the appropriate competent authority, and food business operators (other than primary producers such as farmers and growers) need to have in place and maintain procedures based on Hazard Analysis Critical Control Point (HACCP) principles. The legislation has been structured so this can be applied flexibly depending on the size and nature of the food business in question.

Other implementing and transitional measures have been published, including Commission Regulation (EC) No. 2073/2005 of 15 November 2005, as amended, on microbiological criteria for foodstuffs (*Official Journal of the European Communities* 2005, **48** (L338) 1-26). Although microbiological criteria have been present in different regulatory measures in legislation previously in force, this is the first time a measure has been agreed bringing all of these together with new criteria as deemed appropriate.

General hygiene rules

Regulation (EC) No. 852/2004 takes account of the necessity of establishing microbiological criteria and temperature control requirements based on risk assessment. Food business operators must ensure that at all stages of production, processing and distribution under their control, and that their

products satisfy requirements of the hygiene rules. As appropriate, food business operators must comply with microbiological criteria as specified, and follow procedures to meet targets specified in the Regulation, complying with any temperature control regulations, maintaining the cold chain and undertaking sampling and analysis.

Food businesses must be clean, maintained in good condition and be protected against contamination. Suitable temperature-controlled handling and storage conditions of sufficient capacity for maintaining foods at appropriate temperatures must be available and must be designed to allow temperatures to be monitored and recorded. Where necessary, transport must be capable of maintaining foods at appropriate temperatures and allow monitoring. The risk of contamination must be minimised. Cleaning and disinfection provisions are specified and requirements for personal hygiene are detailed; an adequate supply of potable water must be available. No raw material may be accepted if it is known to be, or reasonably expected to be, contaminated with parasites or pathogens to such an extent that normal sorting/processing procedures would render the final product unfit for human consumption.

Food products that could support the growth of pathogens or toxin formation must not be kept at a temperature that could be a risk to health. The cold chain must not be interrupted but limited periods outside specified temperature control are permitted to accommodate handling practicalities during preparation, transport, storage, display or service of food, provided there is no risk to health. Raw and processed materials must be separated, with sufficient refrigerated storage as required.

If foods are to be stored at chill temperatures, they must be cooled as quickly as possible from any heat processing or final preparation, to a temperature not giving any risk to health. Any thawing must minimise the risk of pathogen growth or toxin formation and temperatures used must not be a risk to health; after thawing, foods or ingredients must be handled in an appropriate manner.

Wrapping and packing of food products must not contaminate the food; in particular cleanliness and integrity of container construction is essential.

For products placed on the market in a hermetically sealed container, parameters such as temperature, pressure, sealing and microbiological criteria, including those set and monitored using automatic devices, must be checked. The process used should be an internationally recognised standard such as pasteurisation, sterilisation or UHT.

Food business operators must ensure food handlers are instructed and/or trained in food hygiene commensurate or appropriate to their food activity. Adequate training in HACCP principles must be given where necessary.

HACCP

Food business operators, other than at the level of primary production, and associated operations must, in accordance to Regulation No. 852/2004, put in place, implement and maintain a permanent procedure or procedures based on principles of the system of hazard analysis and critical control points (HACCP). Emphasis is placed on risk-related control, with responsibility placed on the proprietor of the food business to ensure that potential hazards are identified and systems are developed to control them. Under HACCP food business operators must:

- identify any hazards that must be prevented, eliminated or reduced to acceptable levels
- identify the critical control points at the step(s) at which control is essential to prevent or eliminate a hazard or reduce it to acceptable levels
- establish critical limits at critical control points that separate acceptability from unacceptability for the prevention, elimination or reduction of identified hazards
- establish and implement effective monitoring procedures at critical control points
- establish corrective actions when monitoring indicates that a critical control point is not under control
- establish procedures, that must be carried out regularly, to verify that the measures outlined above are working effectively
- establish documents and records commensurate with the nature and size of the food business to demonstrate the effective application of these measures.

When any modification is made in the product, process, or any step, food business operators must review the procedure and make the necessary changes to it. Food business operators must provide the competent authority

with evidence of their compliance and ensure that any appropriate documents are up to date. Such documents and records must be kept for a specified period.

According to Article 4 of Regulation (EC) No. 852/2004, food business operators are to comply with microbiological criteria. This should include testing against the values set for the criteria through the taking of samples, the conduction of analyses and undertaking any corrective actions needed, in accordance with food law and instructions given by the competent authority. Additionally, Regulation No. 2073/2005, as amended, lays down provisions concerning the analytical methods, including, where necessary, the measurement uncertainty, the sampling plan, the microbiological limits and the number of analytical units that should comply with these limits.

This Regulation specifies the foodstuff to which the criterion applies and the points of the food chain where the criterion applies, as well as the actions to be taken when the criterion is not met. Food business operators may need to consider controls on raw materials, processing criteria, temperature and shelf-life of the product in order to ensure compliance.

Hygiene rules for products of animal origin

As products of animal origin tend to represent the highest risk to public health in food production, additional controls for these products have been introduced by means of Regulation (EC) No. 853/2004, as amended, laying down specific hygiene rules for foods of animal origin. Under this Regulation, hygiene provisions are detailed for products of animal origin in general, including registration and approval of establishments, definitions of products covered by the scope of the Regulations, identification marking and the requirement to comply with specified microbiological criteria.

In Annex III of the Regulations, more specific requirements are detailed for the following categories of products:

- meat of domestic ungulates
- meat from poultry and lagomorphs
- meat of farmed game
- wild game meat
- minced meat, meat preparations and mechanically separated meat (MSM)
- meat products
- live bivalve molluscs

- fishery products
- raw milk, colostrum, dairy products and colostrum-based products
- eggs and egg products
- frogs' legs and snails
- rendered animal fats and greaves
- treated stomachs, bladders and intestines
- gelatin
- and collagen.

Within these requirements are temperature specifications for raw materials and products during processing, packaging and transport; the specified temperatures vary depending on the product. Food business operators must initiate procedures to ensure that raw milk meets the following criteria:

(i) Raw cows' milk must meet the following standards:

Plate count 30 °C (per ml) ≤ 100,000 (1)
Somatic cell count (per ml) ≤ 400,000 (2)

(1) Rolling geometric average over a two-month period, with at least 2 samples per month.
(2) Rolling geometric average over a three-month period, with at least 1 sample per month, unless the competent authority specifies another methodology to allow for seasonal variations in levels of production.

(ii) Raw milk from other species must meet the following standard:

Plate count 30 °C (per ml) ≤ 1,500,000 (1)

(1) Rolling geometric average over a two-month period, with at least 2 samples per month.

(iii) However, if raw milk from species other than cows is intended for manufacture of products made with raw milk by a process that does not involve any heat treatment, food business operators must take steps to ensure the raw milk meets the following criterion:

Plate count 30 °C (per ml) ≤ 500,000 (1)

(1) Rolling geometric average over a two-month period, with at least 2 samples per month.

EC Regulation on microbiological criteria

Regulation (EC) No. 2073/2005 establishes microbiological criteria for a range of food products. Microbiological criteria are considered to give guidance on the acceptability of foodstuffs and their manufacturing, handling and distribution processes. The use of microbiological criteria should form an integral part of the implementation of HACCP-based procedures and other hygiene control measures. The microbiological criteria can define the acceptability of the processes, and food safety microbiological criteria set a limit above which a foodstuff is considered unacceptably contaminated with the micro-organisms for which the criteria are set. Food business operators should decide the necessary sampling and testing frequencies as part of their procedures based on HACCP principles and other hygiene control procedures in order to ensure that their products comply with the applicable microbiological criteria established in this Regulation. However, in some cases harmonised sampling frequencies have been set at Community level, particularly in order to ensure the same level of controls be performed throughout the Community.

International guidelines for microbiological criteria in respect of many foodstuffs have not yet been established. However, the Commission has followed the Codex Alimentarius guideline 'Principles for the establishment and application of microbiological criteria for foods CAC/GL 21 — 1997' and other advice in laying down microbiological criteria. Existing Codex specifications in respect of dried milk products, foods for infants and children and the histamine criterion for certain fish and fishery products have been taken into account. The adoption of Community criteria is intended to benefit trade by providing harmonised microbiological requirements for foodstuffs and replacing national criteria, which were potential barriers to trade. The criteria set will be reviewed to take into account food safety and microbiology developments.

Food business operators will take the necessary measures as part of their procedures based on HACCP principles and good hygiene practice throughout the different stages of food production, processing and distribution, including retail, to ensure the following:

a) that the supply, handling and processing of raw materials and foodstuffs under their control are carried out in such a way that the process hygiene criteria are met

b) that the food safety criteria applicable throughout the shelf-life of the products can be met under reasonably foreseeable conditions of distribution, storage and use.

Additionally, when considered necessary, food business operators will be responsible for the conduction of studies in order to investigate the compliance with the established microbiological criteria during the shelf-life of the product. This applies in particular to ready-to-eat foodstuffs that are able to support the growth of *Listeria monocytogenes*. Further details on these studies are outlined in Annex II of the Regulation.

Article 7 of this Regulation outlines the procedure that business operators will follow when the testing against the corresponding food safety criteria provides unsatisfactory results. As a general rule, when the results of testing are unsatisfactory, the product or batch of a food will be withdrawn or recalled. Some exceptions to this general rule are also explained in this chapter.

Annex I to Regulation (EC) No. 2073/2005 is divided into three chapters, the first one detailing food safety criteria, the second one establishing the process hygiene criteria and lastly the third chapter refers to the rules for sampling and preparation of test samples.

Only an outline of the specified criteria is given here, for full details the official text should be consulted.

Food safety criteria

This chapter details the food categories for which microbiological criteria are specified, names the specific microbes and their toxins and metabolites as relevant, sampling plan (number of units comprising the sample and number of samples giving values between stated limits), limits, analytical reference method and the stage in the process chain where the stated criterion applies.

For example, the following applies for ready-to-eat foods;

Notes to Table I:

1. n = number of units comprising the sample; c = number of sample units giving values over m or between m and M
2. For foods included in this table m = M
3. The most recent edition of the standard shall be used
4. Regular testing against the criterion is not useful in normal circumstances for the following ready-to-eat foods:

- those that have been heat-treated or had other processing effective to eliminate *Listeria monocytogenes* when recontamination is not possible after this treatment (e.g. products heated in their final package)
- fresh, uncut and unprocessed fruit and vegetables, excluding sprouted seeds
- bread, biscuits and similar products
- bottled or packed waters, soft drinks, beer, cider, wine, spirit drinks and similar products
- sugar, honey and confectionery, including coca and chocolate products
- live bivalve molluscs
- food grade salt

5. This criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g during shelf-life. Intermediate limits may be fixed during the process that should be low enough to guarantee that the 100 cfu/g limit is not exceeded at the end of shelf-life.
6. 1 ml of inoculum is placed on a Petri dish of 140 mm diameter or on three Petri dishes of 90 mm diameter
7. This criterion applies to products before they have left the immediate control of the business operator producing them where he cannot show, to the satisfaction of the competent authority, that the product will not exceed the 100 cfu/g limit throughout shelf-life.
8. Products with pH $\leq$ 4.4 or $a_w \leq 0.92$, products with pH ≤ 5.0 and $a_w \leq 0.94$, products with shelf-life less than 5 days are automatically included in this category. Other products can be included following scientific justification.

Table I
Food Safety Criteria for Ready-to-eat Food

Food category	Microorganisms/toxins, metabolites	Sampling plan[1]		Limits[2]	Analytical reference method[3]	Stage where criterion applies
		n	c	m M		
Ready-to-eat food intended for infants and young children, ready-to-eat foods for special medical purposes[4]	*Listeria monocytogenes*	10	0	Absence in 25 g	EN/ISO 11290-1	Products placed on the market during their shelf-life
Ready-to-eat foods able to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g[5]	EN/ISO 11290-2[6]	Products placed on the market during their shelf-life
		5	0	Absence in 25 g[7]	EN/ISO 11290-1	Before the food has left the immediate control of the food business operator who has produced it
Ready-to-eat foods unable to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes[4,8]	*Listeria monocytogenes*	5	0	100 cfu/g	EN-ISO 11290-2[6]	Products placed on the market during their shelf life

The following products have additional food safety criteria specified by Annex 1:

Meat and meat products – minced meat and meat preparations intended to be eaten raw, minced meat and meat preparations from poultry meat intended to be eaten cooked, minced meat and meat preparations from other species than poultry intended to be eaten cooked, MSM, meat products intended to be eaten raw except products where the manufacturing process or composition will eliminate any *Salmonella* risk, meat products from poultry intended to be eaten cooked, gelatine and collagen (*Salmonella*).

Dairy products – cheeses, butter, cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation, milk powder and whey powder, ice cream except products where the manufacturing process or composition will eliminate any *Salmonella* risk (*Salmonella*), cheeses, milk and whey powder that may contain staphylococcal enterotoxins (staphylococcal enterotoxins).

Egg products – egg products except products where the manufacturing process or composition will eliminate any *Salmonella* risk, ready-to-eat foods containing raw egg, except products where the manufacturing process or composition will eliminate any *Salmonella* risk (*Salmonella*).

Fish products – cooked crustaceans and molluscan shellfish (*Salmonella*), live bivalve molluscs and live echinoderms, tunicates and gastropods (*Salmonella* and *E. coli*), fishery products from fish species containing high amounts of histidine, fishery products having undergone enzyme maturation treatment in brine, manufactured from fish species containing high amounts of histidine (histamine).

Fruit and vegetable products – sprouted seeds, pre-cut ready-to-eat vegetables, unpasteurised ready-to-eat fruit and vegetable juices (*Salmonella*).

Infant formulae and dietetic foods – dried infant formulae and dried dietary foods for special medical purposes intended for infants under 6 months of age (*Salmonella* and *Cronobacter* spp. (*Enterobacter sakazakii*)) and dried follow-on formulae (*Salmonella*).

Additional information is given in respect of interpreting the test results.

Process hygiene criteria

In Chapter 2 of Annex I process hygiene criteria are specified for various higher risk products, with an additional requirement for action where unsatisfactory results are obtained. An example from this chapter is as follows:

The following products have process hygiene criteria set:

Meat and meat products – carcasses of cattle, sheep, goats and horses (aerobic colony count, Enterobacteriaceae, *Salmonella*), carcasses of pigs (aerobic colony count, Enterobacteriaceae, *Salmonella*), poultry carcasses of turkeys and broilers (*Salmonella*), minced meat (aerobic colony count, *E. coli*), MSM (aerobic colony count, *E. coli*), meat preparations (*E. coli*).

Milk and dairy products – pasteurised milk and other pasteurised liquid dairy products (Enterobacteriaceae), cheeses from milk or whey that has undergone heat treatment (*E. coli*), cheeses from raw milk, cheeses from milk having undergone a lower heat treatment than pasteurisation, ripened cheeses made from milk or whey having undergone pasteurisation or a stronger heat treatment, unripened soft or fresh cheeses made from milk or whey having undergone pasteurisation or a stronger heat treatment, (coagulase-positive Staphylococci), butter and cream from raw milk or milk having undergone a lower heat treatment than pasteurisation (*E. coli*), milk powder and whey powder (Enterobacteriaceae and coagulase-positive Staphylococci), ice cream and frozen dairy desserts (Enterobacteriaceae).

Infant feed - dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age (Enterobacteriaceae and presumptive *Bacillus cereus*) and dried follow-on formulae (Enterobacteriaceae).

Egg products (Enterobacteriaceae)

Fish products – shelled and shucked products of cooked crustaceans and molluscan shellfish (*E. coli*, coagulase positive Staphylococci).

Fruit and vegetable products – pre-cut ready-to-eat fruit and vegetables and unpasteurised ready-to-eat fruit and vegetable juices (*E. coli*).

Table II
Example of Process Hygiene Criteria

Food category	Microorganisms	Sampling plan[1] n	Sampling plan c	Limits[2] m M	Analytical reference method[3]	Stage where the criteria apply	Action in case of unsatisfactory results
Carcasses of cattle, sheep, goats and horses	*Salmonella*	50[4]	2[5]	Absence in the area tested per carcass	EN/ISO 6579	Carcasses after dressing but before chilling	Improvements in slaughter hygiene, review of process controls and of origin of animals

Notes to table:

1 n = number of units comprising the sample; c = number of sample units giving values over m or between m and M
2 For foods above m = M
3 The most recent edition of the standard shall be used
4 The 50 samples are derived from 10 consecutive sampling sessions in accordance with the sampling rules and frequencies specified in the Regulation
5 The number of samples where the presence of *Salmonella* is detected. The c value is subject to review to take account of progress in reducing *Salmonella* prevalence. Member States or regions having low *Salmonella* prevalence may use lower c values even before the review.

Rules for sampling and preparation of test samples

According to Chapter 3 of Annex I in the absence of specific rules on sampling and test preparation, the appropriate International Organisation for Standardisation (ISO) and Codex Alimentarius guidelines will be used as reference methods.

Specific rules are laid down for the sampling that needs to be carried out in slaughterhouses and establishments producing meat preparations and mince meat.

Additional requirements with regards to the sampling frequency for carcasses, mince meat, meat preparations and MSM are also defined in this Chapter.

Guidance documents

The following guidance documents have been published related to microbiological criteria:

- Guidance Document on official controls concerning sampling and testing of foodstuffs
- Guidance Document on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods. This document is directed to producers of ready-to-eat products that support the growth of *Listeria monocytogenes* and conduct studies in accordance to Annex II of Regulation (EC) No. 2073/2005 of 15 November 2005
- Technical Guidance Document on shelf-life laboratory durability and challenge studies for *Listeria monocytogenes* in ready-to-eat foods. This document is intended for laboratories that collaborate with food business operators in conducting shelf-life studies for *Listeria monocytogenes* in ready-to-eat foods.

Water

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, as amended (*Official Journal of the European Communities* 1998, **41** (L330), 32-54) details measures to focus on compliance with essential health and quality parameters for drinking

water while allowing for the Member States to add other parameters as they see fit, if it is considered necessary to protect public health. The scope extends to water used in the food industry unless it can be shown that the use of such water does not affect the wholesomeness of the finished product. Parametric values have been based on available scientific knowledge. The precautionary principle has also been taken into account; the values have been selected to ensure that water intended for human consumption can be safely consumed on a life-long basis, so giving a high level of health protection.

Member States must ensure that water intended for human consumption is wholesome and clean. For the purposes of the minimum requirements of this Directive, water intended for human consumption is wholesome and clean if it

- is free from any micro-organisms and parasites and free from any substances that, in numbers or concentrations, constitute a potential danger to human health, and
- meets the minimum requirements set out in Parts A and B of Annex I of the Directive

and, in accordance with other articles and with the Treaty, Member States take all other measures necessary to ensure water intended for human consumption complies with the requirements of this Directive. Microbiological parameters according to this Directive include *E. coli*, Enterococci, *Pseudomonas aeruginosa* and colony counts; certain parameters of microbiological concern are also included.

The quality of natural mineral waters and spring waters is covered by Directive 2009/54/EC of the European Parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters (*Official Journal of the European Communities* 2009, **45** (L164). In addition, spring waters must comply with the provisions established by Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.

The Directive includes details of microbiological criteria for natural mineral waters at source and during marketing.

At source and during marketing, natural mineral water will be free from:

a) parasites and pathogenic micro-organisms

b) *Escherichia coli* and other coliforms and faecal streptococci in any 250 ml sample
c) sporulated sulphite-reducing anaerobes in any 50 ml sample
d) *Pseudomonas aeruginosa* in any 250 ml sample

Additional requirements with regards to the revivable total colony count at source, total colony count after bottling and at source are also laid down in this Directive.

Mycotoxin Levels in Food

Commission Regulation (EC) No. 1881/2006 of 19 December 2006, as amended, (*Official Journal of the European Communities*, **49** (L364) 5 -24) sets maximum levels for certain contaminants in foodstuffs. The Regulations' aim is to protect public health and keep contaminants at levels that are toxicologically acceptable and it contains provisions on maximum limits for specified mycotoxins.

Maximum limits apply to the edible part of the foodstuff unless otherwise specified. For products, that are dried, diluted, processed or composed of more than one ingredient, the maximum levels applicable must correspond to those laid down by the Regulation and the following must be taken into account:

(a) changes of the concentration of the contaminant caused by drying or dilution process
(b) changes of the concentration of the contaminant caused by processing
(c) the relative proportions of the ingredients in the product and
(d) the analytical limit of quantification.

The Regulation set maximum limits for the following mycotoxins in specific food/food ingredients as indicated below:

Ochratoxin A

- dried vine fruit
- unprocessed cereals, processed cereal products and cereals intended for direct human consumption
- coffee
- wine, fruit wine, aromatised wine, aromatised wine-based drinks and aromatised wine-product cocktails

- processed cereal-based foods and baby foods for infants and young children and dietary foods for medical purposes specially for infants
- grape juice, grape nectar and grape must
- spices
- liquorice

Aflatoxins
- cereals and products derived from cereals
- maize and rice
- nuts (different types) and processed products thereof
- dried fruit and processed products thereof
- milk (M1)
- processed cereal-based foods and baby foods for infants and young children, infant formula and follow-on formula including infant milk and follow-on milk and dietary foods for medical purposes specifically for infants
- spices

Patulin
- fruit juices and fruit nectars
- spirit drinks, cider, and other fermented drinks derived from or containing apples
- Solid apple products
- Solid apple products and apple juice for infants and young children
- Baby foods other than processed cereal-based foods for infants and young children

Deoxynivalenol
- unprocessed cereals and cereals for direct human consumption, cereal flour, bran and germ
- pasta (dry)
- bread, pastries, biscuits, cereal snacks and breakfast cereals
- processed cereal-based foods and baby foods for infants and young children
- milling fractions of maize

Zearalenone
- unprocessed cereals and cereals for direct human consumption, cereal flour, bran and germ

- refined maize oil
- bread, pastries, biscuits, cereal snacks and breakfast cereals excluding maize-based snacks and maize-based breakfast cereals
- maize for direct human consumption, maize-based snacks and maize-based breakfast cereals
- processed cereal-based foods and baby foods for infants and young children
- milling fractions of maize

Fumonisins
- unprocessed maize
- maize for direct human consumption, maize-based foods for direct human consumption, maize-based snacks and maize-based breakfast cereals
- processed maize-based foods and baby foods for infants and young children
- milling fractions of maize

T-2 and HT-2 toxin
- Unprocessed cereal and cereal products

Up to date scientific opinions regarding contaminant safety levels can be accessed through the European Food Safety Authority Website www.efsa.europa.eu.

Application in UK law

Copies of the UK Statutory Instruments can be accessed via www.opsi.gov.uk/stat.htm, the Office of Public Information website.

Hygiene

The revised hygiene legislation, including requirements for microbiological criteria, are published at European level in the form of regulations, i.e. they are binding in the Member States from the date that they come into force and there is no scope for interpretation. However, before regulations can be applied in the UK, a statutory instrument is required to enable enforcement, including detailing offences, penalties and defences. In the UK the Food Hygiene (England) Regulations, S.I. 2006 No. 14, as amended by S.I. 2007

No. 56 and S.I. 2010 No. 534, and equivalent regulations in Scotland, Wales and Northern Ireland, form the appropriate legislation. In most cases the national law does not reproduce all the technical requirements of the European legislation, but makes reference to it, citing appropriate EC regulations. There are certain areas, for example temperature control requirements, where Member States can make their own provisions and such requirements are included in the UK regulations. The requirement for food to be of the nature, substance or quality demanded by the purchaser and comply with food safety requirements is contained within the Food Safety Act 1990.

The Food Hygiene (England) Regulations, S.I. 2006 No. 14, as amended, implement the following into the national legislation, Regulation (EC) No. 852/2004, Regulation (EC) No. 853/2004, Regulation (EC) No. 854/2004 and Regulation (EC) No. 2073/2005.

Water

The Water Supply (Water Quality) (England and Wales) Regulations 2000, S.I. 2000 No. 3184, as amended by S.I. 2001 No. 2885 and S.I. 2010 No. 991 and the Natural Mineral Waters, Spring Water and Bottled Drinking Water (England) Regulations 2007, S.I. 2007 No. 2785, as amended by S.I. 2009 No. 1598 and S.I. 2010 No. 433 are applicable in the UK.

Mycotoxins

The Contaminants in Food (England) Regulations 2009, S.I. 2009 No. 1223 implement into the national legislation Commission Regulation (EC) No. 1881/2006.

SUPPLIERS

LABORATORY MEDIA SUPPLIERS

BioMérieux (UK) Ltd, Grafton House, Grafton Way, Basingstoke, Hants, RG22 6HY. Tel: +44 (0) 1256 461881. Fax: +44 (0) 1256 816863. http://www.biomerieux.co.uk/servlet/srt/bio/englishuk/home.

Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ 07417, USA. www.bd.com.
UK Sales, The Danby Building, Edmund Halley Road, Oxford Science Park, Oxford, OX4 4DQ. Tel: +44 (0) 1865 748844. Fax: +44 (0) 1865 717313.

Cherwell Laboratories Ltd, 7 & 8 Launton Business Centre, Murdock Road, Bicester, OX26 4XB. Tel: +44 (0) 1869 355500. Fax: +44 (0) 1869 355545. www.cherwell-labs.co.uk.

E & O Laboratories Ltd, Burnhouse, Bonnybridge, FK4 2HH, Scotland. Tel: +44 (0) 1324 840404. Fax: +44 (0) 1324 841314. www.eolabs.com.

LabM, Topley House, 52 Wash Lane, Bury, BL9 6AU. Tel: +44 (0) 161 797 5729. Fax: +44 (0) 161 762 9322. www.labm.com.

Life Technologies Ltd, 3 Fountains Drive, Inchinnan Drive, Paisley, PA4 9RF, Scotland. Tel: +44 (0) 141 814 6100. Fax: +44 (0) 141 814 6317. www.lifetechnologies.com.

Mast Diagnostics Ltd, Mast House, Derby Road, Bootle, Merseyside, L20 1EA. Tel: +44 (0) 151 933 7277. Fax: +44 (0) 151 944 1332. E-mail: sales@mastgrp.com. www.mastgrp.com.

Medical Wire & Equipment Co. (Bath) Ltd, Leafield Ind. Est., Potley, Corsham, Wilts, SN13 9RT. Tel: +44 (0) 1225 810 361. Fax: +44 (0) 1225 810 153. www.mwe.co.uk.

Merck Chemicals Ltd, Boulevard Industrial Park, Padge Road, Beeston, Nottingham, NG9 2JR. Tel: +44 (0) 800 622935. Fax: +44 (0) 115 9430951. www.chemdat.info.

M Tech Diagnostics, Unit-4, Station Road, Latchford, Warrington, Cheshire, WA4 1LB. Tel: +44 (0)1925 416622. Fax: +44 (0)1925 416677 http://www2.m-techmicro.com. E-mail queries@m-techdiagnostics.ltd.uk.

3M Health Care Ltd, 3M House, Morley Street, Loughborough, Leicestershire, LE11 1EP. Tel: +44 (0) 08705 360036. www.solutions.3m.co.uk.

Oxoid Ltd, Wade Rd, Basingstoke, Hampshire, RG24 8PW. Tel: +44 (0) 1256 841144. Fax: +44 (0) 1256 814626. www.oxoid.com.

Sigma-Aldrich Co. Ltd, The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT. Tel: +44 (0) 0800 717181. Fax: +44 (0) 0800 378785. www.sigmaaldrich.com.

Technical Service Consultants Ltd, The Ropewalk, Schofield St, Heywood, Lancs, OL10 1DS. Tel: +44 (0) 1706 620600. Fax: +44 (0) 1706 620445. www.tscswabs.co.uk.

VWR International Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire, LE17 4XN. Tel: +44 (0) 1455 558600. Fax: +44 (0) 1455 558586. http://uk.vwr.com/app/Home.

CULTURE COLLECTIONS

ATCC
The American Type Culture Collection (ATCC) is the World's largest and most diverse culture collection. The catalogues and further information can be obtained from:

American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA. Tel: +1 800-638-6597. Fax: +1 703-365-2750.
E-mail for UK technical enquiries: atcc-tech@lgcpromochem.com.

UKNCC
The United Kingdom National Culture Collection (UKNCC) co ordinates 11 of the UK's collection of microbial organisms. A single search can access information from all included collections. Collections included on the UKNCC are CABI (IMI), NCTC and NCYC.

Website: www.ukncc.co.uk provides links to the various collections.

CABRI
Common Access to Biological Resources and Information (CABRI) is a consortium of European collections and information centres and comprises 28 catalogues with over 100,000 items. Collections accessed from the website can be simultaneously searched.

Website: www.cabri.org.

WDCM
There are around 460 culture collections from 62 countries registered with the World Date Centre on Micro-organisms (WDCM). The WDCM is part of the World Federation for Culture Collections (WFCC). Links to the collections and to further information can be found on http://wfcc.info

KIT/INSTRUMENT SUPPLIERS

Name and Address	Supplies include:
bioMérieux (see mediasuppliers) Tel: +44 (0) 1256 461881 Fax: +44 (0) 1256 816863 www.biomerieux.co.uk	VITEK, VIDAS and API Kits; BACTOMETER Mini API system; SLIDEX (*Staph*. or *Strep*.) kits; GEN PROBE: DNA Probe kits
BioControl Systems Inc. (USA) Tel: +1 425 603 1123 Email: info@biocontrolsys.com www.biocontrolsystems.com	*Salmonella* 1 2 Test, and Assurance EIA for *Salmonella*, *Listeria*, and *E. coli* O157:H7. VIP tests for *E. coli* O157:H7 and *Listeria*. COLITRAK for coliforms and *E. coli* COLICOMPLETE for confirmed detection of coliforms and *E. coli*.
Celsis Ltd Cambridge Science Park Milton Road Cambridge CB4 4FX Tel: +44 (0) 1223 426008 Fax: +44 (0) 1223 426003	A range of systems for the rapid detection of microbial contaminants, for many industries including pharmaceutical, cosmetics, food and drink, dairy and water. Instruments include: M 1800, M 2800, M 4000, CelsisAdvance, systemSURE. Kits include: Milk microbial kit, Meat microbial kit, Fruit Juice test kit, Hygiene monitoring test kit.

Name and Address	Supplies include:
Chemunex The Opas Centre St John's Innovation Park Cowley Road Cambridge CB4 OWS Tel: +44 (0) 1223 420815 Fax: +44 (0) 1223 420844	CHEMSCAN (analyser single cell detection) D COUNT (flow cytometry, automated analysers); FLUORASSURE Reagent kits (viable bacteria and yeast detection).
Cortecs Diagnostics Ltd Newtech Square Deeside Industrial Park Deeside Clwyd CH5 2NT Tel: +44 (0) 1244 288888 Fax: +44 (0) 1244 280221	HYGICULT range of hygiene monitoring (agar contact plate) kits
Becton Dickinson (see media suppliers) Tel: +44 (0) 1865 748844 Fax: +44 (0) 1865 781557	STAPH Latex test (for *Staph. aureus* coagulase); HYCHEK hygiene control dip slide.
Don Whitley Scientific Ltd 14 Otley Road Shipley W. Yorks BD17 7SE Tel: +44 (0) 1274 595728 Fax: +44 (0) 1274 531197 E-mail: info@dwscientific.co.uk www.dwscientific.co.uk	WASP Spiral plater. Agents for PROTOCOT image analyser; RABIT impedance system. Anaerobic Work Stations. (MACS) AES Media preparators. Gravimetric diluters. RAINBOW agar for VTEC. BIOLOG identification products

Name and Address	Supplies include:
Dynal (UK) Ltd 10 Thursby Road Croft Business Park Bromborough Wirral Merseyside L62 5AZ Tel: +44 (0) 151 346 1234 Fax: +44 (0) 151 346 1223	Reagents for selective enrichment of *Salmonella*, *E. coli* O157 and *Listeria* based on DYNABEAD technology Anti *Salmonella*, Anti *cryptosporidium* Dynabeads.
Dynex Technologies Columbia House Columbia Drive Worthing, West Sussex BN13 3HD Tel: +44 (0) 1903 267555 Fax: +44 (0) 1903 267722	Reagents and instrumentation for ELISAs. MICROLITE luminometers. Fluorescence reader.
Foss (UK) Ltd 730 Birchwood Boulevard Birchwood Warrington Cheshire, WA3 7QY Tel: +44 (0) 1925 287700 Fax: +44 (0) 1925 287777	BACTOSCAN automatic rapid microbiology system; EIAFOSS automated ELISA system for rapid pathogen screening.
Hughes Whitlock Ltd Wonastow Road Monmouth Gwent NP5 EAH Tel: +44 (0) 1600 715632 Fax: +44 (0) 1600 715674	BIOPROBE luminometer system for detection of microbial contamination (ATP) on surfaces and in selected products.

Name and Address	Supplies include:
Idexx Laboratories Ltd Milton Court Churchfield Road Chalfont St Peter Bucks SL9 9EW Tel: +44 (0) 1753 891660 Fax: +44 (0) 1753 891520 Technical Services: +44 (0) 800 581 786	Hygiene monitoring (ATP system) LIGHTENING; DWI approved rapid test for coliform and *E. coli* in water COLILERT; defined sub strate technology rapid method for basic micro biology tests SIMPLATE
LabM Topley House 52 Wash Lane Bury Lancashire, BL9 6AU Tel: +44 (0) 161 797 5729 Fax: +44 (0) 161 762 9322	MALTHUS System V for *Salmonella*, *Listeria*, *Campylobacter* and *E. coli*; Latex *Salmonella* confirmation kit.
Life Sciences International (UK) Ltd Unit 5, The Ringway Centre Edison Road Basingstoke Hants RG21 2YH Tel: +44 (0) 1256 817282 Fax: +44 (0) 1256 817292 E-mail: 100436.3576@compuserve.com	Multiplate based MULTISKAN for ELISA type tests; BIOSCREEN automated growth counter; LUMINOMETERS
3M Health Care Ltd 3M House Morley Street Loughborough Leicestershire LE11 1EP Tel: +44 (0) 08705 360036	PETRIFILM range of products (for TVC, coliforms, *E. coli*, including *E. coli* O157, and yeasts and moulds).

N**ame and Address**	**Supplies include:**
Mast Diagnostics (see Media Suppliers) Tel: +44 (0) 151 933 7277 Fax: +44 (0) 151 944 1332	Mast ID & MASTASCAN ELITE; MASTAZYME *Salmonella*; MAST ASSURE bacterial agglutination antisera (*Salmonella*, *Shigella*, *E. coli*, *Vibrio*). *Campylobacter* identification and biotyping systems; CRYOBANK bacterial preservation system. Full range of dehydrated culture media.
Merck Chemicals Ltd, (see Media Suppliers)	Hygiene monitoring equipment (ATP system)
Microgen Diagnostics Ltd 1 Admiralty Way Camberley Surrey GU15 3DT Tel: +44 (0) 1276 600081 Fax: +44 (0) 1276 600151	MICROBACT Identification System MICROSCREEN Rapid latex agglutination (*Salmonella*, *Listeria* *Staph. aureus*, *E. coli* O157, *Campylobacter*). rapid card tests for *Salmonella*, *E. coli* O157 and *Listeria*; biocontrol range of enzyme immunoassay and rapid card tests for *Salmonella*, *E. coli* O157 and *Listeria*.

Name and Address	Supplies include:
Murex Biotech Central Road Temple Hill Dartford Kent DA1 5AH Tel: +44 (0) 322 277711 Fax: +44 (0) 322 273288	WELLCOLEX *Salmonella*, WELLCOLEX *Shigella* and WELLCOLEX *E. coli* for the identification of *Salmonella*, *Shigella* and *E. coli* O157. STAPHAUREX and STAPHAUREX PLUS for the identification of *Staph. aureus*. Agglutinating antisera for *Salmonella*, *Shigella*, *Vibrio* and *E. coli*.
Organon Teknika Ltd Science Park Milton Road Cambridge CB4 0FL Tel: +44 (0) 1223 423650 Fax: +44 (0) 1223 420264	LISTERIA TEK kit; SALMONELLA TEK kit; MICRO ID , incl. MICRO ID LISTERIA; EHEC TEK.
Oxoid Ltd (see Media suppliers) Tel: +44 (0) 1256 841144 Fax: +44 (0) 1256 463388	Latex agglutination kits for bacterial toxins (RPLA). OXOID SALMONELLA RAPID TEST (OSRT). LISTERIA RAPID TEST (OLRT) *Campylobacter* dry spot test. STAPH Latex Tests. *Esch. coli* O157 latex test
Palintest Ltd Palintest House Kingsway, Team Valley Trading Estate Gateshead Tyne and Wear NE11 0NS Tel: +44 (0) 191 491 0808 Fax: +44 (0) 191 482 5372 E-mail: palintest@palintest.com	COLILERT for coliforms and *E. coli*.

Name and Address	Supplies include:
Prolab Diagnostics Unit 7, Westwood Court Clayhill Industrial Estate Neston South Wirral Cheshire L62 3UJ Tel: +44 (0) 151 353 1613 Fax: +44 (0) 151 353 1614	PROLEX O157 Latex test (for *E. coli* O157), PROLEX Rapid Acid Extraction Streptococcal Grouping kit (for Group D streptococci/enterococci), PROLEX Staph. latex agglutination kit (*Staph. aureus*) *Salmonella* and *Clostridium perfringens* antisera and reagents for *Legionella*.
Rhône diagnostics Technologies Ltd West of Scotland Science Park Unit 3.06 Kelvin Campus Glasgow G20 0SP Scotland Tel: +44 (0) 141 945 2924 Fax: +44 (0) 141 945 2925 E-mail: rdt@rhone diagnostics.co.uk	*Salmonella* - diagnostic kits: screen - LOCATE, confirmation - SPECTATE; *Listeria* - screen Hygiene monitoring (ATP system)
Tecra Diagnostics UK Batley Business and Technology Centre Technology Drive Batley West Yorkshire WF17 6ER Tel: +44 (0) 1924 441255 Fax: +44 (0) 1924 441611	Immunoassay kits for *Listeria*, *E. coli* O157, *Salmonella*, BDE toxin and *Staph. aureus* Staphylococcal enterotoxin and *Bacillus* toxin. Also, UNIQUE *Salmonella* and *Listeria* test.
Tepnel Life Sciences Gen-Probe Life Sciences Ltd Heron House Oaks Business Park, Crewe Road, Wythenshawe, Manchester M23 9HZ Tel: +44 (0) 161 946 2200 Fax: +44 (0) 161 946 2211	DARAS instrument for automatic nucleic acid based tests for food pathogens.

ADDRESSES OF AUTHORITIES/SOURCES OF FURTHER INFORMATION, ETC.

Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA)
37-41 Bedford Row
London
WC1R 4JH
Tel: +44 (0) 20 740 4911
www.bccca.org.uk

British Hospitality Association (BHA)
Queens House
55-56 Lincoln's Inn Fields
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Tel: +44 (0) 20 7404 7744
Fax: +44 (0) 20 7404 7799
bha@bha.org.uk
www.bha.org.uk

British Retail Consortium
2nd Floor
21 Dartmouth Street
London
SW1H 9BP
Tel: +44 (0) 20 7854 8900
Fax: +44 (0) 20 7854 8901
www.brc.org.uk

British Standards Institution (BSI)
389 Chiswick High Road
London
W4 4AL
Tel: +44 (0) 20 8996 9001
Fax: +44 (0) 20 8996 7001
cservices@bsigroup.com
www.bsi-global.com

CABi Bioscience (formerly International Mycological Institute)
Nosworthy Way
Wallingford
Oxfordshire
OX10 8DE
Tel: +44 (0) 1491 832111
Fax: +44 (0) 1491 829292
enquiries@cabi.org
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Station Road
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GL55 6LD
Tel: +44 (0) 1386 842000
Fax: +44 (0) 1386 842100
info@campden.co.uk
www.campden.co.uk

Central Public Health Laboratory (CPHL) and Communicable Disease Surveillance Centre (CDSC).
Health Protection Agency
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NW9 5EQ (CDSC)
Tel: +44 (0) 20 8200 4400
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www.hpa.org.uk

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www.codexalimentarius.net

Consumers Association (CA)
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www.which.co.uk

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www.dh.gov.uk

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cfa@chilledfood.org
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Food and Drink Federation (FDF)
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Fax: +44 (0) 20 7836 0580
generalenquiries@fdf.org.uk
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FSA Helpline : +44 (0) 20 7276 8000
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The Stationery Office Publications Centre
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www.ncc.org.uk

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PHLS Central Public Health Laboratory
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www.riph.org.uk

The Royal Society for the Promotion of Health
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Tel: +44 (0) 20 7630 0121
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www.scotconsumer.org.uk

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Aberdeen
AB11 6NJ
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MK4 7PH
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www.sfam.org.uk

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Foodborne Infections
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INTERNET

Introduction

A great deal of information of use to food microbiologists and those with an interest in food safety is now accessible through the Internet. The following section is intended to be a list of some of the most useful sites available at the time of writing. There has been an explosion in the number of Web sites containing food safety and microbiology information, and it would be impossible to list all the relevant sites. Those listed below represent the range of resources to be found, and many of them contain links to other sites.

The pace of change on the Internet is rapid, and site addresses and contents are often modified. Therefore it is worth visiting sites of interest on a regular basis and noting any significant changes. It is also important to realise that there are no effective controls over the information posted on Internet sites, and it is necessary to ensure that any information that you may want to use professionally comes from a reputable source. There are existing sites professing to give food safety information that are at best misleading.

IFST (INSTITUTE OF FOOD SCIENCE & TECHNOLOGY)
http://www.ifst.org

A source of detailed, topical information and with comprehensive links to other sites. Now has a search facility.

FDA CENTRE FOR FOOD SAFETY & APPLIED NUTRITION
http://vm.cfsan.fda.gov/

A very useful site that covers a wide range of information, including the 'Bad Bug Book', a series of summaries of the properties of food poisoning organisms.

Government/international organisations

FSA (Food Standards Agency)
http://www.food.gov.uk/

DEFRA (Department for Environment, Food and Rural Affairs)
http://www.defra.gov.uk

DOH (Department of Health - UK)
http://www.doh.gov.uk

HPA (Health Protection Agency)
http://www.hpa.org.uk

Trading Standards
http://www.tradingstandards.gov.uk/index.cfm

USDA (United States Department of Agriculture)
http://www.usda.gov/

FDA (Food & Drug Administration - US)
http://www.fda.gov

CDC (Centers for Disease Control and Prevention)
http://www.cdc.gov/

WHO (World Health Organisation)
http://www.who.int/en

FAO (United Nations Food & Agriculture Organisation)
http://www.fao.org/

EFSA (European Food Safety Authority)
http://www.efsa.europa.eu/en.html

EUROPA
http://europa.eu/pol/food/index_en.htm

Eurosurveillance
http://www.eurosurveillance.org/index-02.asp

Food Standards Australia New Zealand
http://www.foodstandards.gov.au/

FSIS (Food Safety and Inspection Service)
http://www.fsis.usda.gov/News_&_Events/index.asp

Canadian Food Inspection Agency
http://www.inspection.gc.ca/english/toce.shtml

Institutes, associations and societies

APHA (American Public Health Association)
http://www.apha.org/

AOAC (Association of Official Analytical Chemists)
http://www.aoac.org/

American Food Safety Institute
http://www.americanfoodsafety.com

IFR (Institute of Food Research - UK)
http://www.ifrn.bbsrc.ac.uk/

IFT (Institute of Food Technologists - US)
http://www.ift.org/

IFIC (International Food Information Council Foundation)
http://www.ific.org/index.cfm

National Institutes of Health (US)
http://www.nih.gov/

Society for General Microbiology (UK)
http://www.socgenmicrobiol.org.uk/

Food safety and microbiology

Food Safety Consortium
http://www.uark.edu/depts/fsc

International HACCP Alliance
http://haccpalliance.org

FoodHACCP.com - Food Safety Information Web Site
http://www.foodhaccp.com/indexcopy.html

Outbreak
http://www.outbreak.org

Seafood Network Information Centre
http://seafood.ucdavis.edu/

The Microbiology Network
http://microbiol.org/

USDA Food Safety & Inspection Service
http://www.usda.gov/agency/fsis/homepage.htm

Food and Drink Federation
http://www.fdf.org.uk/

Methods

AOAC
http://www.AOAC.org

FoodHaccp.com – New Control Methods for Food Safety
http://foodhaccp.com/

Health Canada - Compendium of Analytical Methods
http://www.hc-sc.gc.ca

RapidMicrobiology.com
http://www.rapidmicrobiology.com/index.php

Health Canada – Official Methods for the Microbiological Analysis of Food
http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume1/index_e.html

ISO (International Organisation for Standardization)
http://www.iso.org/iso/en/CatalogueListPage.CatalogueList?ICS1=67&ICS2=&ICS3=&scopelist

GLOSSARY OF TERMS

Acidophiles are organisms that have optimum growth pH <6. They usually grow below pH4.0

Aciduric organisms can survive adversely acidic conditions, usually in the form of a spore.

Adventitious infection occurs with organisms from the environment.

Aerobes are micro-organisms that require air / oxygen, usually at or near atmospheric levels of oxygen (21%), in order to produce the necessary energy for growth.

Aerotolerant refers to anaerobic micro-organisms which can survive but not grow in the presence of oxygen or grow sub-optimally when oxygen is present.

Acid substances have a pH of less than 7.

Alkaline substances have a pH of greater than 7.

Anaerobes are micro-organisms that will only grow in an environment in which oxygen is absent and generate cellular energy from fermentation pathways. They are poisoned by oxygen.

Anamorph is the asexual reproductive stage or conidial form of fungi.

Antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi, or protozoa, as well as destroying viruses.

Ascomycetes are a group of yeasts and moulds which form sexual spores in structures known as asci (singular ascus). The spores are known as ascospores. The spores are relatively heat resistant and have a different genetic makeup from the parent strains.

Ascospores are sexual fungal spores which are produced in an ascus by Ascomycete fungi.

Ascus is a thin walled saclike organ where ascospores are formed.

Asexual reproduction is reproduction where the resulting cells have the same genetic makeup as the parent. It is usually faster than sexual reproduction. Cells, buds or spores can be formed from asexual reproduction.

Asexual spores are fungal spores produced from asexual reproduction. Not usually heat resistant. Many spores can be produced.

Asymptomatic carrier is someone who has had a food poisoning illness, and has recovered, but still carries the bacterium inside them. Can occur with *Salmonella* isolates. The carrier can shed the organism in their faeces, potentially cross contaminating food.

ATP (Adenosine Triphosphate) the carrier of energy in the living cell.

Bacteraemia is the presence of bacteria in the blood.

Bacteriocin is an antimicrobial often produced by lactic acid bacteria e.g. nisin. It is mainly effective against Gram positive bacteria, particularly spores.

Bacteriophage is a tiny virus that specifically attacks certain bacteria, killing them.

Bacteria / bacterium are relatively simple, single-celled organisms and are the smallest free-living organisms. They have no internal cellular organisation and no nucleus.

Bacteriology is the study of bacteria.

Basidiospores are sexual fungal spores which are produced in a basidium by Basidiomycete fungi.

Binary fission is a type of cell division where the original cell (mother cell) elongates (increase in size and mass) and then divide into two new cells (daughter cells) both with identical genetic material, by forming a cross wall. Bacteria and some yeasts divide in this way.

Budding is a type of cell division where a weakness produced in the cell wall causes a bud to be extruded. Eventually some genetic material passes into the bud, a cross wall forms, and the bud separates off from the mother cell. The bud has the same genetic material as the parent cell. Most yeast cells multiply in this way.

Capnophiles are organisms which thrive in the presence of high concentrations of carbon dioxide or which require the presence of carbon dioxide to survive.

Capsule is a layer formed outside the cell wall by the extrusion of material produced by the cell. They are firmly attached to the cell wall. If, however, the layer is easily detached and can diffuse into the surrounding giving the immediate bacterial environment a slimy consistency, it is called a *slime layer*. The capsule has many functions some of which include protecting the cell from dehydration, enabling bacteria to stick to surfaces and each other, protecting cells from nutrient loss.

Catalase is an enzyme capable of breaking down hydrogen dioxide into oxygen and water. As a test it is used to identify bacteria on the basis of whether they posses this enzyme or not.

Cell is the basic building block for micro-organisms. They may be individual, or joined together, depending on the type of organism.

Cell membrane/ Cytoplasmic membrane/ Plasma membrane is a thin flexible lipid and protein envelope that define the boundary between the cell cytoplasm and external environment. In also controls movement of materials in and out of the cell.

Cell wall is the outer structure of microbes. It has several functions some of which include providing rigidity for the cell contents, acting as a selective barrier for food and waste materials.

Chlamydospores are asexual fungal spores produced within the fungal mycelium

Classification is the orderly arrangement of organisms into groups which have similar physical, biochemical or genetic characteristics.

Cleistothecia are fungal fruiting bodies produced by ascomycetes which lack a special opening.

Coliforms are a type of bacteria that is often used as an indicator of poor processing, post-process contamination, or poor quality raw materials. They are not as useful an indicator as the Enterobacteriaceae.

Colony forming unit (cfu) is the basic microbial unit that can be measured. An individual colony on an agar plate usually comes from one individual cell that has multiplied on an agar plate.

Commercial sterilisation is the process of killing all pathogens and most spoilage organisms, usually by heating canned and pouched products in a retort, or by heating using the UHT process, if it is a liquid product. Surviving spore formers cannot grow under normal storage conditions in the country concerned. Survivors are usually thermophilic.

Conidia are asexual spores produced in the conidiophore.

Conidiophores are aerial hypha bearing conidiospores.

Cytoplasm is an aqueous mixture in bacterial cells containing proteins, carbohydrates, lipids, inorganic salts and waste products of metabolism.

Decimal Reduction time (D-value) is the time taken for a population to pass through a log cycle (90% of the population is killed).

Diarrhoeal toxin is a toxin that causes excessive and frequent evacuation of watery faeces.

Emetic toxin is a toxin that causes vomiting.

Endospores are bacterial spores formed inside a bacterial cell capable of survival in harsh environments.

Endotoxins are only released upon death or break down/lysis of a micro-organism.

Enterobacteriaceae are a family of indicator organisms.

Enterotoxins are toxins which are released/excreted by micro-organisms which are specific for the cells of the intestine.

Equilibrium relative humidity (ERH) is the water content in the atmosphere above a food at equilibrium with the food and is equal to the a_w x 100%.

Extrinsic factor are external factors affecting microbial growth of organisms e.g. temperature, humidity, gas atmosphere.

Facultative aerobes are organisms which are normally associated with anaerobic conditions but which can also grow in the presence of oxygen.

Facultative anaerobes are organisms that can grow whether the gas atmosphere is aerobic or anaerobic; they are fermentative organisms. They often start in an aerobic atmosphere, and then as the oxygen is used up they switch to anaerobic metabolism.

Fermentation is the conversion of complex organic compounds into smaller substances yielding energy.

Flagellum (pl. flagella) are hair-like appendages that arise from the cell surface of some bacteria. They are involved with the movement of bacteria in liquids.

Fission is the asexual reproduction of some unicellular organisms by division of the cell into two more or less equal parts.

Foodborne disease is disease that results from the ingestion of contaminated food.

Food poisoning is the process of becoming ill due to the consumption of food invaded by food poisoning organisms. It can be due to organisms growing in the body and making the person ill, or due to the release of toxins in the food or the body.

Food spoilage is food that has been damaged by spoilage organisms. The resulting food cannot make you ill, although it may not taste smell, or look nice.

Fungi are a group of organisms that contain a true nucleus.

Gastroenteritis is an infection or irritation of the stomach and intestines.

Generation time / Population doubling time is the time taken for the population of cells to double in size.

Genome refers to an organism's hereditary (genetic) material. It controls cell development and metabolic activities.

Gram stain is an important staining technique that is used to show the shape and arrangements of bacterial cells as well as their Gram reaction.

Gram negative (Gram –ve) bacteria are those that lose a crystal violet iodine complex when a decolouring agent is applied to their cell.

Gram positive (Gram +ve) bacteria are those that retain a crystal violet iodine complex when a decolouring agent is applied to their cell.

Guillain-Barré syndrome is an autoimmune disorder affecting the peripheral nervous system, usually triggered by an acute infectious process for example with *Campylobacter*.

Halophilic organisms prefer to grow in substances containing high levels of salt.

Hazard Analysis and Critical Control Point (HACCP) is a food safety management system used to identify and manage hazards in food.

Heat-labile toxin is a type of toxin which is destroyed or altered by heat.

Heat-stable toxin is a type of toxin which survives heating.

Heterofermentation is fermentation when more than one major product is formed.

Homofermentation is fermentation when only one major product is formed.

Hyphae are branched or unbranched, thread-like tubular structures (filaments) that form the building blocks of fungal growth.

Inorganic acids are acids composed of an inorganic acid containing a non-metallic element or radical and hydrogen. Examples are hydrochloric, nitric and sulphuric acids.

Indicator organisms are common microbes that indicate the presence of another, less common organism, or the failure of a process condition, or cross contamination.

Infection is a type of food poisoning where the organism grows in the animal's body, causing illness.

Infective dose is the minimum number of organisms of a specific type ingested with a food required to cause the symptoms of food poisoning.

Intoxication is a type of food poisoning where the organism produces a toxin in the food or the body, making the person unwell. The organism is relatively harmless, without the toxin.

Intrinsic factors are growth factors within the food matrix that affect the growth of bacteria e.g. water activity, nutrient content, pH, redox potential.

Ionising radiation is a form of radiation that via ionisation can kill micro-organisms by disrupting and destroying individual cells at the molecular level.

Mesophiles (organisms adapted to grow in the middle temperature zone) are organisms that have an optimum growth temperature at 28 - 43 °C. They can, however, grow in temperatures ranging from 5 - 52 °C.

Microaerophiles are microbes that require conditions of reduced oxygen levels, but not anaerobic conditions. They will not grow in a normal atmosphere.

Microbiology is the branch of biological sciences that deals with micro-organisms i.e. bacteria, fungi, protozoa, some algae, viruses, viroids and prions.

Micrometre (μM) is the unit of measurement of length or size of a cell. A micrometre is one millionth of a metre (1/1000 of a cm).

Motility is the ability of a micro-organism to move by itself, often via the use of flagella.

Moulds are filamentous/mycelial organism contained within the fungal kingdom.

Mycelium is a group or mass of discrete hyphae.

Mycology is the study of fungi.

Mycotoxin is a poison (toxin) produced by moulds. Only some moulds are mycotoxin producers.

Neurotoxin is a toxin which is poisonous or destructive to nerve tissue or cells.

Nucleus is a double membrane structure with numerous pores through which proteins and RNA can move to control the biochemical processes in the cell cytoplasm. It contains the genetic material involved with the synthesis of ribosomal RNA.

Obligate aerobes are organisms which are only capable of growth at atmospheric levels of oxygen.

Obligate anaerobes are organisms which cannot grow in the presence of oxygen.

Obligate psychrophiles (cold loving organisms) are organisms that have an optimum growth temperature at 10 - 15 °C. They can, however, grow in temperatures ranging from -10 to 20 °C.

Organic acids are acids containing carbon atoms for example citric and acetic acid.

Osmophilic organisms prefer to grow in substances containing high levels of sugar.

Osmotolerant organisms are able to withstand high concentrations of solutes (sugar or salt) but may not grow.

Oxidase test is used for the identification of bacterial strains. It is a measure of whether a bacterium produces cytochrome oxidase and can therefore use oxygen for energy generation.

Parasites are microbes that can only grow and reproduce inside the host, but may be able to survive in the environment for some time.

Pasteurisation is a heat process designed to kill all vegetative cells of pathogens and many spoilage organisms, but not overcook the food product.

Perithecia are flask shaped fruiting bodies which open at maturity to release ascospores.

Peritrichous flagella are flagella arranged over the whole cell surface.

pH scale is a measure of the concentration of hydrogen ions in a solution using a logarithmic scale.

Phycology (algology) is the study of algae.

Pili are tubular structures that originate from the cell membrane and protrude from the surface of cells of some bacteria. They enable bacteria to stick to surfaces and anchor themselves in an environment suitable for growth.

Plasmids are small pieces of hereditary material distinct from the main chromosome. They carry information that is not essential to survival of the cell but may help it to adapt to changing environmental circumstances.

Polar flagella are flagella arranged at the ends of the cell only.

Proton is a hydrogen ion; one of two parts that is released when the acid dissociates. It is the part that makes an acid effective as an antimicrobial and is poisonous to microbial cells.

Protozoa are single celled microbe with internal cellular organisation. Some are parasitic, some free living.

Protozoology is the study of protozoa.

Psychrophiles are cold loving organisms which have an optimum growth temperature of 15 °C and cannot grow at 30 °C.

Psychrotrophs are organisms which are capable of growth at cold temperatures that have an optimum growth temperature at 20 - 30 °C. They can, however, grow in temperatures ranging from -10 to 42 °C.

Pycnidia are flask shaped fruiting bodies which superficially resemble perithecia but contain conidiophores and conidia.

Putrefaction is the process in which protein-rich material are broken down releasing offensive smells and tastes.

Redox potential / Oxidation reduction potential (EH or OR) is a measure of whether a material has a tendency to gain electrons (become reduced) or lose electrons (become oxidised).

Ribosomes are small rounded bodies made up of proteins and RNA. They are responsible for protein synthesis.

Saprophytes are organisms which survive by living off dead or decaying plant material.

Serotype is a grouping of micro-organisms or viruses based on their cell surface antigens.

Sexual reproduction is the process of exchanging genetic material between two dissimilar strains of a micro-organism.

Sexual spores are spores produced from sexual reproduction on yeasts and moulds. In the food industry, this is usually an ascospore.

Species (spp.) are one of the smallest taxonomic groups. A species can be categorised as a group of individuals who display a high level of mutual similarity.

Sporangium (pl. sporangia) are closed intracellular structures in which asexual spores are produced.

Spore is a type of reproductive structure produced by particular organisms when unfavourable conditions are encountered. They then germinate when suitable conditions return and produce vegetative cells. Some types of spore are very heat resistant, others much less so.

Strain is a genetic variant or subtype of a species of micro-organisms.

Taxonomy is the practise and science of classification of living organisms.

Teleomorph is the sexual reproductive stage of fungi producing ascospores or basidiospores.

Thermoduric/Thermotolerant are organisms able to tolerate high temperatures, though not necessarily able to grow at high temperatures.

Thermophiles (organisms loving high temperatures) are organisms that have an optimum growth temperature at 50 - 65 °C. They can, however, grow in temperatures ranging from 30 to 70 °C.

Toxin is a poison produced by a micro-organism either in the body or in the food, and causes food poisoning.

Trophoziote is the active, motile feeding stage of a protozoan parasite.

Ultra high temperature (UHT) processing is one type of process designed to render food commercially sterile.

Undissociated acid is an acid that is a complete molecule; it has not broken down into the acid and base parts. An acid must be in this state in order to enter a microbial cell.

Vegetative cell is a growing microbial cell. Usually refers to the actively growing bacterial cell, as opposed to a bacterial spore.

Virology is the study of viruses.

Virus is a very small micro-organism that only grows inside a host cell. It takes over the host cell and causes it to produce more copies of itself, eventually being released from the host cell, to start the process all over again.

Water activity (a_w) is the amount of water available in a food for microbial growth. It is mathematically represented by the following equation

Water activity = Vapour pressure of a substance or solution / Vapour pressure of water at the same temperature

It can also be calculated from the relative humidity of the air that is in equilibrium with the substrate and is $1/100^{th}$ of that value. There a RH% of 95% would correspond to and a_w of 0.95.

Yeast is a single celled type of fungus.

z-value is the change in temperature required to achieve a tenfold change in the D-value.

Xerophiles are yeasts and moulds which are capable of growth in dry conditions in water activities less than 0.85.

Xerotolerant organisms are able to survive but not grow in dry conditions.

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