Control of bacterial spores

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Bacterial spores are much more resistant than their vegetative counterparts. The most dangerous spore-former is *Clostridium botulinum* which produces a potent neurotoxin that can prove fatal. The most common food poisoning from a spore-former is caused by *C. perfringens*. Other food poisoning spore-formers include *Bacillus cereus, B. subtilis* and *B. licheniformis* There are a number of non-pathogenic spore-formers including butyric and thermophilic anaerobes that cause significant economic losses to food producers. Some unusual spoilage complaints have been reported, for example, *B. sporothermodurans* in UHT milk, *Alicyclobacillus acidoterrestris* in apple and orange juice and *Desulfotomaculum nigrificans* in hot vending machines. Control of spore-formers requires an understanding of both the resistance and outgrowth characteristics of the spores.

Bacterial spores are much more resistant to heat, chemicals, irradiation and desiccation than their vegetative cell counterparts. The main food poisoning spore-formers are *Clostridium botulinum*, *C. perfringens* (formerly known as C. *welchii*) and *Bacillus cereus*. Occasionally *B. subtilis* and *B. licheniformis* have been implicated in food poisoning incidents. The most common food poisoning in the UK caused by a spore-former is from *C. perfringens*[1]. Between 1985 and 1994, the number of reported cases of *C. perfringens* food poisoning ranged from 446 to 1466 each year, whereas *Bacillus* spp. only accounted for 31 to 418 cases a year. By comparison, in the UK, outbreaks of botulism caused by the deadly *C. botulinum* are very rare with only 10 recorded outbreaks this century[2].

Non-food poisoning spore-formers can also produce spoilage in food products resulting in commercial loss, which can be substantial. Spoilage should be carefully investigated because it may be an indication that there is a fault in the processing or that hygiene standards are insufficient. Today's spoilage outbreak may be tomorrow's food poisoning incident.

Because spore-formers are inherently more resistant than vegetative cells, methods of control need to be chosen carefully. The reader is referred to *ICMSF Book 5* for the most comprehensive recent text on the characteristics and control of food pathogens[3].

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One of the most common methods of control of spore-formers is by heat. The most recent and comprehensive text on thermal processing is that by Holdsworth[4]. The UK Department of Health has also produced guidelines for the safe production of heat preserved foods[5].

Information on control of spore-formers by disinfectants is best obtained from the manufacturers of these chemicals. Formulation of disinfectants is constantly evolving to meet the demands of the food industry and to meet international disinfectant tests. The most widely used disinfectant is probably chlorine, but it is only slowly sporicidal and is readily inactivated by organic matter, although there are organic chlorine release agents which are more effective in the presence of soiling.

UV light is finding increased use in the food industry for the destruction of micro-organisms on surfaces and in water and air. UV lamp technology has improved considerably in the last 8–10 years and it is now possible to obtain very powerful lamps, which can produce significant reduction of spore-formers. Much of the recent work is not yet published and is held by manufacturers of the lamp systems or their customers. Multi-lamp arrays are also being developed to fit around conveyor systems so that the surfaces of product and packaging can be 'sterilised' before transfer to high care areas.

Spore-formers causing food poisoning

Clostridium botulinum

The most important spore-forming pathogen is *C. botulinum* because of the potent neurotoxin that it produces. The strains of *C. botulinum* are divide into seven types, A–G, based on the serological specificity of the toxins. There is also a further division into proteolytic and non-proteolytic strains. Only types A, B, E and F have been implicated in human food-borne botulism[6]. Types C and D produce botulism in birds and cattle, while type G has not been implicated in an outbreak.

In the US, where home canning is common, there were 688 reported outbreaks of botulism between 1899 and 1973 resulting in 978 deaths[7]. Hauschild[6] surveyed botulism outbreaks in 18 countries and found that 60–100% of the recorded outbreaks were from home-prepared foods. Between 1978 and 1991 in the US, types A, B and E accounted for 60%, 13% and 24%, respectively, of the 371 cases of food-borne botulism (Table 1)[8]. Only one case of type F, from home-prepared pig's feet was recorded in the same period.

Products associated with outbreaks of botulism are very diverse and include meat, fish, vegetables, dairy and honey products. The pH of

Table 1 *Yearly incidence of botulism in the United States*

Year	Foodborne type				Total	Infant type			Total	Wound type			Total	Un-	Year
	A	B	E	?	cases	A	B	?	cases	A	B	?	cases	known	total
1978	54	4	0	0	58	20	18	1	39	0	0	0	0	14	111
1979	4	2	2	0	8	13	10	1[a]	24	2	0	1[b]	3	5	40
1980	12	4	2	0	18	31	34	1[c]	66	2	0	0	2	1	87
1981	5	6	9	2	22	40	31	0	71	3	2	0	5	1	99
1982	27	3	0	0	30	29	31	0	60	0	0	1	1	1	92
1983	38	4	1	0	43	33	46	0	79	0	0	0	0	3	125
1984	12	1	4	1	18	42	54	1[b]	97	2	1	0	3	3	121
1985	10	8	14	0	32	36	35	0	71	0	1	0	1	1	105
1986	10	5	8	1[a]	24	53	35	0	88	1	0	2	3	0	115
1987	10	2	3	5	20	42	44	0	86	2	1	0	3	0	109
1988	14	3	26	2	45	32	42	4	78	2	0	0	2	1	126
1989	17	1	5	0	23	28	45	1	74	2	1	1	4	0	101
1990	8	6	8	0	22	29	45	0	74	3	1	0	4	0	100
1991[d]	0	1	7	0	8	21	23	1	45	0	0	0	0	0	53
Total	221	50	89	11	371	449	493	10	952	19	7	5	31	30	1384

Table compiled from summaries of botulism cases reported to CDC[8]
[a]Type F, [b]types A/B, [c]types B/F, [d]1 January – 30 June 1991

these products is normally above 4.5. However, there were 34 reported outbreaks in the US of food-borne botulism between 1899 and 1975 from acid products including pears, apricots, tomato products, pickles, apple sauce, okra, peaches, huckleberry and blackberries[9]. Spores of *C. botulinum* have been shown to survive for long periods of time at low pH. The lowest pH associated with two cases of botulism in Kentucky was 3.5. In order for *C. botulinum* to grow in products with pH values below 4.5, it is necessary for initial spoilage caused by other micro-organisms to occur first. The initial spoilage organism raises the pH (*e.g.* beneath a pellicle of mould growth) above 4.5 which then allows the spores of *C. botulinum*, which have survived the pasteurisation step, to germinate and produce toxin.

Early researchers, such as Weiss[10] and Esty and Meyer[11], were not aware that *C. botulinum* was unlikely to grow in acid products and, therefore, did many experiments to determine the heat resistance of the spores in fruit products with pH values as low as 2.1. Once pH 4.5 became established in the 1930s as the minimum pH for growth and toxin production, little attention has been paid to resistance of spores at pH values lower than this. Recently, however, there are many examples of pasteurised fruit products being mixed with food components with pH above 4.5 (*e.g.* fruit pulp in dairy desserts, pasteurised fruit pieces mixed with cooked meat toppings). The only recent study into the heat resistance of spores of *C. botulinum* in fruit has been by Smelt[12] who

Table 2 Recorded incidents of food-borne botulism in the UK

Year	Cases	Deaths	Food implicated	*C botulinum* type
1922	8	8	Duck paste	A
1932	2	1	Rabbit and pigeon broth	?
1934	1	0	Jugged hare	?
1935	5?	4?	Vegetarian nut brawn	A
1935	1	1	Minced meat pie	B
1947	5	1	Macaroni cheese	?
1955	2	0	Pickled fish from Mauritius	A
1978	4	2	Canned salmon from US	E
1987	1	0	Kosher airline meal	A
1989	27	1	Hazelnut yoghurt	B

Compiled from Gilbert *et al*[2]

specifically addressed the elimination of the spores in strawberries which were to be pasteurised and mixed with a sterilised dairy product. The decimal reduction time of spores of proteolytic strains at 90°C ranged from 2.3 to 7.5 min. He concluded that spores of proteolytic *C. botulinum* could not be eliminated by heat without impairing the quality of the strawberries.

The most recent, and largest, outbreak of botulism in the UK in which 27 people were affected and one died was associated with hazelnut yoghurt[13]. This was caused by the failure to apply a botulinum cook to the canned hazelnut used to flavour the yoghurt. This example of botulism from a final product with pH below 4.5 emphasises the need for vigilance at all stages of food manufacturing.

The 10 recorded outbreaks in the UK began in 1922 with the Loch Maree outbreak in Scotland caused by *C. botulinum* type A in potted duck paste (Table 2)[2]. This had been processed at 113.9–115.6°C for 2 h, removed from the steriliser, filled into glass containers which were capped and cooked at 99°C for 40 min. Eight people died in this outbreak. The incidents between 1932 and 1947 all involved home prepared foods. The incident in 1955 involved pickled fish from Mauritius, in 1978 the outbreak was caused by Alaskan canned salmon, in 1987 an airline meal was involved and in 1989 the hazelnut yoghurt incident occurred.

In the US, the most common form of human botulism is infant botulism[14]. Between 1976 and 1989 over 850 laboratory-confirmed hospitalised cases had been reported in the US. The illness results when ingested spores of *C. botulinum* germinate and colonise the large intestine of infants. Most early cases are due to types A and B (proteolytic group 1) strains. Cases have also been attributed to *C. butyricum* producing type F toxin and to *C. barati* producing type E toxin. Infant botulism has been reported in 14 countries. Because honey fed to infants has been identified

Table 3 Wet heat resistance of spores of *C. botulinum*

Type	Typical D value (min)			Typical z value	Reference
	100°C	121°C	140°C	(°C)	
A proteolytic	29 2*	0 05–0 13	0 001*	8 2–9 1	15
B proteolytic	10 5*	0 13*	0 002	11 0	16
	20 7–23 5				17
B non-proteolytic	0 08*	0 0003*	–	8 6–9 8	18
C mildly or non-proteolytic					19
Terrestrial	3 4*	0 003*	–	10 0–11 5	
Marine	0 6*	0 0002*	–	10 7–10 8	
D mildly or non-proteolytic	–	–	–	–	20†
E non-proteolytic	0 03*	0 0002*	–	6 1–8 4	21
F proteolytic	8 8–17 8*	0 14–0 22*	0 003–0 004	9 3–12 1	22
F non-proteolytic	0 0001–0 0002*	–	–	9 5–14 8	23
G proteolytic	1 1–1 3*	0 14–0 19	0 02–0 04*	20 9–27 3	24

*Calculated from published data

†No data has been found for type D but reported[20] to have similar resistance to type C

as a source of spores of *C. botulinum*, it is not recommended to give honey to infants until they reach one year of age.

The wet heat resistance of spores of *C. botulinum* is summarised in Table 3[15–24]. The reader should also consult *ICMSF Book 5*[3] and Brown[25] for reviews of heat resistance data on *C. botulinum*.

A third type of botulism, wound botulism, caused by germination and outgrowth of spores of *C. botulinum* in wounds accounted for 31 cases of botulism between 1978 and 1991 in the US[8]. A number of cases of wound botulism were a result of intravenous drug use.

C. botulinum can grow and produce toxin with ease in many foods. It is important to ensure that not only are the correct thermal processes and preservative regimens followed, but also that all parts of the food are under control. Several instances of growth of *C. botulinum* in unusual circumstances are cited in *ICMSF Book 5*[3]. These include chopped garlic in oil that was unheated, contained no preservatives and relied solely on refrigeration for safety, preserved peanuts, and 'kapchunka' – a salt-cured, air-dried, whole uneviscerated whitefish. 'Kapchunka' preservation relies on the salt concentration inside all parts of the flesh of the fish reaching an inhibitory level quickly enough to prevent *C. botulinum* growing. Botulism outbreaks have also been reported from potato salad prepared from foil wrapped baked potatoes which had been stored at room temperature after baking and sautéed onions where the spores had survived the frying step and germinated in the anaerobic conditions provided by the layer of margarine on the surface of the onions. Non-proteolytic *C. botulinum* can grow down to

3.3°C and, therefore, presents a risk to chilled foods. The proteolytic strains can grow down to 10°C.

Clostridium perfringens

C. perfringens was the cause of 18,970 cases of food poisoning between 1970–1980 in England and Wales[26] and 982 cases between 1985–1994[1]. It poses a world-wide problem.

Food poisoning from this organism is typically characterised by acute diarrhoea and severe abdominal pain 8–24 h after ingestion of food containing large numbers of vegetative cells. Full recovery within 24–48 h is normal.

C. perfringens is grouped into 5 types, A–E, depending on the exotoxin produced (as distinct to the enterotoxin). Type A is the usual strain associated with food poisoning in the UK and the West. This strain can also cause gas gangrene and septicaemia as well as food poisoning.

In New Guinea, a type of food poisoning called 'pig-bel' is caused by *C. perfringens* type C. During pig roasts at tribal celebrations, spores of this strain germinate and proliferate during the slow cooking and cooling of the meat which is often cooked as whole carcasses in shallow pits in the ground. Consumption of sweet potatoes, which contain a heat stable trypsin inhibitor, at the same time prevents destruction of the bacterial toxin and is believed to be responsible for the number of fatalities from 'pig-bel'.

Spores of *C. perfringens* type A are widespread in the environment and are present in a wide variety of foods including meat, fish, poultry, vegetables, dairy products and dried foods[3]. Outbreaks of food poisoning from *C. perfringens* have often followed consumption of meals prepared for large numbers of people in schools, factories, hospitals and social functions.

An outbreak investigated by the author serves as a typical example. Guests at a wedding reception were served with sliced turkey by the hotel. A number of guests, including the bride and groom and close relatives, became ill later that evening. Only about a third of the guests became ill even though most had eaten turkey. The hotel had cooked two large pieces of turkey roll the previous day. The joints, produced by a local butcher, were formed by pressing pieces of turkey into a roll thus ensuring that any contaminating spores of *C. perfringens* were distributed to the centre. The size of the two rolls of meat and the cooking details were not definitely established but each roll weighed 20 lb and was cooked for 20 min/lb. The cooking temperature was not definitely established either, but due to the size of the roll and the moisture content of the meat, it was calculated that the temperature at

the centre could have been anything from 67–117°C at the end of the cooking time. It is not generally appreciated that when products containing a high moisture content are cooked or deep fried, the centre temperature does not rise much above 100°C until the water is driven off. At normal atmospheric pressure, water boils at 100°C whether it is deep fried, oven baked or boiled in a kettle.

After cooking, which was insufficient to kill the spores of *C. perfringens*, (decimal reduction time up to 17 min at 100°C)[27] the rolls of meat were allowed to cool at room temperature for 1 h, wrapped in cling film and placed in a chiller at 15°C for another hour followed by refrigeration at 0–2°C for 12 h. Simulations of the centre temperatures during cooling demonstrated that they would have been at 15–50°C for 5–6 h. This would have been more than sufficient for each individual surviving spore to germinate and increase to over 2 x 10^6 cells assuming an average generation time of 17 min (generation times as short as 7 min have been reported[27]). The joints of meat were then removed from the refrigerator, sliced and overlapped on plates for ease of serving. The meat was sprinkled with water, covered with foil and kept 'hot' in a fan oven set at 180°C for up to 2 h before serving. The reason only some of the guests became ill was believed to be because only the centre of one of the two meat rolls was thought to contain sufficient of the organism. Since the slices were served sequentially, most of the guests affected were sitting together. The organism was isolated from some of the turkey roll taken home by one of the guests for her cat. Fortunately for the cat, the meat was sent instead to the local environmental health officer for analysis when its owner succumbed to the food poisoning.

Cooking and cooling the meat in smaller portions, which would have heated and cooled more rapidly, would have significantly reduced the risk of *C. perfringens* food poisoning. The reheating of the meat slices in the fan oven was not effective because of the thickness of the overlapping slices. A temperature above 70°C would be necessary to destroy the vegetative bacteria before consumption.

Bacillus cereus, B. subtilis and *B. licheniformis*

The causes of B. cereus food poisoning are similar in many respects to those of *C. perfringens*. The conditions that favour the growth of *B. cereus* include cooking procedures that activate the spores followed by slow cooling and storage of food at 10–50°C[3]. *B. cereus* and the other food poisoning bacilli, *B. subtilis* and *B. licheniformis*, are widespread in the environment. Rice, cereals and spices are commonly contaminated with spores of *B. cereus*. Following cooking, in the absence of a competitive microflora, the surviving spores germinate and proliferate

rapidly. There are two forms of illness produced by B. cereus. One is caused by a diarrhoeal toxin, which is produced during the exponential phase of growth. The onset of diarrhoea occurs 8–24 h after ingestion of large numbers of bacteria or toxin. This toxin is heat sensitive and is inactivated by heating at 56°C for 5 min. The other is caused by an emetic toxin, which is produced during the stationary phase of growth. Emesis occurs 1–6 h after ingestion of the toxin. This toxin is heat stable and can withstand normal cooking procedures[3].

The spores of *B. cereus* appear to vary widely in heat resistance. However, when the published data are plotted as log decimal reduction or D value against temperature, most of the data clusters together[25] with only the ileal loop 2 strain of Bradshaw *et al.*[28] standing out as a particularly heat resistant strain. A D value of 2.35 min at 121.1°C was reported[28] for this strain.

B. licheniformis spores are similar in resistance to typical *B. cereus* spores with D values at 100°C around 4–8 min. *B. subtilis* spores typically have D values at 121.1°C of approximately 0.5 min. Compilations of thermal resistance data can be found in *ICMSF Book 5*[3], Holdsworth[4] and Brown[25].

Control of *B. cereus, B. subtilis* and *B. licheniformis* depends on adequate heat processing to destroy the spores and rapid cooling of product after cooking. Holding of food at temperatures of 10–50°C will allow the organism to proliferate provided other growth conditions, such as pH, are favourable.

Under dry heat conditions, spores of *B. subtilis* can be extremely resistant with D values at 160°C of 0.1–3.5 min being reported by various researchers[29].

Spore-formers causing spoilage

Clostridium butyricum, C. beijerinckii and *C. pasteurianum*

There are several spore-formers which have not been associated with food poisoning but which can produce significant economic spoilage of foodstuff.

Butyric anaerobes, for example, *C. butyricum, C. beijerinckii* and *C. pasteurianum* produce gas and butyric odours in canned foods, particularly those with pH values between 3.9 and 4.5 (*e.g.* tomatoes and pears)[30]. During the storage and ripening of hard cheeses such as Gouda, Edam and Emmentaler, *C. butyricum* and *C. tyrobutyricum* can cause spoilage and gas production ('blowing'), the spores often occurring in milk from cows fed silage during winter months. Spores of *C. butyricum*

have been reported to have D values as high as 23 min at 85°C and pH 7[31]. At pH 4.4, the thermal death time may be 10–15 min at 100°C[30].

For destruction of spores of *C. pasteurianum*, it has been suggested that a centre temperature of 95°C should be reached for products with a pH between 4.2 and 4.5 and a centre temperature of 84°C for products with a pH below 4.2[30].

C. beijerinckii is a close relative of *C. butyricum* with D values of 2–4 min at 85°C and pH 7[32].

Control of butyric anaerobes requires thorough washing of the raw material together with pH and process temperature control. Failure to control spore-formers in products with pH values 3.9–4.5 may result not only in spoilage but also a rise in pH which could allow spores of *C. botulinum*, which had survived pasteurisation, to germinate and produce toxin.

Clostridium sporogenes

C. sporogenes is closely related to the proteolytic strains of *C. botulinum*, but produces spores which are approximately 5 times as resistant with D values up to 1.5 min at 121°C[25]. Spoilage from this organism produces typically blown or burst packs with a strong putrefactive odour. If spoilage from *C. sporogenes* is experienced, all suspect packs should be recalled and investigations into the cause of spoilage undertaken. A process fault that allows *C. sporogenes* to survive and proliferate may also have been serious enough to allow spores of *C. botulinum* to survive, germinate and produce toxin.

Bacillus sporothermodurans

This is a mesophilic spore-former which produces highly heat-resistant spores. It was first detected in UHT milk in 1985 in southern Europe and in UHT milk in Germany in 1990[33]. Since then, there have been several reports from other European and non-European countries[34]. The spores survive the heat process and then multiply to a maximum of about 10^5/ml of milk during incubation at 30°C for 5 days, but cause no noticeable spoilage and are non-pathogenic.

The decimal reduction times of spores from 3 dairies ranged from 19–34 s at 121°C[35]. Raw milk must be autoclaved to enrich for the spores and eliminate competitive microflora. According to Meier *et al.*,[35] the spores of *B. sporothermodurans* are more resistant than the spores of many thermophiles.

Clostridium thermosaccharolyticum

The most heat resistant spores are those of *C. thermosaccharolyticum*. D values as high as 195 min at 121°C have been recorded[36]. The author investigated a spoilage outbreak in canned mushrooms caused by heat resistant spores of *C. thermosaccharolyticum* that had grown in the composted forest bark used on the mushroom beds[37]. D values of these spores were 68 min at 121°C.

Spoilage from this organism manifests itself by blown or burst packs with a strong butyric or cheesy odour. The spores survive thermal processing to germinate and grow when the product is stored at elevated temperatures around 30–60°C (*e.g.* in pallets of inadequately cooled cans). Spoilage by *C. thermosaccharolyticum* is not uncommon.

Desulfotomaculum nigrificans

In contrast, spoilage caused by *D. nigrificans*, another thermophile, is now quite rare although, in the 1920s, an entire season's production of canned sweet corn could be lost from this organism. *D. nigrificans* causes 'sulphur stinker' spoilage often resulting in blackened product when the steel in cans reacts with the H_2S produced. D values as high as 55 min at 121.1°C have been recorded[38]. An unusual outbreak of spoilage caused by *D. nigrificans* and *Clostridium thermoaceticum* in Japan was reported by Matsuda *et al.*[39]. The spoilage occurred in canned coffee and 'Shiruko' (a soft drink made from red beans and cane sugar) produced for retail in hot vending machines at temperatures above 50°C.

Bacillus stearothermophilus and B. coagulans

B. stearothermophilus is a common thermophilic spoilage organism that normally produces acid but no gas in spoiled packs that have been held at elevated temperatures around 50–55°C. If readily fermentable sugars are in limited supply, the author has found that this organism can elevate pH. The minimum pH for growth is around 5.3. The D value at 120°C can be as high as 16.7 min[40]. Prevention of spoilage is achieved by holding product below 30–60°C because it is often impracticable to try to process product for long enough to destroy the spores.

Under dry heat conditions at 121°C, the D value of spores of *B. stearothermophilus* can be as high as 936 min[41].

B. coagulans is also a thermophile but differs from *B. stearothermophilus* in being able to grow at pH values down to 4.0[30]. It is less heat

resistant having a D-value at 98.9°C of 3.1 min[30]. It produces off-flavours and souring of product during spoilage.

Alicyclobacillus acidoterrestris

Traditionally, pasteurised acidic fruit juices with pH values below 4.0 have been considered unlikely to support the growth of spore-forming bacteria[30,42]. However, in Germany in 1982. a spoilage outbreak in apple juice was caused by an acid tolerant, heat resistant, thermophilic spore-forming bacterium[43]. The organism was identified as a strain of *Alicyclobacillus acidoterrestris* that could grow over the pH range 2–6 and at temperatures between 35–55°C. The decimal reduction time for the spores at 90°C was 15 min. The organism produces a 'disinfectant' or 'antiseptic' taint in apple and orange juice. The most recent reports of spoilage from this organism were in 1994/5 with unusual taint development in aseptically packed orange juice and concentrate in the UK and North America[44].

The most likely cause of contamination of the fruit is from soil contamination during harvesting. The heat resistance of the spores is such that pasteurisation will not guarantee freedom from the organism. Thorough washing of the fruit prior to processing appears to be the only control measure at present. There is no evidence that the organism is pathogenic, and since it does not change the pH of the fruit, there should be no risk of secondary growth of spore-forming pathogens such as *C. botulinum*. Brown[45] has reviewed *A. acidoterrestris* and others in the same genus which have been isolated from hot springs.

Clostridium putrefaciens

In the early 1900s, *C. putrefaciens* was of considerable concern to the ham curing industry. Studies by Roberts and Derrick[46] demonstrated that this organism was able to grow in 4% NaCl + 100 p/m of $NaNO_2$ at pH 7.0 even at 5°C. The spores were not particularly heat resistant however (D-value 8–14 min at 80°C). Modern processing trends are to use lower levels of salt and nitrite, increased pH levels of 6.8–7.0 and chill storage which would tend to favour the growth of *C. putrefaciens*.

Key points for clinical practice

Bacterial spores are resistant to heat, chemicals, freezing, desiccation and irradiation. Resistance can vary widely from species to species. The

Table 4 Growth range temperatures of some spore-formers

Organism	Temperature (°C)			Reference
	Minimum	Optimum	Maximum	
A. acidoterrestris	26	42–53	80	45
B cereus	3–4	30	48	47
	4	30–40	55	3
	5		50	48
B coagulans	30	45	60	47
		50–55		42
B licheniformis	30	–	60	49
B sporothermodurans	10	–	50	34
B stearothermophilus	28	55	72	47
B subtilis	10	28	51	47
	12	43–46	55	49
C beijerinkii	–	30	–	49
C botulinum (proteolytic)	10	38–40	48	47
C botulinum (non-proteolytic)	3 3	25–30	40	3
C butyricum	10	–	45	49
C pasteurianum	–	30	–	50
		25-35		42
C perfringens	12	43–47	50	3
	15		50	27
C putrefaciens	5	15–22	35	46
	0	20	35	47
C sporogenes	–	28–30	–	42
	18		45	49
C thermosaccharolyticum	30	55	71	47
		50–55		42
D nigrificans	30	55	71	47

most dangerous food poisoning species is *C botulinum* and the most common is *C. perfringens*. *B. cereus, B. subtilis* and *B. licheniformis* have also been implicated in food poisoning. *C. perfringens, C. botulinum* and *C. sporogenes* can cause deep wound infections and, in the case of the first two, cause wound botulism and gas gangrene. The most common type of botulism is infant botulism. Several non-pathogenic spore-formers can cause significant spoilage in food products. A number of spoilage spore-formers are thermophilic and these proliferate when the food is held at elevated temperatures (30–60°C). Other spore-formers, such as non-proteolytic *C. botulinum*, can grow down to 3.3°C and can, therefore, present a risk to chilled foods. Many foods receive heat treatments that do not eliminate all bacterial spores, and rely upon chilled storage for their shelf-life and safety. Examples of the range of temperatures for growth, including the minimum, are given in Table 4 (*see also* chapter on 'Control of vegetative micro-organisms'). Bacterial spores are several orders of magnitude more resistant under dry heat than wet heat conditions. Control of spore-formers requires an understanding of both the resistance and outgrowth characteristics of the spores.

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