Bacillus cereus

Family of *Bacillaceae*
Genus *Bacillus*

Bacterium

Characteristics and sources of *Bacillus cereus*

Main microbiological characteristics

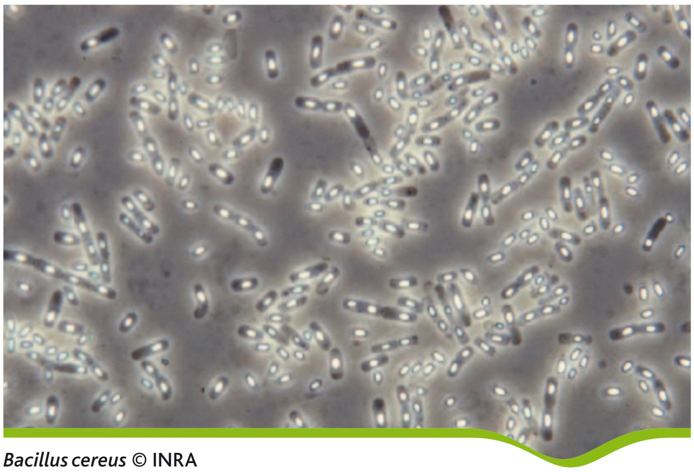
Bacillus cereus is responsible for outbreaks of foodborne illness causing diarrhoea and intoxication[1] characterised by emetic symptoms. It is a rod-shaped, Gram-positive, spore-forming, facultatively anaerobic bacterium.

It belongs to a group of related species, often found together in the literature under the term "*Bacillus cereus sensu lato*" which are traditionally broken down into:

- *Bacillus cereus sensu stricto* (haemolytic);
- *Bacillus thuringiensis*, differentiated from *B. cereus sensu stricto* by its production of parasporal crystals that are toxic to insects;
- *Bacillus anthracis* (non-haemolytic), the causative agent of anthrax;
- *Bacillus weihenstephanensis*, made up of certain psychrotrophic strains of *B. cereus*;
- *Bacillus mycoides* and *Bacillus pseudomycoides* which form rhizoid colonies on agar media.

B. cereus sensu stricto, *B. anthracis* and *B. thuringiensis* in fact constitute a single species but can be distinguished from one another by their virulence properties, generally carriedby plasmids.

When analysing food or investigating outbreaks, no attempt is usually made to distinguish between *B. thuringiensis*, *B. cereus sensu stricto* and *B. weihenstephanensis*. Thus, information on *Bacillus* spp. foodborne illness



Bacillus cereus © INRA

rarely differentiates between *B. cereus sensu stricto*, B. *thuringiensis* and *B. weihenstephanensis*. In this datasheet, unless otherwise stated, the term *B. cereus* encompasses all three species.

B. cereus sensu lato was recently split into seven genetic groups, with the traditional species found in one or more groups. The seven genetic groups have different capacities for adapting to thermal conditions, ranging from psychrotrophic to heat-tolerant species, and are also associated with foodborne illnesses to varying degrees. Group VI is the most psychrotrophic

(1) Foodborne disease resulting from the ingestion of enterotoxins pre-formed in the food.

Table 1. Characteristics concerning growth and toxigenesis of *B. cereus* (data vary by genetic group)

Parameters	Growth (vegetative cells)			Toxins				
				Production			Stability	
	Min	Opt	Max	Min	Opt	Max	Min	Max
Temperature (°C)	4 (for a majority of emetic strains, the minimum temperature is 10)	30-37	55	10	20-25	40	/	/
pH	4.3	6-7	9.3	/	/	/	Emetic toxin: 2	Emetic toxin: 9
a_w	0.92	0.99-1	/	/	/	/	/	/
NaCl (g/L)	/	/	50	/	/	50 (very low production of cereulide)	/	/

CO_2 can inhibit growth of *B. cereus* if the atmospheric content is greater than 40-50%.

Data sheet on foodborne biological hazards
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and has thus far never been associated with food poisoning. Groups II, V and IV are psychrotrophic to mesophilic and Groups III and VII are mesophilic to heat-tolerant.

Currently, three enterotoxins (Hbl, Nhe and CytK) have been described in *B. cereus*. The enterotoxins Hbl (hemolysin BL) and Nhe (non-haemolytic enterotoxin) are each made up of three proteins. There are two forms of Cytotoxin K (CytK), CytK1 and CytK2, the first being more cytotoxic than the second. Moreover, certain strains of *B. cereus* can produce the emetic toxin known as cereulide (a cyclic peptide). These are relatively rare, generally accounting for 1% or less of the isolates taken from food or the environment, and mostly belong to Group III.

Hazard sources

Spores of *Bacillus cereus* are found in soil at concentrations of the order of 10^4 to 10^5 spores per gram of soil. Spores may be dormant in soil and develop within soil organisms, such as in the digestive tracts of insects, arthropods and earthworms. *B. cereus* may be a gut commensal in insects that only proliferates when its host is weakened. *B. cereus* spores are also present in the digestive tracts of warm-blooded animals. *B. cereus sensu stricto* can also be responsible, albeit rarely, for mastitis in cattle and abortions in cattle and sheep. *B. thuringiensis* is an insect pathogen and, like *B. cereus sensu stricto*, is often found in food; it can cause the same illnesses as *B. cereus* in humans.

Transmission routes

The main transmission route to humans for this bacterium is food. Due to its abundance in soil and the resistance of its spores, *B. cereus* can contaminate practically all foods, especially plant matter.

Some infections with *B. cereus* have been described that were unrelated to food. The portals of entry for the infection include contaminated wounds or catheters, or the injections practiced by drug addicts.

Human foodborne illness

Nature of the disease

B. cereus causes intoxications leading to emetic symptoms (Table 2) and poisoning characterised by diarrheal symptoms (Table 3). Illnesses with emetic symptoms are caused by the ingestion of a toxin, cereulide, produced in the food during the growth of *B. cereus*. Illnesses with diarrheal symptoms are thought to be caused by ingestion of cells and/or spores of *B. cereus*, followed by the production of enterotoxins in the intestine. However, *B. cereus* produces several other proteins with toxic effects *in vitro* or in animal models, and some of these proteins may also contribute to the diarrhoeal symptoms.

Susceptible population[(2)]**:** there is no identifiable susceptible population for intoxication with *B. cereus*. Serious forms of infection (septicaemia, necrotising enterocolitis, acute liver failure, encephalopathy, brain abscess, death) have been observed in premature infants, newborns, patients suffering from haematological malignancies, cirrhosis, or Crohn's disease treated by immunosuppressors. However, no link with consumption of foods has been demonstrated for these infections, which are of a different nature to those described in Tables 2 and 3.

Dose-effect[(3)] and dose-response[(4)] relationships

Diarrhoeal-type *B. cereus* infections are most often associated with a bacterial count of at least 10^5 cfu/g of food ingested, although outbreaks associated with food containing only 10^3 cfu/g have also been reported. Not all *B. cereus* strains have the same ability to cause diarrhoea, and some are even used as probiotics.

According to studies in monkeys and based on the analysis of foods involved in foodborne intoxication in humans, from 5 to 10 µg of cereulide per kg of body weight is necessary to induce the emetic symptoms. This quantity of cereulide can be found in food when a *B. cereus* strain reaches a concentration of 10^6 cfu/g or greater.

No dose-response curves have been determined for *B. cereus* or cereulide.

Epidemiology

In France, foodborne outbreaks are notifiable diseases. In France, as in Europe, illnesses caused by *B. cereus* are probably underreported. Moreover, the diarrhoeal and emetic symptoms of *B. cereus* food poisoning are very similar to those caused by *Clostridium perfringens* and *Staphylococcus aureus* respectively. From 1996 to 2005, *B. cereus* was the fourth cause of foodborne outbreaks in France with 1,766 cases spread over 94 confirmed outbreaks (i.e. 3.5% of confirmed foodborne outbreaks). In 2008, *B. cereus* was the third cause of foodborne outbreaks in France in which the presence of the microorganism in food was confirmed or suspected. In France, between 2006 and 2008, *B. cereus* accounted for 4.5% of confirmed outbreaks and was suspected in another 15% of outbreaks. Still in France over the same period, 3,127 foodborne outbreaks were reported, involving 33,404 patients, 2,302 hospitalisations and 15 deaths, of which one was related to *B. cereus* infection. In France, in 2009, 4 hospitalisations were reported for which infection with *B. cereus* was confirmed and

(2) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used in ANSES data sheets].

(3) Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.

(4) For a given effect, the relationship between the dose and the response, i.e., the probability of this effect appearing in the population.

Table 2. **Characteristics of illnesses with emetic symptoms**

Mean incubation period	Target population	Main symptoms	Duration of symptoms	Duration of contagious period	Complications
30 min - 6 h	The entire population, irrespective of age	Nausea Vomiting Discomfort Diarrhoea and occasional abdominal pain	Less than 24 hours	Non-transmissible (toxins)	Toxic to several types of human cells, and can induce reversible liver failure. The emetic toxin has been responsible for at least 8 serious cases reported in the literature, including the deaths of 5 children or young adults with different types of organ dysfunction (heart, liver, kidneys or brain).

Table 3. **Characteristics of illnesses with diarrheal symptoms**

Mean incubation period	Target population	Main symptoms	Duration of symptoms	Duration of contagious period	Complications
8 - 16 h	The entire population, irrespective of age	Watery diarrhoea Abdominal pains Occasional nausea	24 heures	Non-transmissible (toxins)	Complications are rare in illnesses with diarrheal symptoms. In France, in 1998, 3 deaths were recorded of fragile elderly residents of a retirement home that suffered an outbreak of food-poisoning. It should be noted, however, that this outbreak was due to a very rare strain of *B. cereus* producing a cytotoxin that had never been observed previously.

24 hospitalisations for which *B. cereus* was suspected. Between 2006 and 2008, more than 80% of food poisoning outbreaks caused by *B. cereus* and *C. perfringens* occurred in mass catering situations. These two agents were implicated in 23% of outbreaks occurring in mass catering.

The role of food

Main foods to consider

Spores of *B. cereus* are present in almost all categories of food. Dried or dehydrated food products, such as spices, herbs, certain vegetables, cereals and flours, are often contaminated to different degrees by *B. cereus*. Whenever these raw materials are among the ingredients of finished products they are potential sources of contamination. Furthermore, the spores of *B. cereus* can adhere to stainless steel surfaces particularly well and can accumulate in food processing equipment, which can thus become reservoirs for spores.

The risk to consumers is most often related to the multiplication of *B. cereus* when foods are exposed to unsuitable temperatures. Foods associated with foodborne outbreaks implicating *B. cereus* have often, though not always, been heat-treated and/or insufficiently cooled after preparation and before consumption. Several foodborne outbreaks with emetic symptoms were caused by products rich in starch (rice- or pasta-based dishes).

Amongst other foods, prepared meals, products with added spices, herbs or aromatics, dehydrated foods reconstituted by adding hot water (dried soups, mashed potatoes prepared from potato flakes, powdered milk, etc.) or cooked in water (pasta, rice, semolina) kept at a temperature at which *B. cereus* can grow (temperatures between 4°C and 55°C), and not consumed immediately, may all be counted as risk foods, regarding the *B. cereus* hazard.

Inactivation treatments in industrial environments

See information in Table 4.

Monitoring in food

European regulations do not provide food safety criteria for *B. cereus*. However, Regulation (EC) no. 2073/2005 (amended) defines a process hygiene criterion applicable to *B. cereus* in dried preparations intended for children under six months.

The French NF EN ISO 7932 standard covers the identification and counting of presumptive viable *B. cereus*. Scanning for and counting presumptive viable *B. cereus* present in small numbers is covered by the French NF EN ISO 21871 standard. Furthermore, methods for detecting *B. cereus* by real-time PCR have recently been developed.

Various techniques are available for scanning for diarrheal toxins: ELISA, reverse passive latex agglutination (RPLA), immunodiffusion or immunoelectrophoresis. The scientific community is in agreement that the diarrheal toxins are produced during the growth of *B. cereus* in the digestive tract. Screening for them in food therefore does not provide an indication of the risk of diarrheal poisoning. These tests are more useful for characterising the toxigenic potential of the different strains of *B. cereus*.

Detecting cereulide in food requires cellular cytotoxicity tests and subsequent confirmation by mass spectrometry, and is not currently practised in France. Cereulide is very stable and can survive in food after the bacteria have been deactivated, by heat treatment, for example, or can be introduced in the food *via* one of its ingredients in which *B. cereus* may have developed. The *B. cereus* count in a food at the moment of consumption is not therefore a sufficient indicator of the risk of intoxication.

Table 4. Inactivation treatments of *B. cereus* in foods

Disinfectants	Effects of temperature
Vegetative cells are sensitive to all disinfectants authorised in the agro-food sector, on condition that the recommended procedures for use are followed. Cleaning procedures using heat and caustic soda can achieve several log reductions in the number of spores adhering to surfaces. Approximately 4 log reductions in the number of spores can be achieved with chlorine disinfectants containing at least 100 to 200 mg/L of active chlorine (pH and the presence of organic soiling reduce the efficacy of disinfection). The germination of spores and subsequent development are inhibited by nisin (not a sporicide).	Cooking food does not guarantee the elimination of spores of *B. cereus*, but can be sufficient to deactivate the most heat-sensitive genetic groups, although all strains of *B. cereus* are deactivated by canning. **D* and z** values for spores of *B. cereus*, examples found in the literature** $D_{95°C}$: 2 min (pork) $D_{100°C}$: 1.2-7.5 min (rice) $D_{120°C}$: 3.4 min (soy oil) z: 8-12.5°C **$D_{90°C}$ values for spores of *B. cereus* by genetic group** Group VI: 1.7 min Groups II, V, IV: 20-30 min Groups III, VII: 40-90 min 6 log reductions are obtained, for example, by heat treatment: • at 70°C for 12 s (on vegetative cells in pork); • at 105°C for 36 s (on spores in pork). NB: the spores are less heat-resistant at acid pH values (pH < 4.3). Diarrheal toxins are destroyed by treatment at 56°C applied for 5 min. The emetic toxin is destroyed by treatment at 126°C applied for 90 min. In addition, it has been shown that at neutral pH, treatments at 121°C for 120 min are not sufficient to deactivate cereulide.
Irradiation	**High pressure**
For spores: D_{10}*** = 1.6 – 2.6 kGy	Spores of *B. cereus* are highly resistant to pressure. They can be deactivated by a combination of heat treatment (60-70°C) and high pressures treatment (550-600 MPa).

* D is the time needed to divide by 10 the initial population of a microbiological hazard.

** z is the variation in temperature (°C) corresponding to variation by a factor of 10 of the decimal reduction time.

*** D_{10} is the dose (in kGy) needed to reduce a population to 10% of its initial strength.

Recommendations to operators

- Good hygiene practices and good manufacturing practices are indispensable to prevent the formation of spores. Systematic cleaning is essential to prevent contamination of the equipment used to carry food between the different production stations.
- It is necessary to prevent *B. cereus* from attaining a level that would be dangerous to consumers, which, depending on the growth capacity of *B. cereus* in the food, may mean monitoring the number of spores (and vegetative forms) present in the raw materials, at the end of production and/or during the product's shelf-life, as well as in the equipment used for production.
- Especially in mass catering, it is important to cool cooked foods rapidly to prevent spores from germinating and vegetative cells from multiplying. Foods that are not consumed immediately after preparation and in which *B. cereus* might be present and develop must be kept at temperatures above 63°C or either deep-frozen to prevent its growth or refrigerated to slow it. In the latter case, the shelf life should be determined as a function of the growth rate of *B. cereus* in the food, based on reasonably predictable refrigeration temperatures.

Domestic hygiene

Recommendations to consumers

- Washing vegetables to remove soil, and cleaning surfaces, refrigerators and kitchen tools reduce but do not eliminate contamination of foods by spores of *B. cereus*.
- Cooked foods or rehydrated dried foods that are not consumed immediately must be cooled rapidly and placed in a refrigerator. Foods with a high water content (prepared meals, mixed salads, etc.) that have been kept for several hours at room temperature (e.g. picnic leftovers) must be thrown away and not consumed, even after reheating.

References and links

General references

- Opinion of the French Food Safety Agency on a request for an additional opinion on the references applicable to foodstuffs as process hygiene criteria. 10 April 2010.
- Dromigny, 2008. *Bacillus cereus*. Collection "Monographies de microbiologie". Editions Lavoisier Paris.
- EFSA 2005. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. The EFSA Journal, 175, 1-48.
- Guinebretière *et al*. 2008. Ecological diversification in the *Bacillus cereus* Group. Environ. Microbiol. 10, 851-865.
- *Les toxi-infections alimentaires collectives en France entre 2006 et 2008*. [Food poisoning outbreaks in France between 2006 and 2008] BEH No.31-32. 27 July 2010.

Useful links

- ANSES: www.anses.fr
- EFSA: www.efsa.europa.eu
- French Institute for Public Health Surveillance (InVS): http://www.invs.sante.fr/surveillance/tiac