



Bacillus cereus in Milk and Dairy Products

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The genus *Bacillus* is the largest genus within the family *Bacillaceae*, presently consisting of at least 226 species most of which are saprophytes widely distributed in the environment, and commonly isolated from soil, air, water, plants and animals.

The majority of *Bacillus* species are commensal and have rarely been associated with diseases in humans or animals; some species are used as probiotics or additives and others have been identified as the cause of food spoilage. The principal exceptions to this are the members of the *B. cereus* group that contain different species of key medical importance, and are the focus of this fact sheet. It should be noted that *B. anthracis*, an animal pathogen that is not associated with dairy products, is excluded from this overview.

Bacteriological characteristics

Bacillus cereus sensu lato (or *B. cereus* group) consists of eight formally recognized species: *B. anthracis*, *B. pseudomycooides*, *B. mycooides*, *B. thuringiensis*, *B. weihenstephanensis*, *B. cytotoxicus*, *B. toyonensis* and *B. cereus sensu stricto* (the *B. cereus* species). Most of these species are very difficult to distinguish, even with 16S rDNA sequencing. Many publications that refer to *B. cereus* actually refer to the *B. cereus* group.

The *B. cereus* group represents spore forming Gram-positive bacteria that are optionally motile and facultatively anaerobic saprophytes. Some are food pathogens and can cause both diarrheal and emetic human gastrointestinal syndromes. If present in raw milk, their spores can survive the pasteurization process and contaminate product where they have the following growth characteristics:

- Growth temperature: optimally at 30-37°C but also at $\geq 4^{\circ}\text{C}$ and $\leq 50^{\circ}\text{C}$, depending on the genetic group.
- pH range: growth at a wide pH range from 4.3 to 9.3.
- Water activity range: growth within the range 0.912 to 0.995

Some species of this group appear to be non-pathogenic: *B. thuringiensis* is a well-known insect pathogen used as a biocontrol agent. *B. weihenstephanensis*, *B. pseudomycooides*, *B. mycooides* have not been described as food poisoning agents. *B. toyonensis* is used as a feed additive.

The origin of contamination

The *B. cereus* group are ubiquitous and abundant as spores in the soil. They are also found in large quantities in silage, feces and litter. *B. cereus* group are not responsible for zoonosis but due to their high presence in the environment, they can be carried by animals, including cows. They can contaminate raw milk by simple transfer during milking, when hygiene conditions are not fully observed, and some strains of the species may cause mastitis in very rare cases.

Pasteurization induces sporulation of *B. cereus* group species, the spores can subsequently survive the pasteurization process and, therefore, contaminate dairy products, causing quality and safety issues.

B. cereus sensu stricto has been identified in virtually all categories of foods. However, the main foods involved in food poisoning are heat-treated food, mainly food based on rice and pasta for the emetic syndrome and pasta for diarrheal syndrome.

Frequency and severity of illness

Bacillus cereus is the cause of two kinds of foodborne disease, an emetic intoxication and a diarrheal infection. There is epidemiological evidence that a high proportion of strains of *B. cereus sensu stricto*, *B. cytotoxicus* and a few strains of other *Bacillus* spp. can cause foodborne illness. However, many strains among these species are presumably not foodborne pathogens. Very little is known about the virulence mechanisms of other *Bacillus* spp. Foodborne illness caused by other *Bacillus* spp. has always been linked to high numbers of cells/spores in the food (equal or more of 6 log cfu per g).

Few data are available on the contamination of milk and dairy products by *B. cereus sensu stricto*. The available data indicates the presence of vegetative cells and spores, up to 10^2 - 10^3 spores / L raw milk.

Spores are also found in the finished products like pasteurized milk, as well as ultra-high temperature (UHT) processed milk powder (10^2 to 10^3 spores / g) and cheeses such as Cheddar (10^2 - 10^3 cfu/ g).

The emetics syndrome is caused by the emetic toxin or cereulid, which is highly resistant to temperature (90 min at 126 °C) and highly stable (at least two months at 4° C), pH resistant ($2 \leq \text{pH} \leq 11$) and resistant to proteolytic enzymes. It is pre-formed in the food and therefore will not be destroyed either by cooking or by digestion. The dose-response of the emetic toxin is 8 mg per kg of body weight. If a food contains between 10^5 and 10^6 cells of *B. cereus sensu stricto*, it is considered as potentially dangerous.

The diarrheal syndrome is caused by three enterotoxins; HBL, Nhe and/or CYTK formed in the intestine by a high number of cells or spores ingested. The enterotoxins are heat-labile and sensitive to acid conditions or proteolysis. They are destroyed during cooking or gastro-intestinal digestion. Food containing at least 10^3 - 10^5 cfu⁻¹ is considered to provide too many cells which will not be destroyed by digestion, but instead colonize the gut of the host and produce sufficient enterotoxin to cause disease.

Foodborne illness caused by *B. cereus sensu stricto* is rarely fatal, although cases have been attributed to *B. cytotoxicus* (Guinebretière et al., 2013) and to the emetic type of *B. cereus sensu stricto* (Dierick et al., 2005; Naranjo et al., 2011).

Detection methods

Methods to enumerate and detect *B. cereus* group are available (ISO 7932, ISO 21871), but it is important to be aware that they give no indication of the ability of the bacteria to produce toxins, and are inadequate for the differentiation of *B. cereus sensu stricto* from closely related *B. cereus* group species that have not been associated with illness. To distinguish some of the species, there are genetic methods that can be used based on the presence of certain genes (*B. anthracis*) or genetic signatures (*B. weihenstephanensis*). Additionally, visual or microscopic observations can be used for *B. thuringiensis*, *B. mycoides*, and *B. pseudomycoides*, or maximum growth temperature for *B. cytotoxicus*.

Methods for detection of toxins and strain characterization for the production of toxins exist but are not standardised. They are not used routinely by industry

Regulation

Some regulatory authorities have set *B. cereus* group food safety limits of 10^3 cfu/g in dairy products for the general population and 10^2 cfu/g in infant formula. Alternatively, many countries have chosen not to impose *B. cereus* group standards for dairy products. Where standards exist, they tend to be restricted to products that are destined for consumption by infants and 'at risk' consumers.

An amendment to European Regulation No 2073/2005 dated 2007 (Corrigendum No. 1441/2007) includes *B. cereus* group as a microbiological process hygiene criterion for powdered formulas and dried dietary foods for infants less than 6 months ($n=5$; $c=1$; $m=50$ cfu/g; $M=500$ cfu/g).

In Europe, there are no specific regulations concerning *B. cereus* group in other milk and dairy products. However, dairy companies manufacturing powder for infant formula generally have specifications of less than 100 cfu/g of *B. cereus* group strains. In order to meet this requirement, the corresponding specification for raw milk should be as low as 10 cfu/ml for *B. cereus* group. Methodology to determine this low number in a timely manner is lacking.

Prevention methods

B. cereus group organisms are present in most raw materials in the form of spores. Adoption of measures to minimise contamination of food from the very outset, even before processing is the best way to avoid the growth of *B. cereus* group organisms to dangerous levels (10^3 to 10^5 cfu/g).

At milk production level

Good milking hygiene and equipment maintenance (milking, milk storage) protocols are essential to reduce contamination of raw milk at milking. Strict compliance with the hygiene rules will control the risk of contamination despite the large presence of the bacteria in the environment.

At the manufacturing level

B. cereus group spores originating from contaminated udders and/or milking equipment not alone transfer readily into milk used for manufacture of dairy products, but will also survive pasteurization due to their heat resistance. Consequently, raw milk handling and dairy processing conditions (temperature, time) must be managed to control growth of *B. cereus* group organisms and prevent them from reaching unsafe levels that could permit toxin formation. This is achieved by ensuring a low level of contamination at the farm and reducing any opportunity for growth during processing or storage of products prior to consumption.

The major control measures are to control temperature and to establish an HACCP system. Only heat treatments used for canning of low-acid foods will ensure a complete destruction of spores of *B. cereus*. For ultra-high temperature (UHT) milk, heating at 140°C for one to two seconds will inactivate most spores. The number of spores in processed foods other than canned foods and UHT milk must be kept as low as possible by proper cleaning and disinfection of equipment. Rapid cooling is necessary to prevent germination and growth of *B. cereus* spores. Low pH (below 4.5), reduction in aw (below 0.92) would inhibit *B. cereus*. In other cases, refrigeration below 4°C is necessary to prevent growth of all types of *B. cereus*, including psychrotrophic strains. However, between 4 and 10°C, the lag and generation times are very significantly increased, particularly if other factors (i.e. pH, aw or nutrient content of the food) are not optimum for *B. cereus*. This should be verified by microbiological testing. Control measures adopted for *B. cereus* are also applicable for the control of other *B. cereus* group species. (EFSA, 2005).

Growth of *B. cereus* group organisms present in raw milk can arise during processing because of growth and biofilm formation on the surface of processing equipment. Biofilms containing viable spores can be particularly difficult to remove from process surfaces between production runs. The vegetation of surviving spores can thus lead to further cell propagation and contamination of dairy products; for example, increased contamination of pasteurized and spray dried milks may be due to *B. cereus* group strains persisting in pasteurizing and drying equipment.

Product Formulation and Control

Food processing innovations, i.e. changing an ingredient used to manufacture a product, changing a product formulation or changing a manufacturing step, may escalate food safety risks posed by *B. cereus* group organisms present in traditional products. For example, formulation modifications that reduce/remove 'hurdles' to microbiological growth in traditional foods (e.g. changing the pH, moisture and/or salt level) could potentially favour growth of *B. cereus* group species to the point of forming toxins. As such, caution should be used when introducing formulation changes to products.

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