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Review Article

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# *Bacillus cereus* Characteristics, Virulence Factors Profiles and Toxin Production

Aya R. Mohammed, Esmat I. El-Said, Salah F. Abd ElAal, Rania M. Kamal*

Department of Food Control, Zagazig University, Zagazig City, 44511, Sharkia Governorate, Egypt.

***Correspondence**
Corresponding author: Rania M. Kamal
E-mail address: raniakamal79@gmail.com

## Abstract

*Bacillus cereus* is a Gram positive, facultative anaerobic bacterium characterized by large rod- shaped cells and an ability to form heat-resistant endospores. Because this bacterium is so widely distributed throughout nature and is frequently found in soil, it is naturally present in a wide variety of food products of both animal and plant origin. The presence of *B. cereus* and its virulence factors in dairy products may result in food poisoning and other illnesses. *B. cereus* causes two types of intestinal illness: emetic (vomiting) and diarrheal. Furthermore, the bacterium causes a variety of systemic and local infections in both immunosuppressed and immunocompetent persons. Different toxins and pathogenic factors like nonhemolytic enterotoxin Nhe, hemolytic enterotoxin Hbl, enterotoxin FM and cytotoxin K are accountable for diarrheal syndrome, meanwhile the depsipeptide cereulide toxin causes emetic syndrome. Because of its genetic similarity to *Bacillus* anthracis and *Bacillus* thuringiensis, the ability to detect this pathogen in food is frequently difficult. We reviewed characters, virulent attributes, toxins profiles of this bacterium. Current control methods are limited so future control strategies must be developed.

KEYWORDS
*B. cereus*, Cereulide, Emetic syndrome, Diarrheal toxins.

## INTRODUCTION

One of the most challenging public concerns is food safety. Food pathogens and their toxins are responsible for a variety of food-related health threats that cause disease and death worldwide (Assefa, 2019). *Bacillus cereus* (*B. cereus*) is an example of these foodborne pathogens (Soleimani *et al*., 2018). This species is commonly known for food pathogenicity in the *Bacillus* genus (Soleimani *et al*., 2018).

*Bacillus cereus* is a Gram-positive, rod shape, motile, spore-forming bacterium that related to the genus *Bacillus* (Montville and Matthews, 2005). Under adverse environmental conditions, the microorganism produces oval endospores that can survive for extended periods of time, even in extreme conditions (Delbrassinne *et al*., 2012). Because of the bacterial ability to grow at temperatures ranging from 4°C to 50°C and resist heat and chemicals, this pathogen is popularly found in soil, air, grains, rice (row and cooked), vegetables, meat, and milk (Setlow, 2006).

### Taxonomy and characteristics of *B. cereus*

The *B. cereus* group species complex, also known as *B. cereus* sensu lato (s.l.), is a subgroup of strongly related *Bacillus* species. Members of this group are Gram-positive, spore-forming, and widely spread throughout the environment (Carroll *et al*., 2020). This *B. cereus* group includes at least twelve species that are closely related: *B. cereus*, *B. thuringiensis, B. anthracis, B. mycoides, B. pseudomycoides, B. cytotoxicus, B. weihenstephanensis, B. wiedmanni, B. toyonensis,* and the recently identified, *B. albus, B. tropicus, B. paranthracis, B. pacificus, B. mobilis, B. Luti, B. proteolyticus, B. nitratireducens, B. paramycoides, B. gaemokensis, B. bingmayongensis, B. manliponensis and B. fungorum* (Bianco *et al*., 2021).

*Bacillus cereus* strains can grow in a moderately wide temperature range; most lineages seem to be mesophilic (with an optimal growth temperature of 37°C and survival below 10°C), while psychotropic strains (with an optimal growth temperature below 10°C and slow growth at 37°C) have also been described, this allows them to be easily isolated from foods even when refrigerated. Some lineages are linked to milk and dairy production environments, including psychrotolerant strains (Guinebretiere *et al*., 2008).

### Ecology of *B. cereus*

Because *B. cereus* is so ubiquitous, its presence in most raw foods is considered inescapable. The original source of *B. cereus* spore contamination in food is soil. Similar genotypic strains, for example, have been found in milk and dairy farm soil (Christiansson *et al*., 1999). The soil is *B. cereus*' principal environmental reservoir, where it can finish its saprophytic life cycle (Vilain *et al*., 2006). When conditions are favourable, *Bacillus cereus* spores germinate in soil, grow on decomposing organic matter in the multicellular filamentous phenotype for translocation through the soil, and eventually resporulate when nutrients are diminished, and conditions are no longer favourable.


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## Contamination routes

*Bacillus cereus* can easily contaminate a variety of foods and it is substantially impossible to obtain raw materials free from *B. cereus* spores (Johnson, 1984; Te Giffel *et al*., 1996). concerning food products, this microorganism has been obtained from the air in a cow shed (Ehling-Schulz *et al*., 2019), rice, spices, milk and dairy products, vegetables, meat, starchy foods, desserts, and cakes (Ghelardi *et al*., 2002). This organism is a significant issue for the food industry because it is difficult to be removed from food products due to its ability to tolerate a wide range of circumstances despite environmental stresses that would normally restrict bacterial survival (Andersson *et al*., 1995). The milk production system is highly complicated, with dead ends, pockets, and traps that have difficulty in cleaning process and can serve as a constant reservoir for spoilage microorganisms (e.g., valves, shafts, and gaskets). Milking equipment can also be a source of contamination because spores can attach and germinate in dairy equipment (for example, pasteurizers, tanks, and packaging machines), resulting in post-treatment contamination of milk (Te Giffel *et al*., 1996; Svensson *et al*., 2000; Eneroth *et al*., 2001). Milk contamination by *Bacillus cereus* group members is critical not only because of their ability to spoil milk, but also because of their potency to cause diseases in humans. (Andersen Borge *et al*., 2001; Janštová *et al*., 2006).

## *Bacillus cereus* Culture and Isolation

The agar plate-based counting is the conventional method for *B. cereus* detection, which is guided by ISO 7932:2004, and was last revised nearly 15 years ago. For each dilution, the plating steps must be duplicated using certain media. Then the plates must be incubated at 30°C for 24 to 48 hours. The selective media mannitol yolk poly-myxin B agar (MYPA) and polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA) is frequently used for its selective isolation and count from food products. *B. cereus* grows fast on these media (within just 24 hours) forming colonies surrounded by an opaque halo caused due to the ability of the microorganism's lecithinases to degrade the lecithin present in the egg yolk. On PEMBA medium, Colonies are crenated, 5mm diameter, turquoise to peacock blue with a zone of egg yolk precipitation after 18-24 h incubation. *B. cereus* colonies on MYP medium have a pink-purple color, surrounded by a distinctive halo formed of pink precipitation, allowing their identification. Moreover, when assessing *B. cereus* counts, the presence of vegetative cells, spores, or a mixture of both forms should be considered. A thermal treatment (80°C for 10 minutes) is performed to selectively detect spores (Reyes *et al*., 2007; Mohamed *et al*., 2016; Spanu *et al*., 2016) This step produces a dual effect, inactivating vegetative cells and activating spore germination.

Recently, alternative approaches for enumerating presumptive *B. cereus* in food based on the NF EN ISO 7932 standard have been confirmed. They use a selective chromogenic medium, COMPASS and BACARA respectively. *B. cereus* colonies appear in green (COMPASS) or orange surrounded by an opaque halo (BACARA) after hydrolysis of the chromogenic substrate. They have comparable specificity, selectivity, and accuracy to the standard reference. Furthermore, they save time and do not require a confirmation action.

## Toxins and Virulence factors

Consuming food contaminated by *B. cereus* may lead to gastrointestinal diseases and non- gastrointestinal diseases including respiratory tract infections, bacteremia, endocarditis and central nervous system infections (Fricker *et al*., 2007).The diarrheal disease characterized by abdominal pain and non-bloody diarrhea that occur 4–16 h after eating. These symptoms are caused by the bacterial production of diarrheal toxins hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe) (Ehling-Schulz *et al*., 2006b) and cytotoxin CytK (Fagerlund *et al*., 2004) in the human small intestine. All three enterotoxins are cytotoxic and cell membrane pore- forming toxins (Ramarao and Sanchis, 2013: Kumari and Sarkar, 2016). Hbl and Nhe each consist of three different protein components, named L2, L1, and B, and NheA, NheB and NheC, respectively, while CytK is a single-component toxin (Stenfors Arnesen *et al*. 2008; Fagerlund *et al*. 2010). Emetic disease, caused by a thermo- and acidic-stable non-ribosomal peptide called cereulide (emetic toxin), and can occur within 0.5-5 hours of ingesting food that has been contaminated then the symptoms appear as an acute attack of nausea, vomiting, and copious abdominal cramping (Ehling- Schulz *et al*., 2005; 2015).

*Bacillus cereus* not only produces toxins but also releases several compounds (cytotoxic factors, degradation enzymes, proteases, hemolysins, cell-surface proteins) that may play a role in its virulence (Ramarao and Sanchis, 2013; Guillemet *et al*., 2016). For example, InhA1 and NprA are synthesized metalloproteases that disturb the body immune defense system and contribute to *B. cereus* pathogenicity (Charlton *et al*., 1999; Ramarao and Lereclus, 2005; Guillemet *et al*., 2010; Haydar *et al*., 2018). Enterotoxin T (BcET) and enterotoxin FM (EntFM) are other enterotoxins that believed to play a role in foodborne illness. The pore-forming toxin Hemolysin II (HlyII) stimulates macrophage apoptosis, enabling *B. cereus* to evade immune defense of the host (Tran *et al*., 2010; 2011). Further virulence factors including sphingomyelinase and exoproteases have been reported to support the pathogenicity of *B. cereus* (Doll *et al*., 2013; Jeßberger *et al*., 2015).

*Cereulide*

Melling *et al*. made the initial suggestion in 1976 that emetic syndrome is caused by a different toxin. They suggested this by feeding Rhesus monkeys two distinct isolated strains, one from a diarrheal outbreak and the other from a vomiting outbreak. The first strain resulted in fluid retention, whereas the second did not. This indicated that vomiting symptoms are caused by a distinctive toxin (Melling *et al*., 1976). Then, (Agata *et al*., 1995) identified cereulide as the emetic toxin factor.

Cereulide is a toxic cyclic dodecadepsipeptide (1.2 kDa) produced enzymatically by a large protein complex in a process called non ribosomal protein synthesis (NRPS) (Horwood *et al*., 2004).The cyclic and lipophilic dodecadepsipeptide containing three repetitions of four amino acids, D-Oxy- Leu—DAla—L-Oxy-Val—L-Val, which resembles valinomycin, a well-known antibiotic formed by Streptomyces with the sequence D-Oxy-Hyi—D-Val—L-Oxy-Lac—L-Val (Agata *et al*., 1994). The ces gene locus, which is located on a 270-kb megaplasmid known as pCER270 (or pCERE01) has coding for the enzymes concerned with cereulide synthesis. This plasmid is significantly linked to the toxin plasmid pX01 from *Bacillus* anthracis (Ehling-Schulz, *et al*., 2006a). CesA (10 kb) and cesB (8 kb) genes encode the cereulide synthetase. CesA is mainly accountable for the d-Ala-d-O-Leu fragment, while the CesB is necessary for the generation of l-Val-l-O-Val fragment. (Marxen *et al*., 2015).

Cereulide, which is produced by emetic *B. cereus* strains, can be found in foodstuffs, creating a significant risk of food poisoning. Furthermore, the toxin has been shown to be heat, low pH, and trypsin digestion resistant, allowing passage through

the stomach into the digestive tract. The fact that cereulide can withstand heat treatment at 12°C for 2 hours, roasting, frying, microwave cooking, and pH values ranging from 2 to 10 demonstrates why traditional food processing methods fail to eliminate its presence in consumer foods (Agata *et al*., 2002; Rajkovic *et al*., 2006).

Cereulide formation in the digestive tract is rarely observed, if it occurs at all. Intoxication is usually caused by the ingestion of toxin preformed in food by ces-positive *B. cereus* strains prior to consumption (Ehling-Schulz *et al*., 2015).

*Enterotoxins*

Diarrhea is caused by at least five *B. cereus* enterotoxins. Two of them are protein complexes (hemolysin BL and non-hemolytic enterotoxins). The other three (enterotoxin FM, enterotoxin T, and cytotoxin K) are single protein substances (Beecher *et al*., 1995; Lund *et al*., 2000). Diarrhea occurs after enterotoxins are produced in the intestine where invasion and outgrowth of cells and/or most likely spores occur after ingesting contaminated foods (Ceuppens *et al*., 2012).

*Hemolysin BL*

Hbl is an enterotoxin developed by diarrheal *B. cereus* strains. It is a three-partite protein consisting of a binding protein B encoded by the hblA gene and two lytic components L1 and L2 encoded by the hblC and hblD genes, respectively. The first enterotoxin recognized in *B. cereus* was Hbl (Beecher and Wong, 1994). All three components are required for Hbl's biological activity, additionally, Hbl must be combined with Nhe to be biologically active (Sastalla *et al*., 2013). The Hbl potential was comparable to that of cholera toxin, thus supposing that it could be regarded as a principal virulence factor in diarrheal cases caused by *B. cereus* (Beecher *et al*., 1995).

*Non-hemolytic enterotoxin (Nhe)*

The three-component enterotoxin Nhe was discovered for the first time in a *B. cereus* strain that was associated with a large food poisoning outbreak in Norway (Lund and Granum, 1996). This three-component pore-forming toxin is made up of the proteins NheA (41.0 kDa), NheB (39.8 kDa), and NheC (36.5 kDa), which work together to form a pore in the cell membrane and induce cell lysis (Lindback *et al*., 2004). The proteins were distinguishable from Hbl components and exhibited no hemolytic activity. About 92 to 100% of *B. cereus* isolates produce Nhe toxin. In comparison, only 42 strains were able to synthesize the enterotoxin Hbl, revealing the predominance of Nhe in *B. cereus* strains, which is found more frequently than Hbl production (Schoeni and Wong, 2005; Moravek *et al*., 2006). This demonstrates that Nhe serves as the most predominating toxin in diarrheal food poisoning, proving that *B. cereus* cytotoxicity is strongly related to the concentration of Nhe and weakly related to Hbl concentration (Fagerlund *et al*, 2008).

*Cytotoxin K*

CytK was the first toxin proved to be the only cytotoxic, necrotic, and hemolytic toxin of the *B. cereus* strain NVH391/98, which was implicated in a serious food poisoning outbreak in France in 1998. When six people suffered from bloody diarrhea, and three died, during this time, none of the known *B. cereus* enterotoxins could be discovered, and CytK has been shown to be toxic towards Vero cells (Lund *et al*., 2000). This 34-kDa protein was also isolated from fractionated supernatants of an endophthalmitis-associated *B. cereus* isolate in the same year and termed "hemolysin IV". (Beecher *et al*., 2000).

CytK is a single protein, β-barrel pore-forming toxin. There are two variants of CytK that share 89% of identity: CytK-1, and CytK2. CytK1 was identified in 1998 from a strain of *B. cereus* that caused a large foodborne outbreak (FBO) in France and that conducted to the death of three elderly people (Lund *et al*., 2000). Although CytK2 is five times less toxic than CytK-1, it seems more frequently associated with strains causing FBO (Ramarao and Sanchis, 2013).

## Applicable Control Strategies of *B. cereus*

Recent research has shown that high hydrostatic pressures (HHP) could be effective in inactivating bacterial spores when applied in combination with moderate temperature heat treatments (>60°C) (Soni *et al*., 2016). According to (Schneider *et al*., 2017), and based on the National Institutes of Health (NIH), the National Food Processors Association (NFPA), and the FDA Food Code 2013, the following strategies are helpful for destroying *B. cereus*. Both vegetative cells and spores can be destructed by steaming food at ≥145°F (63°C) under pressure, roasting, frying, and grilling. Cooking at 145°F (63°C) and reheating at 165°F (74°C) for 15 seconds kills the vegetative cells. However, if the toxin was produced in food, it is hazardous to consume. Therefore, the best way to prevent spore germination is to cool foods quickly after heating them.

## CONCLUSION

This organism is the primary cause of the majority of food-related diseases because of the high prevalence of *B. cereus* food contamination and active production and secretion of HBL, Nhe, and CytK enterotoxins. *B. cereus*' ability to form highly resistant spores, permitting it to spread in a wide range of environments, is what makes it a successful food pathogen. As a result, *B. cereus* presents in a broad range of foodstuffs, posing a serious food safety risk. Current FDA and other regulatory agencies control practices are inadequate for removing *B. cereus* from food. New techniques for controlling or eliminating *B. cereus* from food matrices are needed.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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