

MiniReview

Bacillus cereus and its food poisoning toxins

Per Einar Granum *, Terje Lund

Department of Pharmacology, Microbiology and Food Hygiene, Norwegian College of Veterinary Medicine, P.O. Box 8146 Dep., N-0033 Oslo, Norway

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Abstract

Bacillus cereus is becoming one of the more important causes of food poisoning in the industrialised world. It produces one emetic toxin and three different enterotoxins. The emetic toxin is a ring-shaped structure of three repeats of four amino and/or oxy acids: [D-O-Leu-D-Ala-L-O-Val-L-Val]₃. This ring structure has a molecular mass of 1.2 kDa, and is chemically closely related to the potassium ionophore valinomycin. Two of the three enterotoxins have been shown to be involved in food poisoning. They both consist of three different proteins that act together. One of these enterotoxins is also a haemolysin. This haemolytic enterotoxin is transcribed from one operon. The third enterotoxin is a single component protein, but has not been shown to be involved in food poisoning.

Keywords: *Bacillus cereus*; Food poisoning

1. Introduction

Bacillus cereus is a Gram-positive, spore-forming, motile, aerobic rod that also grows well anaerobically. *B. cereus* causes two different types of food poisoning: the diarrhoeal type and the emetic type. The diarrhoeal type of food poisoning is caused by complex enterotoxins [1,2], produced during vegetative growth of *B. cereus* in the small intestine [3], while the emetic toxin is produced by growing cells in the food [4]. For both types of food poisoning the food involved has usually been heat-treated, and surviving spores are the source of the food poisoning. *B. cereus* is not a competitive microorganism, but grows well after cooking and cooling

(<48°C). The heat treatment will cause spore germination, and in the absence of competing flora, *B. cereus* grows well. *B. cereus* is a common soil saprophyte and is easily spread to many types of foods, especially of plant origin, but is also frequently isolated from meat, eggs and dairy products [4]. The development of psychrotrophic strains in the dairy industry has led to increasing surveillance of *B. cereus* in recent years [5]. *B. cereus* food poisoning is underreported as both types of illness are relatively mild and usually last for less than 24 h [4,5]. However, occasional reports have described a more severe form of the diarrhoeal type of *B. cereus* food poisoning [5].

The closely related *B. thuringiensis* is reported to produce enterotoxins [6,7], and has been shown to cause food poisoning when given to human volunteers [7]. This may develop into a serious problem, as

* Corresponding author. Tel.: +47 22 96 48 45; Fax: +47 22 96 48 50; E-mail: Per.E.Granum@veths.no

spraying of this organism to protect crops against insect attacks has become common in several countries. *B. thuringiensis* has recently been reported to cause an outbreak of food poisoning [6]. However, since the normally used procedures for confirmation of *B. cereus* would not differentiate between the two organisms, undetected outbreaks may have occurred. To assure safe spraying with *B. thuringiensis*, the organism in use should be unable to produce food poisoning toxins.

2. Foodborne outbreaks

The dominating type of disease caused by *B. cereus* differs from country to country. In Japan the emetic type is reported about 10 times more frequently than the diarrhoeal type [4], while in Europe and North America the diarrhoeal type is the most frequently reported [4]. Since *B. cereus* food poisoning is not a reportable disease in any country, there are very few figures given of the total number of these kinds of food poisoning. Even if the two syndromes were reportable one would expect dramatic underreporting, since few seek medical help during the active phase of the disease, and the patients recover quickly thereafter. Only a few countries in Europe have published convincing data in recent years. *B. cereus* is the cause of 33% of the total cases of food poisoning (excluding virus) in Norway (1988–1993) [3], 47% in Iceland (1985–1992), 22% in Finland (1992), 8.5% in The Netherlands (1991), and 5% in Denmark (1990–92) [8]. Much lower numbers have been reported before from other countries,

such as England and Wales (0.7%), Japan (0.8%), USA (1.3%) and Canada (2.2%) [4].

3. Characteristics of disease

There are two types of *B. cereus* food poisoning. The first type, caused by an emetic toxin, results in vomiting, while the second type, caused by enterotoxins, gives diarrhoea [4]. In a small number of cases both types of symptoms are recorded [4], probably due to production of both types of toxins. There has been some debate about whether or not the enterotoxin(s) can be preformed in foods, and cause an intoxication. Reviewing the literature it is obvious that the incubation time is a little too long for that (> 6 h; average 12 h) [4], and in model experiments it has been shown that the enterotoxin(s) is degraded on its way to the ileum [3]. Although the enterotoxin(s) can be preformed, the number of *B. cereus* cells in the food would be at least two orders of magnitude higher than that necessary for causing food poisoning [5], and such products would no longer be acceptable to the consumer. The characteristics of the two types of *B. cereus* food poisoning are given in Table 1.

4. Infectious dose

Counts ranging from 200 to 10^9 g⁻¹ (or ml⁻¹) *B. cereus* [5] have been reported in the incriminated foods after food poisoning, giving total infective doses ranging from about 5×10^4 to 10^{11} . Partly

Table 1
Characteristics of the two types of disease caused by *Bacillus cereus*^a

	Diarrhoeal syndrome	Emetic syndrome
Infective dose	10^5 – 10^7 (total)	10^5 – 10^8 (cells g ⁻¹)
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein	Cyclic peptide
Incubation period	8–16 h (occasionally > 24 h)	0.5–5 h
Duration of illness	12–24 h (occasionally several days)	6–24 h
Symptoms	Abdominal pain, watery diarrhoea and occasionally nausea	Nausea, vomiting and malaise (sometimes followed by diarrhoea, due to additional enterotoxin production?)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/ sauces and milk/milk products	Fried and cooked rice, pasta, pastry and noodles

^aBased on [3,4,9].

Table 2
Characteristics of the three enterotoxins from *B. cereus*^a

	Enterotoxin HBL	Enterotoxin NHE	Enterotoxin T
Number of components	3	3	1
Size of active component(s)			
L ₂	46 kDa	45 kDa	41 kDa
L ₁	38 kDa	39 kDa	
B	37 kDa	105 kDa	
Haemolytic	Yes	No	No
Toxicity in cell tests	80 ng	70 ng	Not determined
Shown to be involved in food poisoning	Yes	Yes	No
Cloned and sequenced	Yes	No	Yes

^aBased on [2,10,14,16,19,20].

due to the large differences in the amount of enterotoxin produced by different strains [5], the total infective dose seems to vary between about 10⁵ and 10⁸ viable cells or spores. Thus, any food containing more than 10³ *B. cereus* g⁻¹ cannot be considered completely safe for consumption.

5. Virulence factors/mechanisms of pathogenicity

The two types of *B. cereus* food poisoning are caused by very different types of toxins. The emetic toxin, causing vomiting, has recently been isolated and characterised [9], while the diarrhoeal disease is caused by at least three different enterotoxins [1,2,10].

5.1. The emetic toxin

The emetic toxin causes emesis (vomiting) only and its structure has for a long time been a mystery, as the only detection system involved living primates [4]. The recent discovery that the toxin could be detected (vacuolation activity) by the use of HEP-2 cells [11] has led to its isolation and determination of its structure [9]. Although there has been some doubt as to whether the emetic toxin and the vacuolating factor are the same component [4], there is no doubt that it is the same toxin [12,13]. The emetic toxin has been named cereulide, and consists of a ring structure of three repeats of four amino and/or oxy acids: [D-O-Leu-D-Ala-L-O-Val-L-Val]₃. This ring structure (dodecadepsipeptide) has a molecular mass of 1.2 kDa, and is chemically closely related to the potassium ionophore valinomycin [9]. The emetic

toxin is resistant to heat, pH and proteolysis but is not antigenic [4] (Table 1). The biosynthetic pathway and mechanism of action of the emetic toxin still have to be elucidated, although it has recently been shown that it stimulates the vagus afferent through binding to the 5-HT₃ receptor [12]. It is not clear if the toxin is a modified gene product or if it is enzymatically produced through modification of components in the growth medium. However, with such a structure it is most likely that cereulide is an enzymatically synthesised peptide and not a genetic product.

5.2. Enterotoxins

The number of enterotoxins and their properties have also been debated for a long time [4,5], but at least three different enterotoxins have been characterised [10,14–16]. However, there is no evidence that the recently reported enterotoxin T [10] causes food poisoning. The early studies on the enterotoxin [4,5] suggested a single or a multicomponent enterotoxin. Further work has now shown that *B. cereus* produces two different three-component enterotoxins [1,2,14–16]. A three-component haemolysin (HBL; consisting of three proteins: B, L₁ and L₂) with enterotoxin activity has been purified and characterised [1,14,15]. This toxin also has dermonecrotic and vascular permeability activities, and causes fluid accumulation in ligated rabbit ileal loops. HBL has therefore been suggested to be a primary virulence factor in *B. cereus* diarrhoea [15]. Convincing evidence has shown that all three components are necessary for maximal enterotoxin activity [15]. A non-haemolytic three-component enterotoxin (NHE) was recently

characterised by Lund and Granum [16]. The three components of this toxin were different from the components of HBL. The characteristics of the two three-component enterotoxins are given in Table 2 together with the information available on enterotoxin T.

The three components of NHE enterotoxin were first purified from a *B. cereus* strain isolated after a large food poisoning outbreak in Norway in 1995. This strain was used to characterise the NHE mainly because it did not produce the L₂ component [3,16,17]. We expected to show that the L₂ component was unnecessary for biological activity of the HBL enterotoxin, but instead we found another three-component enterotoxin. Binary combination of the components of this enterotoxin possesses some biological activity, but not nearly as high as when all the components are present [2].

Some strains produce both of the three-component enterotoxins, while other strains contain genes for only one of them [2,17]. At present we do not know the distribution of the two enterotoxin complexes among strains, or how important each of them is in relation to food poisoning. However, it seems as if they are both important. The enterotoxin T gene has recently been shown to be absent in 57 of 95 strains of *B. cereus*, and in five out of seven strains involved in food poisoning [17].

It has been suggested, from studies of interactions with erythrocytes, that the B protein is the component that binds HBL to the target cells, and that L₁ and L₂ have lytic functions [18]. Recently another model for the action of HBL was proposed, suggesting that the components of HBL bind to target cells independently and then constitute a membrane-attacking complex resulting in a colloid osmotic lysis mechanism [1]. Studies of interactions between NHE and Vero cells have shown that the 105-kDa protein might be the binding component of that complex [2].

Table 3
N-terminal sequences of the 45-kDa and 39-kDa proteins of the NHE

45-kDa	AQNVIAPNTLS NSIRMLGSQSPLIQAYG
39-kDa	AESTVKQAPVXAVAKAYNDYEE YSLGPEGLK- D AMEXTGSNALVMD

The residues given in bold represent identical residues in L₂ (for the 45-kDa protein) and L₁ (for the 39-kDa protein).

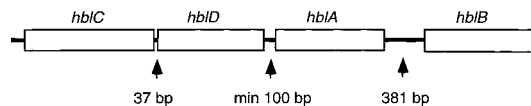


Fig. 1. The map of the *hbl* operon [19,20]. The operon includes the three known proteins of the HBL: L₂ protein encoded by *hblC*, L₁ protein by *hblD*, and B protein by *hblA* and the B' protein by *hblB*, with 73% identity to the B protein in the first 158 amino acids [5].

The two other components are probably not able to bind to these cells alone. It is still not clear if the two different toxin complexes only damage the plasma membranes of the targets cells or if some of the components are translocated to the cytosol and have lytic activity inside the cell.

There is a high degree of identity between the N-terminal part of L₁ and the corresponding part of the 39-kDa protein, and high identity also exists between parts of L₂ and the 45-kDa protein (Table 3). It has also been reported that antibodies against the B protein recognise a protein of about 100 kDa [19]. If this protein is the same as the 105-kDa protein of NHE, parts of the 105-kDa protein may be very similar to parts of the B protein. Together these observations indicate some similarities between HBL and NHE.

5.3. Other toxins

Some reports on other proteins with enterotoxic activity have been published [4], but little is known about them. One enterotoxin protein had a molecular mass of about 57 kDa and another about 100 kDa [4]. The largest could be the 105-kDa component of the NHE.

5.4. Gene organisation of the enterotoxins

All three proteins of the HBL are transcribed from one operon (*hbl*) [19], and Northern blot analysis has shown an RNA transcript of 5.5 kb (Fig. 1). *hblC* (transcribing L₂) and *hblD* (transcribing L₁) are only separated by 37 bp and encode proteins of 447 aa and 384 aa. L₂ has a signal peptide of 32 aa and L₁ a signal peptide of 30 aa. The B protein, transcribed from *hblA*, consists of 375 aa, with a signal peptide of 31 aa [20]. The exact spacing between *hblD* and *hblA* is at least 100 bp (overlapping sequence not

published), but is claimed to be approximately 115 bp [19]. The spacing between *hblA* and *hblB* is 381 bp, and the length of *hblB* is not known [20]. However, based on the length of the Northern blot it is tempting to suggest a similar size to *hblA*. The B and the putative B' protein are very similar in the first 158 aa (the known sequence of B' protein based on the DNA sequence). The function of this putative protein is not yet known, but it is possible that it may substitute for the B protein. We have, however, shown by PCR analysis that not all strains containing the *hbl* operon have *hblB* present with the known sequence. The *hbl* operon is mapped to the unstable part of the *B. cereus* chromosome [21].

The two smallest proteins of the NHE complex are similar to the L₁ and L₂ proteins of HBL, at least in parts of the known sequences (Table 3). We have just sequenced from the beginning of the 45-kDa protein gene into the gene of the 39-kDa protein, showing that the genes are next to each other, probably in one operon. The gene of the 105-kDa protein seems to be somewhat further away, based on PCR analysis using primers constructed from the N-terminal sequences of the proteins of NHE (Table 3) (Granum et al., unpublished work).

5.5. Regulation of enterotoxin production

Very little is yet known about the regulation of enterotoxin transcription. It seems as if maximal enterotoxin activity is found during late exponential or early stationary phase, and indeed it has been shown for the 45-kDa protein of the NHE that it is under regulation of *plcR* [22], a gene first described to regulate *plcA* (phospholipase C) [23]. The binding sequence for this regulatory protein (TATG₈NCATG) is not found upstream of the *hbl* operon.

5.6. Commercial methods for detection of the *Bacillus cereus* toxins

None of the two available commercial immunoassays will quantify the toxicity of the enterotoxins from *B. cereus*. The assay measuring L₂ (Oxoid) will not detect strains containing only the NHE while the other kit, detecting mainly the 45-kDa protein (Tecra), will leave out the HBL complex [5,16]. However, if one or both of the commercial kits re-

acts positively with proteins from *B. cereus* supernatants it is likely that the strain is enterotoxin-positive. If the supernatants are also shown to be cytotoxic they can be regarded as enterotoxin-positive.

6. Conclusions

B. cereus is a normal soil inhabitant and is frequently isolated from foods. Psychrotrophic strains have become an increasing problem for the dairy industry.

B. cereus food poisoning is not a reportable disease and is therefore highly underestimated in official statistics.

B. cereus causes two different types of food poisoning, one emetic (intoxication) due to a preformed small cyclic peptide, and one diarrhoeal (infection) due to enterotoxins.

The minimal infective dose for the diarrhoeal type ranges between 10⁵ and 10⁷ *B. cereus* cells.

At least two different enterotoxins involved in food poisoning have been characterised. Both are three-component proteins, one haemolytic and one non-haemolytic.

The haemolytic enterotoxin is transcribed in one operon, and the same is probably true also for the non-haemolytic enterotoxin. The non-haemolytic enterotoxin is regulated by *plcR*.

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