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Review

Bacillus cereus, the causative agent of an emetic type of food-borne illness

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Bacillus cereus is the causative agent of two distinct forms of gastroenteritic disease connected to food-poisoning. It produces one emesis-causing toxin and three enterotoxins that elicit diarrhea. Due to changing lifestyles and eating habits, *B. cereus* is responsible for an increasing number of food-borne diseases in the industrial world. In the past, most studies concentrated on the diarrhoeal type of food-borne disease, while less attention has been given to the emetic type of the disease. The toxins involved in the diarrhoeal syndrome are well-known and detection methods are commercially available, whereas diagnostic methods for the emetic type of disease have been limited. Only recently, progress has been made in developing identification methods for emetic *B. cereus* and its corresponding toxin. We will summarize the data available for the emetic type of the disease and discuss some new insights in emetic strain characteristics, diagnosis, and toxin synthesis.

Keywords: *Bacillus cereus* / Cereulide / Emetic toxin / Food-poisoning / Review

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Contents

1 Introduction

Bacillus cereus is a facultative anaerobic, spore-forming, motile microorganism that is commonly isolated from food. Increasingly, it is recognized as a causative agent of gastrointestinal as well as nongastrointestinal diseases. Two types of gastrointestinal diseases caused by *B. cereus* can be distinguished: emesis and diarrhea. The diarrhoeal type, caused by heat-labile enterotoxins, is mainly associated with meat products, soups, vegetables, sauces, and milk products, whereas emetic outbreaks, associated with a heat-stable peptide toxin, have mainly been linked to farinaceous foods like rice, noodles, pasta, and pastry [1, 2]. Besides its food-poisoning potential, *B. cereus* has been shown to be responsible for wound and eye infections, as well as systemic infections and periodontitis [3 – 5]. It has been identified as the cause of serious or even life-threatening infections in neutropenic and immunosuppressed patients and premature neonates [6, 7]. There are only a few reports of fatal *B. cereus* bacteremia in adults with acute leukemia, but all patients in these reports demonstrated gastrointestinal symptoms immediately preceding death [8, 9]. Recently, it has been reported that systematic complications of *B. cereus* infection in premature neonates might be at least partly related to enterotoxins [10]. However, further research will be necessary to clarify the role of *B. cereus* toxins in systemic infections.

The emetic syndrome is mainly characterized by vomiting 0.5 – 6 h after ingestion of the contaminated food. In the diarrhoeal syndrome symptoms appear 8 – 16 h after ingestion and include abdominal pain and diarrhea. In general, both types of food-borne illness are relatively mild and usually do last not more than 24 h. Nevertheless, more severe cases have occasionally been reported, including one

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Abbreviations: Hbl, hemolysin BL; **Nhe**, nonhemolytic enterotoxin

Table 1. Properties of emesis-causing toxins from *B. cereus* and *S. aureus*

Characteristics	Cereulide, the emetic toxin from *B. cereus*	*Staphylococcus aureus* enterotoxins (SEs)
Type and size	Depsipeptide, 1.2 kDa	Proteins, 25 – 29 kDa
Synthesis	Nonribosomal	Ribosomal
Mechanism	Ionophore, mitochondrial toxin	Superantigens
Symptoms	Nausea and vomiting; neurotoxic activity?	Nausea and vomiting, abdominal cramps; mitogenic activity
Minimal emetic dose (according to animal studies)	30 µg toxin/kg	100 µg toxin/kg
Incubation period	0.5 – 6 h	
Duration of illness	6 – 24 h	
Production	Preformed in food	
Stability	Heat-stable, acid-stable	
Trypsin digestion	Not digested by trypsin	
Prevalence in foods	Rice, noodles, pasta, pastries	Dairy products, meat products, pasta, pastries

Source: [2, 49, 50, 51, 69, 70, 71, 72]; Ehling-Schulz *et al.*, in press

death after the ingestion of food contaminated with high amounts of emetic toxin and three deaths caused by a necrotic enterotoxin [11, 12]. However, the true incidence of *B. cereus* food-poisoning is unknown for a number of reasons, including misdiagnosis of the illness, which is symptomatically similar to other types of food-poisoning. The symptoms of the diarrhoeal type of the disease mimic those of *Clostridium perfringens* food-poisoning while symptoms of the emetic type parallel those caused by *Staphylococcus aureus* food-borne intoxication (Table 1).

In this article, we will discuss various aspects of the emetic type of food-borne disease caused by *B. cereus* while a more detailed discussion on the diarrhoeal syndrome is provided elsewhere [13, 14].

2 Characteristics of *B. cereus*

B. cereus belongs to the *Bacillus cereus* species group. This group comprises six species, namely *B. anthracis*, *B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis,* and *B. weihenstephanensis* [15 – 18]. While a high degree of diversity concerning the virulence factors is found within this group, a close genetic relationship was observed between all members [19, 20]. It was therefore suggested that the entire group represents a single species.

Enterotoxin production is broadly distributed among the different members of this bacterial group [21, 22] and also found in other *Bacillus* sp. [23], whereas emetic toxin formation has been reported to be restricted to a certain group of *B. cereus* [24]. Sequencing of rDNA genes (16S rDNA, ITS, and partial 23S rDNA) from several strains belonging to the different *B. cereus* group members revealed identical sequences for all emetic *B. cereus* strains; even the degree of polymorphism observed in the rDNA operon was exactly the same for all emetic strains (Ehling-Schulz, unpublished data). It has been reported previously, based on a few emetic toxin-producing *B. cereus* isolates, that they possess similar ribotypes [25]. Those data may indicate a clonal population structure of the emetic lineage.

Several characteristics have been suggested for the differentiation and identification of the *B. cereus* group members (see, *e.g.*, [4, 13]) and many different plating media have been developed. Key diagnostic features of *B. cereus* strains are their ability to provoke hemolysis and to hydrolyze lecithin, but an inability to ferment mannitol. Media most commonly used and also recommended by the IDF (International Dairy Federation) for isolation of *B. cereus* from foods are based on the latter two characteristics. *B. cereus* typically forms peacock blue colonies with a visible zone of egg yolk precipitate due to lecithin hydrolysis on polymyxin-egg yolk mannitol-bromothymol agar (PEMBA) [26] after 24 h at 37°C. However, some (mainly emetic) *B. cereus* strains do not show the typical precipitate and the characteristic blue colony color (Fig. 1A). In addition, emetic strains show only weak hemolysis or no hemolysis at all on Columbia (containing 5% sheep blood) based blood agar plates incubated 24 h at 30°C (Oxoid; Fig. 1B). Mutations in the pleiotropic regulator PlcR might be the reason for these untypical characteristics (Ehling-Schulz, unpublished data). The *plc*R gene encodes a pleiotropic regulator that controls the transcription of several extracellular proteins, including phospholipases, proteases, and hemolysins in *B. cereus* and *B. thuringiensis* [27, 28]. A nonsense mutation in the *plc*R gene has been shown to be responsible for the hemolysis negative phenotype of *B. anthracis* [27]. Recently, it has been reported that distinct mutations in the *plc*R regulator, that have been detected in a few strains belonging to the *B. cereus* group (1%, 4 out of 400 tested

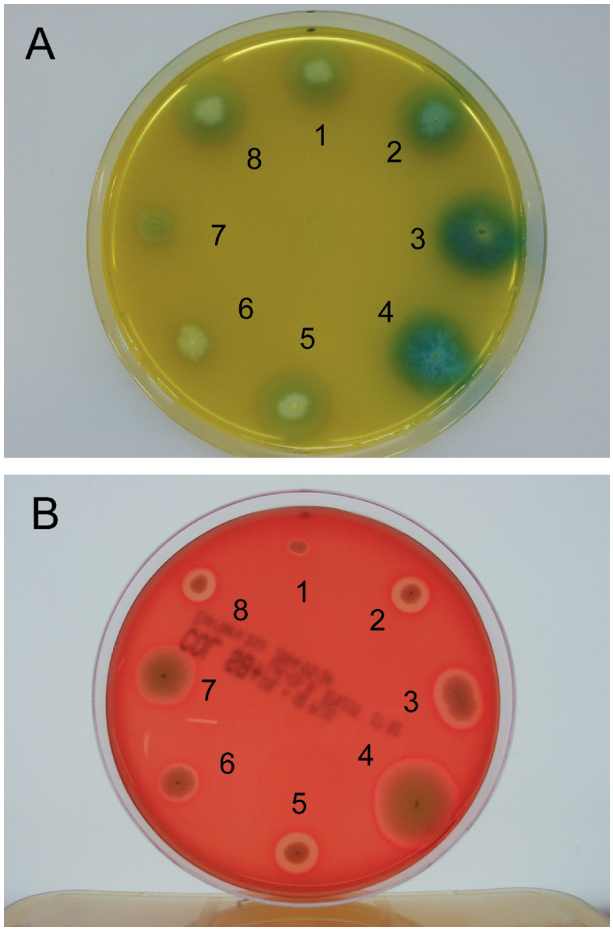

Figure 1. Growth of *B. cereus* strains on selective media. (A) Spot inoculation of selected *B. cereus* isolates on polymyxin-egg yolk mannitol-bromothymol agar (PEMBA [26]); plates were incubated 24 h at 37°C. Isolates 2–4 show the lecithinase reaction and peacock blue colony color typically reported for *B. cereus* on PEMBA, while the emetic isolates 1, 5–8 show an atypical phenotype. (B) Spot inoculation of selected *B. cereus* isolates (same isolate order as in (1A)) on 5% sheep blood agar; plates were incubated 24 h at 30°C. Isolates 2, 3, 5, and 8 show the characteristic clearing zones around the colonies typically found in *B. cereus* due to the activity of hemolysins, while isolates 1, 4, 6, and 7 show a reduced hemolysis or no hemolysis at all. Strain designation: 1, 2, 5, 6, 8, Emetic strains connected to food-poisoning; 7, emetic strain from food environment; 3, nonemetic *B. cereus* type strain ATCC 14579^T; 4, diarrhoeal strain connected to food poisoning.

strains), result in a hemolysis-negative and lecithinase-negative phenotype [29]. Whether emetic toxin producers were present among these strains is not known since the authors did not provide any data on toxin production. Using media for diagnostic purposes that are based on detection of lecithin hydrolysis or hemolysis could lead to substantial misidentifications, and might be one of the reasons for underestimating (especially the emetic type of) food-borne illness caused by *B. cereus*.

All emetic *B. cereus* isolates so far analyzed for starch hydrolysis have been reported to be starch-negative and 70% of the emetic strains belong to the serotype H1 [1, 24, 25]. However, since only 10% of starch-negative strains are emetic and serotype H1 generally is the most common serotype found among *B. cereus*, specific detection methods for the identification of emetic strains are essential.

3 Food-borne illness caused by *B. cereus*

B. cereus was recognized to be the causative agent of food-borne illness in 1950. The diarrhoeal type of illness was described following the consumption of highly contaminated vanilla sauce. Haug isolated *B. cereus*, inoculated sterile vanilla sauce with 10^6 organisms per mL and consumed it. Within 16 h he suffered from abdominal pain, nausea, and watery diarrhea [30]. After Haug's remarkable experiments, *B. cereus* was established as a diarrhoeal disease-causing agent. However, it took about 20 years until *B. cereus* was recognized as an emesis causing staphylococcus type of gastrointestinal disease. In 1971 several incidences associated with *B. cereus* in fried rice were reported in the UK. The illness was characterized by nausea and vomiting 1–6 h after the consumption of fried rice from Chinese restaurants [31].

Bacillus food-poisoning usually occurs because spores survive cooking or pasteurization and then germinate and multiply when food is inadequately refrigerated. The minimal level required to provoke both types of diseases was estimated to be around 10^5–10^8 cfu/g of ingested food (cfu = colony-forming units) [13]. However, there are some reports of emetic syndrome associated with foods containing only 10^3 cfu/g food [2].

The emetic type of food borne disease is often connected to rice and pasta [1, 2]. It frequently occurs if boiled rice is held for prolonged periods at ambient temperatures and then quickly fried before serving, or if precooked noodles are stored at ambient temperature before they are shortly boiled or fried before serving. The traditional Asian cooking involves the precooking of large quantities of rice which are stored at room temperature (RT) to avoid clumping that occurs during refrigeration. Nichols *et al.* [32] analyzed 4162 samples of cooked rice taken from restaurants in the UK and found a significantly higher prevalence of *B. cereus* in precooked stored rice than in point-of-sale cooked rice. Besides the frequently reported incidences caused by emetic toxin-contaminated rice, emetic toxin producers have been isolated from infant formulas, skim milk powders, and other foods [33–35] as well as from environmental sources

like spruce trees [25] and soil (Ehling-Schulz, unpublished).

The trend towards refrigerated processed foods of extended durability (REPFEDs) and the increasing percentage of elderly and immunocompromised people will raise the importance of *B. cereus* as an etiological agent of food-borne illness. Due to the heat and acid resistance of its spores, *B. cereus* is not eliminated by pasteurization or normal sanitation procedures and is therefore a major problem in mass-catering. It was the most common pathogen found in 3% of the samples taken from hot meals served in aircraft from 1991 to 1994 [36] and has been reported to be the main cause of food-borne disease in the mass catering of the German Federal Armed Forces from 1985 to 2000 [37]. In general, the incidence of *B. cereus* food-poisoning is underestimated since *B. cereus* is not a reportable disease and reporting procedures vary among countries. For instance, between 1980 and 1997, 2715 cases of *B. cereus* food-poisoning in England and Wales were reported to the Public Health Laboratory Services (PHLS) and it was the most common microbe isolated from food-borne illness in 1990 in Norway [38]. While in Norway, Finland and Hungary the diarrhoeal type of food-poisoning was predominant, the emetic type was prevalent in the UK, Japan, and the United States [2, 39].

4 Pathogenicity factors

Very different types of toxins are responsible for the two types of gastrointestinal disease caused by *B. cereus*. The emetic syndrome is caused by a single heat-stable peptide toxin, called cereulide, that is, like staphylococcal enterotoxins, preformed in food. On the other hand, different enterotoxins contributing to the diarrhoeal syndrome have been described. The emetic syndrome is presumably the result of intoxication by toxin contaminated food, whereas the diarrhoeal syndrome is thought to be the consequence of a food-borne infection with enterotoxic *B. cereus*.

4.1 Heat-labile enterotoxins causing diarrhea

Diarrhoeal poisoning is caused by heat-labile enterotoxins produced during vegetative growth of *B. cereus* in the small intestine [40]. At present, three different enterotoxins involved in food-poisoning outbreaks have been described: two protein complexes, hemolysin BL (Hbl) [41] and non-hemolytic enterotoxin (Nhe) [42], and the single protein cytotoxin CytK [11]. These toxins are comparatively well-characterized at the molecular level (for review see [13]) and molecular diagnostic assays at an immunological and a PCR level are available (*e.g.*, [21, 43, 44]). In addition, two other proteins, referred to as enterotoxin T and FM [45, 46], have been described. However, these proteins presumably do not contribute to food-borne illness [47]. The enterotoxin T has been shown to be the result of cloning artifact [48] while the role of EntFM is cryptic since no biological studies have been performed on this protein.

4.2 Heat-stable depsipeptide causing emesis

4.2.1 Toxin structure and function

The emetic toxin of *B. cereus*, termed cereulide, is a small cyclic dodecadepsipeptide (Fig. 2) that is chemically closely related to valinomycin produced by *Streptomyces griseus* [49]. Like enterotoxins from *S. aureus* (SEs), cereulide is heat- and acid-stable and toxic at low dose. It shares many characteristics with SEs and both toxins are provoking emesis shortly after ingestion of contaminated food, nevertheless, these toxins are structurally unrelated (Table 1). Cereulide has been shown to be toxic to mitochondria by acting as a potassium ionophore like valinomycin, but its toxic potential is much higher than that reported

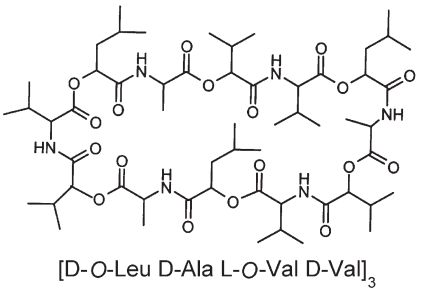

Figure 2. Structure of cereulide (according to [49]).

for the latter ionophore [50 – 52]. From animal feeding tests using house musk shrews it has been suggested that a serotonin 5-HT_3 receptor mediated mechanism is involved in the emetic syndrome [50]. Since it is currently unknown if house musk shrews and humans share a common mechanism of response to emesis provoking toxins, further research will be necessary to elucidate the mechanism of the emetic reaction caused by cereulide. However, cereulide can cause cellular damage in animal models [50, 53, 54] and it was involved in fulminate liver failure in a human case [12]. It has recently been reported that cereulide inhibits human natural killer cells and might therefore have an immunomodulating effect [55].

4.2.2 Emetic toxin expression

The highest amounts of the emetic toxin cereulide have been reported during the beginning of stationary phase growth [56]. In general, the amount of cereulide expressed

Table 2. Emetic toxin production of *B. cereus* in foods and media

Media	Productivity
Skim milk media (10% skim milk)	+++
Rice slurry	++
Brain hearth medium (BHI)	+
Trypticase soy broth (TSB)	+
Plate count broth (PCB)	(+)
Nutrient broth	(+)
Peptone broth	–
Food	
Fried and boiled rice	+++
Milk (aerated)	+++
Noodles and pasta	++
Potatoes	++
Bread	+
Meat pastry	+
Meat	–

Productivity: +++ high; ++ average; + low; – none
Source: [24, 57, 58, 59, 60, 73]; Ehling-Schulz *et al.* (unpublished data)

depends on the incubation temperature and the culture medium (see Table 2), as well as on other extrinsic factors like pH, aeration and the presence of specific amino acids. The highest toxicity titers were observed in 10% skim milk media compared to other culture media like tryptose broth or brain heart infusion broth. No cereulide production was found in peptone broth [57]. The presence of glucose tends to support cereulide production while excessive amounts of leucine, isoleucine, and glutamic acid significantly repress cereulide formation [58]. In food systems, the highest rates have been observed in rice and pasta while toxin production in bread and cakes was low [59, 60]. Negligible amounts of cereulide have been reported from artificially contaminated egg and meat products as well as from nonaerated milk. However, in aerated milk the toxicity titers were even higher than those reported from rice [59]. In contrast to enterotoxins that have been reported to be expressed at refrigerated temperatures, the minimum temperature for cereulide formation was found to be 12°C [56]. While enterotoxin production has been reported from psychrotolerant strains, emetic toxin production is restricted to mesophilic strains with a lower growth limit between 10°C and 15°C ([56]; Ehling-Schulz, unpublished data), which explains why no toxin expression was observed below 10°C. In general, emetic strains show a growth shift to higher temperatures compared to diarrhoeal strains (Ehling-Schulz, unpublished data), but no toxin formation was observed above 40°C [56, 61].

The pathogenicity of emetic *B. cereus* arises from preformed toxin in the food because cereulide is, like the enterotoxins from *S. aureus*, heat-, acid- and trypsin-stable (Table 1) and therefore not degraded in the food or in the gastrointestinal tract. Cereulide will accumulate in food and will not be inactivated or destroyed by reheating of the food [59]. The symptoms are thought to occur from consumption of the preformed toxin. Whether cereulide, like the heat-labile enterotoxins, is produced in the host is still unknown but is probably not the case, because symptoms of the emetic syndrome occur much faster than those observed during the diarrhoeal disease.

The ability for cereulide production varies between different emetic *B. cereus* strains by 3 log units (from 0.02 to 230 $\mu g \cdot mL^{-1}$) [61]. Nevertheless, there is some evidence that the emetic strains belong to one genetic lineage ([25]; Ehling-Schulz *et al.*, submitted). Since no data on the regulation of cereulide production are currently available, it is unknown why emetic strains show such high variations in their toxigenic potential.

4.2.3 Molecular basis of cereulide production

According to its chemical structure (Fig. 1), one could expect cereulide to be synthesized enzymatically by a nonribosomal peptide synthetase (NRPS). Alternating peptide and ester bonds, as well as D-amino acids and a cyclic structure are often found among NRPSs products [62]. NRPSs are large multifunctional proteins that have a modular organization. One module contains all catalytic activities which are necessary for incorporation of one amino acid residue into the peptide product. Within these modules highly conserved core motifs are found, that can be used for a universal PCR approach to identify parts of unknown NRPS [63]. Degenerated primers, targeting such conserved sequences, were successfully applied to emetic *B. cereus* strains to amplify parts of putative NRPS genes. Sequence analysis of one of the amplicons revealed a DNA fragment that was predicted to encode a valine activation NRPS module (Ehling-Schulz *et al.*, in press). Since valine represents one of the four monomers of cereulide, the gene fragment identified was suspected to belong to the genetic locus responsible for cereulide synthesis. Disruption of the corresponding gene by insertion mutagenesis produced cereulide deficient mutants (Ehling-Schulz *et al.*, in press). This is the first time that unequivocal evidence was provided for a nonribosomal assembly of the emetic toxin cereulide.

5 Methods for detection of food-poisoning *B. cereus*

With the increase in reports of food-borne infections, great attention has been given to the development of methods for detection of microbial pathogens. For enterotoxic *B. cereus* strains molecular diagnostic PCR assays have been

described (*e.g.*, [21, 22, 64]) and immunological assays are commercially available (*e.g.*, for detection of the L2 component of Hbl (HblC) the BCET-RPLA *B. cereus* Enterotoxin Test Kit (Oxoid, Basingstoke, UK) and for detection of NheA the Tecra BDE kit (Tecra Diagnostics, Frenchs Forest, Australia)), whereas commercial kits are not yet available for emetic strains.

Three methods for detection of the emetic toxin have been described during the last years: a cytotoxicity assay, LC-MS analysis, and a sperm-based bioassay. The latter biological assay is based on the loss of motility of boar sperm cells upon exposure to the emetic toxin produced by *B. cereus* [65, 66]. Due to its ionophoric nature, cereulide damages mitochondria and blocks the oxidative phosporylation that is required for the motility of boar spermatozoa. Purified cereulide has been shown to produce vacuoles in Hep-2 cells. This observation led to the development of cell culture assays employing HEp-2 cells (see, *e.g.*, [34, 57]). These *in vitro* assays are rather difficult to perform on a routine basis and need one day to one week with precultivation and laborious sample preparations. In addition, these assays do not specifically detect cereulide; *e.g.*, the bioassay is also sensitive to other mitochondrial toxins like gramicidin [65]. Currently, a conclusive identification of cereulide is only provided by LC-MS analysis [61]. However, the latter technique requires laborious sample preparations, costly equipment and specially trained personnel which renders it unattractive for routine diagnosis. Until now, attempts to develop detection systems at an immunological level, as commercially available for *B. cereus* enterotoxins, failed because the antigenic potential of cereulide itself is very low [67].

Quite recently, the first PCR-based assay for the identification of emetic strains has been described [68]. The specificity of the assay was assessed using a panel of 178 bacterial strains; neither false-positive nor false-negative signals were detected, and the general applicability of this primer system was successfully tested in routine laboratories (Dietrich Mäde, Halle, Germany, Jochen Bockemühl, Hamburg, Germany, personal communication). The potential of the developed PCR system for a direct detection of *B. cereus* in foods is currently under investigation.

For the simultaneous screening of multiple samples the slot blot assay presented in Fig. 3, using the DNA probe described by Ehling-Schulz *et al.* [68], provides an interesting alternative to the published PCR assay. In addition, the PCR primers described may contribute to the development of real-time PCR systems and the development of multiplex systems for a one step detection and differentiation of multiple food-poisoning organisms.

The rDNA operon might also be suitable for the development of specific molecular assays for emetic strains.

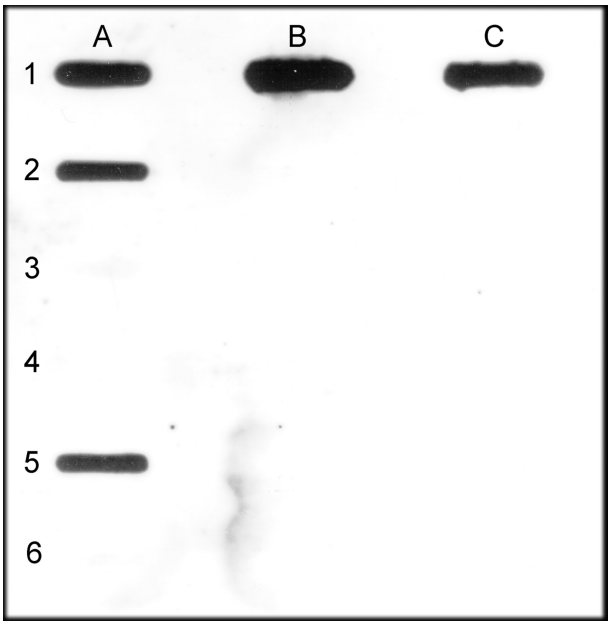

Figure 3. Slot blot analysis of emetic and non emetic *B. cereus* strains. Chromosomal DNA was extracted using the Puregene DNA Purification Gram positive kit (GENTRA, USA), blotted onto nitrocellulose, and hybridized with a digoxigenin-labeled 260 bp probe, which targets an emetic strain-specific chromosomal DNA fragment that has been identified recently [68]. Strain designation: 1A, F4810/72, the emetic reference strain [65]; 2A, emetic isolate from food; 1C, 2A, 5A, emetic strains connected to emetic food-poisoning outbreaks; 2B, 2C, 3A, 3C, 4A–C, 5B, 5C, non emetic strains connected to diarrhoeal food poisoning outbreaks; 3B, 6B, 6C, non emetic isolates from foods.

Sequencing of the ITS region of several emetic strains revealed one nucleotide difference from sequences of non-emetic mesophilic *B. cereus* and *B. anthracis*, and two nucleotides from *B. thuringiensis*, and 11 nucleotides from psychrotolerant *B. weihenstephanensis* and *B. mycoides*, respectively (Fig. 4). Emetic strains showed a heterologous base W at position 19 (70% A and 30%T), while a C was observed in all *B. cereus* group strains without an emetic toxin profile. This nucleotide difference could be used to set up a molecular assay.

6 Concluding remarks

Gastrointestinal diseases caused by *B. cereus* are more common than once thought and the incidence is probably much higher than reported. However, the incidence of gastrointestinal disease, especially the emetic syndrome, can be reduced by proper hygiene and food preparation practices, particularly in restaurants and mass-catering. Emetic strains are unable to grow at temperatures below 10°C ([57]; Eh-

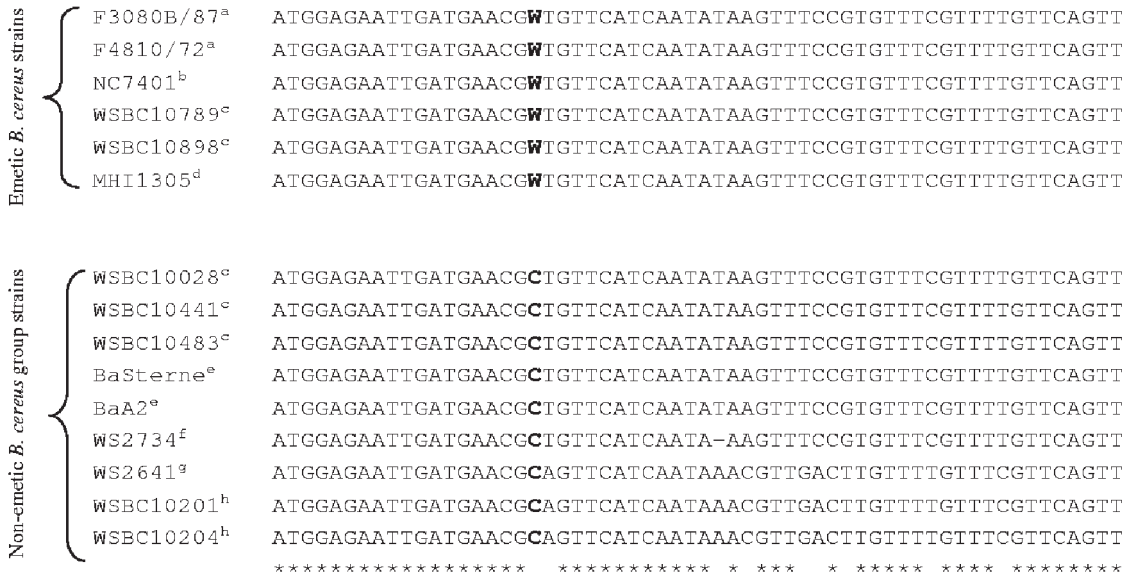

Figure 4. Sequences of 16S – 23S rDNA ITS of *Bacillus cereus* group strains. Bases in boldface print indicate sequence differences. (W = A or T), (H = A, T or C); (a) cereulide producing strains connected to emetic food-poisoning outbreaks (Public Health Laboratory Service, London, UK (PHLS); (b) cereulide-producing strain connected to emetic food-poisoning in Japan (Nagoya City Public Health Research Institute, Nagoya, Japan); (c) strains from foods and environment (Weihenstephan *Bacillus cereus* collection, Weihenstephan, Germany); (d) cereulide-producing *B. cereus* isolate connected to an emetic food-poisoning outbreak in Germany (E. Märtelbauer, personal communication); (e) *B. anthracis* Sterne CIP 7702 (BaSterne) and *B. anthracis* CIP A2 (BaA2); an human isolate from Iran (G. Vergnaud, personal communication); (f) *B. thuringiensis*-type strain; (g) *B. mycoides*-type strain; (h) *B. weihenstephanensis*.

ling-Schulz unpublished) and toxin formation seems to be restricted to certain temperatures, ranging from 10°C to 40°C. Therefore, temperature is a key factor to prevent the emetic type of food-poisoning caused by *B. cereus*. Especially, care has to be taken when rice and pasta are prepared: (i) only small quantities as needed should be prepared; (ii) prepared rice and noodles should be kept hot (> 55°C), and (iii) cooked rice and pasta should be cooled quickly (<10°C). Nevertheless, one should always keep in mind that reheating of precooked foods does not inactivate the potentially preformed emetic toxin cereulide.

Due to the ubiquitous presence of *B. cereus* and resistance of its spores to pasteurization or simple sanitation processes, contamination of foods will always occur. Since the pathogenic potential of the different *B. cereus* isolates is highly variable, from nontoxic to highly toxic strains, methods for a rapid identification of hazard strains are necessary. During recent years reasonable progress has been made in the development of suitable detection systems for emetic strains. An improved bioassay that allows a simultaneous screening of many samples for the emetic toxin [66], and the first molecular assay for detection of emetic *B. cereus* strains have been described [68]. With the latter assay, molecular tools are now available for the detection of all known *B. cereus* toxins contributing to food-poisoning. It is expected that multiplex PCR systems to detect the full set of toxin genes involved in gastrointestinal diseases caused by *B. cereus* and the development of real-time PCR systems will improve diagnosis and food safety substantially. In addition, such molecular assays might also be useful to clarify the role of enterotoxins and the emetic toxin producing *B. cereus* strains in nongastrointestinal infections.

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