Food Microbiology 46 (2015) 195–199

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Short communication

A case of intoxication due to a highly cytotoxic *Bacillus cereus* strain isolated from cooked chicken

Ana C. López [a], Jessica Minnaard [b, d, 1], Pablo F. Pérez [b, c, 1], Adriana M. Alippi [a, *]

[a] Unidad de Bacteriología, Centro de Investigaciones de Fitopatología, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, cc 31, Calle 60 y 119, 1900 La Plata, Argentina
[b] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CONICET. La Plata), Calle 47 y 116-B1900AJI, La Plata, Argentina
[c] Cátedra de Microbiología, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 115-B1900AJI, La Plata, Argentina
[d] Área Farmacia, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 115-B1900AJI, La Plata, Argentina

ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form
11 August 2014
Accepted 14 August 2014
Available online 27 August 2014

Keywords:
Bacillus cereus
Epithelial cells
Virulence
Genes
Detachment
Necrosis
Food poisoning

ABSTRACT

Outbreaks of *Bacillus cereus* infection/intoxication are not commonly reported because symptoms are often mild, and the disease is self-limiting. However, hypervirulent strains increase health risks. We report a case, which occurred in Argentina, of severe food poisoning illness on a healthy adult woman associated to *B. cereus* strain MVL2011. The studied strain was highly cytotoxic, showed high ability to detach Caco-2 cells and was positive for the hblA, hblB, and hblC genes of the hbl complex, bceT, entS and ces. As it is considered that *B. cereus* emetic cluster evolved from a panmictic population of diarrheal strains, *B. cereus* MVL2011 could constitute an intermediate strain between diarrheal and emetic strains.

1. Introduction

Bacillus cereus is a spore-forming rod-shaped bacterium, commonly present in food. It is an opportunistic microorganism widely recognized as the etiological agent of food-borne outbreaks (emetic and diarrheic syndromes) as well as non-intestinal pathologies (Kramer and Gilbert, 1992; Stenfors Arnesen et al., 2008). Emesis is caused by cereulide (Ehling-Schulz et al., 2005a), whereas diarrhea probably involves diverse extracellular factors (Stenfors Arnesen et al., 2008). The virulence of *B. cereus*, whether intestinal or non-intestinal, is intimately associated with the production of tissue destructive/reactive proteins (Bottone, 2010). Two of these virulence factors are protein complexes, i.e. hemolysin BL (HBL) and the non-hemolytic enterotoxin NHE (Guinebretiere et al., 2002). Other virulence factors are single proteins, i. e. entFM (enterotoxin FM), cytK (cytolysin K) and bceT (*B. cereus* enterotoxin) (Fagerlund et al., 2004; Kramer and Gilbert, 1992; Stenfors Arnesen et al., 2008), phosphatidylinositol specific phospholipase (PI-PLC), enterotoxin S (EntS), sphingomyelinase (SMase), cereolysin O (Clo), InhA1, NprA and HlyII (Cadot et al., 2010; Kramer and Gilbert, 1992; Stenfors Arnesen et al., 2008). Moreover, other factors such as adhesion to and invasion of epithelial cells also play a role in the biological effects of *B. cereus* strains (Minnaard et al., 2004, 2007, 2013).

B. cereus symptoms are usually mild (Kramer and Gilbert, 1992) and the pathology is not commonly reported. However more severe cases including fatal outcomes have increased in the last few years (Al-Abri et al., 2011; Bottone, 2010; Dierick et al., 2005; Mahler et al., 1997; Naranjo et al., 2011; Saito et al., 2010; Shiota et al., 2010). In Argentina, diagnostic testing for *B. cereus* is not routinely performed for patients with gastrointestinal diseases. Nevertheless, statistical data provided by the Health Ministry (2008–2013) showed 5783 cases of food-borne disease, of which 26 were positive for *B. cereus* and 9 occurred in 2011 (year of highest incidence).

In this context the aim of the present work was the isolation, identification and characterization of the etiologic agent of a food poisoning episode associated to consumption of chicken by a young healthy woman.

* Corresponding author. Tel.: +54 221 4236758x423; fax +54 221 4352346.
E-mail addresses: rodrika@biol.unlp.edu.ar (A.C. López), jm@biol.unlp.edu.ar (J. Minnaard), pfp@biol.unlp.edu.ar (P.F. Pérez), alippi@biol.unlp.edu.ar, adrianaalippi@gmail.com (A.M. Alippi).
[1] Tel.: +54 221 424 9287; fax: +54 221 425 4853.

http://dx.doi.org/10.1016/j.fm.2014.08.005
0740-0020/

2. Materials and methods

2.1. Case presentation

In Buenos Aires, Argentina, a 39-year-old healthy woman was hospitalized due to dehydration. She had purchased chicken stuffed with carrots, eggs, pepper and cheese which had been refrigerated immediately and the following day (for lunch) warmed in the microwave oven. One hour after eating, she left home for sport activities (5 km running). Ten hours after eating, she started vomiting and had 5 episodes of watery diarrhea. After 3 h from onset, the symptoms had not subsided, and the doctor ordered oral rehydration and rest. However, diarrhea and vomiting continued for further 5 h, which resulted in severe dehydration and required 7 h hospitalization for reposition of water and electrolytes. Analyses performed on faeces and vomitus did not detect the presence of *Escherichia coli*, *Shigella* spp, *Staphylococcus aureus* or *Salmonella* spp.

The rapid onset of symptoms, in conjunction with the results of the microbiological analysis, suggested *B. cereus* as the most likely etiological agent for this case.

2.2. Isolation of bacteria

Two chicken samples (10 g) were diluted 1:10 in sterile distilled water and homogenized. Ten microliters were spread on polymyxin–pyruvate–egg-yolk–mannitol agar (PEMBA) plates (Holbrook and Andersson, 1980), which were incubated at 32 °C for 48–96 h until bacterial growth was detected (Lancette and Harmon, 1980). Gram staining and determination of catalase activities were performed. Colony appearance regarding shape, color, type of growth and presence of opaque halos due to lecithinase activity were also evaluated.

2.3. Bacterial identification

The identity of the bacterial isolates was confirmed by using API 20E and API 50CH strips plus API 50CHB medium (Biomerieux, France) and data base Apiweb (Biomerieux, www.biomerieux.com). In addition, starch hydrolysis, hemolytic activity and production of a discontinuous hemolytic pattern on blood agar plates according to standard protocols (Beecher and Wong, 1994) were tested.

2.4. Detection of enterotoxin genes

Total genomic DNA was isolated from 24 h-cultures grown on TSA using the procedure previously described by López and Alippi (2007). Presence of sequence associated to virulence genes were assessed as previously reported (Minnaard et al., 2007). Genes encoding for enterotoxin-T (bceT) (Guinebretiere et al., 2002), cytotoxin K (cytk) (Ehling-Schulz et al., 2006), sphingomyelinase (sph), enterotoxin FM (entFM), enterotoxin S (entS), phosphatidylinositol, phospholipase C (piplC) (Ghelardi et al., 2002), the components of HBL (hemolysin BL: hblA, hblB, hblC, hblD) and NHE (non-hemolytic enterotoxin: nheA, nheB, nheC) complexes (Guinebretiere et al., 2002) were studied. In addition, the isolated was assessed for the *ces* gene related to the production of cereulide (Ehling-Schulz et al., 2005a). DNA amplifications were performed in a thermal cycler (Mastercycler personal; Eppendorf Hamburg, Germany). Amplicons were analyzed by 1.6% (W/V) agarose gel, in TBE buffer, stained with Gel Red® (Biotium, U.S.A) for 2 h at 80 V.

2.5. Culture of epithelial cells

Caco-2 cells (Fogh and Orfeo, 1977) were routinely grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) (25 mM glucose) (Life Technologies, Cergy, France), supplemented with 15% (v/v) heat-inactivated (30 min, 56 °C) fetal calf serum (FCS) (PAA Laboratories GmbH, Pasching, Austria) and 1% (v/v) non-essential amino acids (Life Technologies, Cergy, France). Monolayers were prepared in 24-tissue culture plates (Greiner Bio One, Frickenhausen, Germany) by seeding 2.5×10^4 cells per well. Experiments and cell maintenance were carried out at 37 °C in a 5% CO_2/95% air atmosphere. Cells at late post confluence (15 days in culture) were used.

Hep-2 cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM, 25 mM glucose, Life Technologies, Carlsbad, CA, USA), supplemented with 10% (v/v) heat-inactivated (56 °C, 30 min) fetal calf serum (FCS, PAA Laboratories, Pasching, Austria), 12 IU/ml penicillin-12 μg/ml streptomycin (Life Technologies) and 1% (v/v) non-essential amino acids (Life Technologies). Cells were seeded at 6.2×10^4 cells per well in 48-well tissue culture plates (Greiner Bio One, Frickenhausen, Germany) and incubated for 48 h at 37 °C in 5% CO_2.

2.6. Detachment of Caco-2 cells

Detachment of enterocyte-like cells was performed as previously reported (Minnaard et al., 2001). Briefly, differentiated Caco-2 monolayers were incubated at 37 °C for 1 h with 0.5 ml of serial dilutions of culture filtrate supernatants (CFS, pH 6.8) from 16 h-old bacteria cultures at 32 °C in BHIG (BHI broth (BIOKAR Diagnostics) supplemented with 0.1% (w/v) glucose). Cells were washed twice with phosphate buffered saline (PBS) (pH 7.2), fixed at room temperature for 1 min with 2% (v/v) formaldehyde in PBS and washed again with PBS. Afterward, cells were stained by incubating for 20 min at room temperature with 500 μl of a crystal violet solution (0.13% (w/v) crystal violet, 5% (v/v) ethanol and 2% (v/v) formaldehyde in PBS). After washing to remove stain excess, samples were treated with freshly prepared 50% (v/v) ethanol at room temperature for 1 h. Absorbance was measured in a microplate reader at 620 nm (Biotek Instruments, Winooski, USA). Percentage of cell detachment was calculated as follows:

$$\text{Cell detachment } \% = 100 \times (\text{Ac} - \text{As})/\text{Ac},$$

where Ac : A_{620} of control cells and As : A_{620} of sample cells.

2.7. Necrosis

Assessment of necrosis was performed as reported previously (López et al., 2013). Briefly, Caco-2 monolayers were incubated with serial dilutions of CFS as describe above. After 1 h of incubation, wells with monolayer not detached were washed twice with binding buffer containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 125 mM NaCl, 2.5 mM $CaCl_2$, (pH 7.2), and 0.2% (p/v) gelatin. Afterward, 1 μg of propidium iodide was added in 100 μl of binding buffer per well, and cells were incubated on ice for 15 min. Then, samples were mounted in 50% (v/v) glycerol in PBS and analyzed by conventional fluorescence microscopy using a Leica DMLB microscope coupled to a Leica DC 100 camera (Leica Microscopy Systems, Heerbrugg, Switzerland).

2.8. Mitochondrial dehydrogenase activity

Activity of mitochondrial dehydrogenases was determined by assessing the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium (MTT) by means of modification of a previously reported protocol (Finlay et al., 1999; Minnaard et al., 2007).

Briefly, autoclaved (15 min at 121 °C) or non-heated CFS diluted in DMEM with 2% methanol were added to each well of Hep-2 cells and incubated 24 h at 37 °C in 5% CO_2. CFS (autoclaved or non-

Table 1
Screening by PCR of genes encoding for components of HBL (hemolysin BL: *hblA*, *hblB*, *hblC*, *hblD*) and NHE (non-hemolytic enterotoxin: *nheA*, *nheB*, *nheC*) complex; enterotoxin-T (*bceT*); cytotoxin K (*cytk*); sphingomyelinase (*sph*); enterotoxin FM (*entFM*); enterotoxin S (*entS*); cereulide (*ces*) and phosphatidylinositol phospholipase C(*piplC*).[a]

Strain	Origin	*hbl* complex				*nhe* complex			*bce*T	*ctyK*	*sph*	*entFM*	*entS*	*ces*	*piplC*	Reference
		hblA	*hblB*	*hblC*	*hblD*	*nhe*A	*nhe*B	*nhe*C								
MVL2011	Chicken (food-borne illness)	+	+	+	−	−	+	−	+	+	+	+	+	+	+	This study
B10502	Food-borne illness	−	−	−	−	+	−	−	−	+	+	+	−	−	+	Minnaard et al., 2004, 2007
T1	Unknown	+	+	+	+	+	+	+	+	+	+	+	+	−	+	Minnaard et al., 2007
2	Skim milk powder	−	−	−	+	−	+	+	+	+	+	−	−	−	−	Minnaard et al., 2001, 2007
M2	Skim milk powder	−	−	−	−	−	+	−	−	−	+	+	−	−	+	

[a] Toxin gene presence was assessed by PCR analysis. The "+" and "−" symbols refer to the presence and absence of the gene, respectively.

heated) from a *ces* (−) *B. cereus* strain (B10502) were also evaluated. The medium was removed and DMEM (without phenol red) containing 0.5 mg/ml of MTT was added to each well; plates were incubated at 37 °C for 4 h and then, the medium was removed. Intracellular formazan was solubilized with 0.25 ml of isopropyl alcohol/0.1 N HCl and absorbance at 570 nm was measured in a microtiter plate reader (Bioteck Instruments, Winooski, USA).

2.9. Cell association assays

Bacterial from a 3 h-old cultures (32 °C) in BHIG were centrifuged and pellets were suspended in DMEM containing 100 μg/ml chloramphenicol. As reported (Minnaard et al., 2004), in these conditions bacterial viability is preserved but no bacterial growth occurs. Cell monolayers were washed twice with PBS before the infection assays and bacterial suspensions were added to the monolayers (Multiplicity of infection, MOI = 100 bacteria per cell) and incubated for 2 h at 37 °C in a 5% CO_2/95% air atmosphere. To evaluate association (adhering plus invading bacteria), monolayers were exhaustively washed with PBS and incubated with 1 ml distilled water per well to lyse eukaryotic cells. Serial dilutions of the samples were plated onto nutrient agar (BIOKAR Diagnostics, Beauvais, France) and incubated at 37 °C for 16 h. Invasion was assessed by the aminoglycoside protection assay as previously reported (Minnaard et al., 2004). All the infection assays were performed in DMEM FCS-free.

3. Results

After 48 h of incubation, typical mannitol negative and lecithinase positive colonies were observed in PEMBA plates. Gram staining showed Gram-positive rods with subterminal spores and no significant swelling of the sporangia. The isolate was named MVL2011. Using the API 20E and API 50CH strips and data base Apiweb, MVL2011 matched as *B. cereus*. In addition, the strain hydrolyzed starch and lead to a discontinuous hemolytic pattern.

The presence of sequences related to genes associated to the virulence of *B. cereus* was determined. As compared with 4 well characterized *B. cereus* strains, the strain under study shows a very different pattern of virulence genes (Table 1). Strain MVL2011 was positive for the sequence corresponding to the genes *bceT*, *cytK*, *sph*, *entFM*, *entS*, ces and *piplC* (Table 1); moreover it presented 3 of the sequences of the hemolyisn BL (*hbl A*, *B* and *C*) and one of the sequences of the non hemolytic enterotoxin (*nhe B*) (Table 1). All the genes gave amplification fragments of the expected sizes.

Biological activity of the strain MVL2011 was studied on epithelial cells.

Coincubation of Caco-2 monolayers with CFS of MVL2011 leads to cell detachment and dose–response behavior was observed (Fig. 1). Low doses of CFS did not detach cells but led to significant necrosis as assessed by propidium iodide staining (Fig. 2). Interestingly, when CFS was heated for 15 min at 121 °C, the ability to detach Caco-2 cells was abrogated (data not shown).

Hep-2 bioassay has been employed to analyzed cereulide. Results showed that, after thermal treatment, 80% of the biological activity was retained as compared to non-heated CFS. In contrast, biological activity of CFS from strain B10502 was completely abolished after thermal treatment.

Infection of enterocyte-like Caco-2 cells was performed with vegetative culture of MVL2011. Association and invasion values were $1.16 \pm 0.03 \times 10^6$ cfu/ml and $1.15 \pm 0.07 \times 10^2$ cfu/ml, respectively. The ratio of associated/initial bacteria was 0.01 ± 0.003 and invading/associated bacteria was $9.95 \pm 0.61 \times 10^{-5}$.

4. Discussion

Results presented in this case study provided evidence that *B. cereus* strain MVL2011 isolated from cooked chicken and related to a case of serious food poisoning in a healthy adult woman, was highly cytotoxic and unable to invade Caco-2 cells in spite of adhesion ability similar to other *B. cereus* strains. Along with the clinical symptoms, evidence suggest that the strain is an emetic isolate since it is positive for this sequences in the PCR assay and the biological activity of the extracellular factors on Hep-2 cells is not completely abrogated by thermal treatment (Stark et al., 2013). Even though levels of cereulide lower than 8 μg/kg body weight can be lowered with proper hydration, these concentrations can be fatal in children (Saito et al., 2010). Some strains of *B. cereus* are psychrotrophic since they lead to the highest production of the emetic toxin between 12 °C and 15 °C (Dierick et al., 2005). Fatal outcomes have been reported for these emetic strains (Dierick et al., 2005). Note that the meal had been refrigerated for 24 h after being purchased, and the same food had been eaten 24 h before without symptoms of food-poisoning. Usually *B. cereus* symptoms

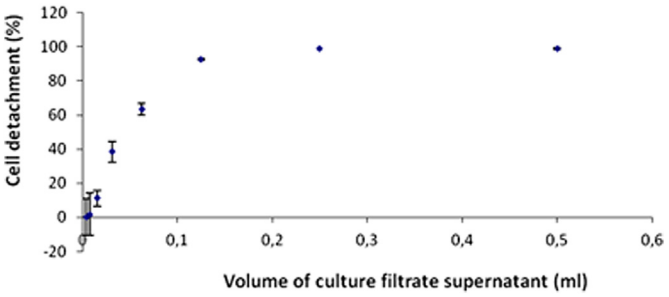

Fig. 1. Detachment of enterocyte-like cells (Caco-2) after incubation with different concentrations of culture filtrate supernatants of *Bacillus cereus* strain MVL2011. Values represent averages from 3 determinations ± standard deviation.

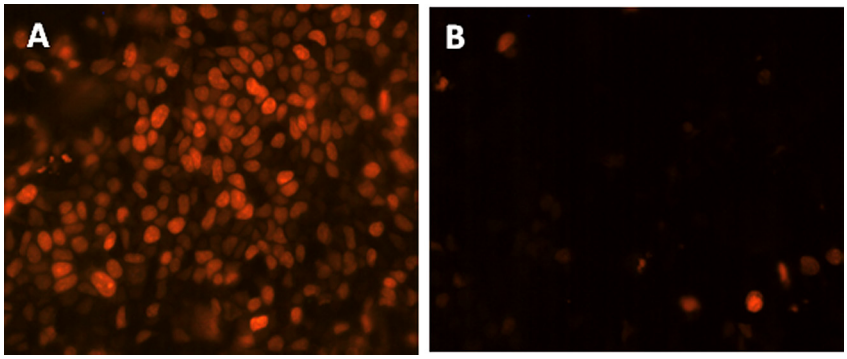

Fig. 2. Fluorescence microscopy of Caco-2 cells co-incubated with culture filtered supernatants of *Bacillus cereus* MVL2001 (A) and control co-incubated with BHIG medium (B). Cells were labeled with propidium iodide indicating red nuclei in necrotic cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are mild (Kramer and Gilbert, 1992) and the pathology is not commonly reported. However cases involving more serious health risks and fatal outcomes (Al-Abri et al., 2011; Dierick et al., 2005; Mahler et al., 1997; Naranjo et al., 2011; Saito et al., 2010; Shiota et al., 2010) have increased in the last few years (Bottone, 2010).

In Argentina, diagnostic test for *B. cereus* is not routinely performed for patients with gastrointestinal diseases. As compared with other non-invasive *B. cereus* strains previously studied (Minnaard et al., 2007) stated in Table 1, strain MVL2011 is the sole strain showing high ability to detach Caco-2 cells. In addition, it is positive for the hblA, hblB and hblC genes of the hbl complex, bceT, entS and ces (cereulide synthase). According to our results and the clinical presentation of the case, *B. cereus* MVL2011 is an emetic strain. In early reports on strains positive for the gene *ces* it has been proposed that, on the basis of gene analysis, they belong to a well-defined "emetic cluster" (Ehling-Schulz et al., 2005b). Noteworthy, our strain is positive for 3 out of 4 genes of the hbl complex that is common in non-emetic strains (Ehling-Schulz et al., 2005b). These apparently contradictory findings are in agreement with current knowledge on the virulence of *B. cereus* (Castiaux et al., 2014; Messelhäusser et al., 2014). Indeed, it has been demonstrated that the distribution of the *ces* gene is not homogeneous along *B. cereus* populations and strains positive both for *ces* and *hbl* complex have been isolated (Kim et al., 2010; Rahmati, T. and Labbe, R., 2008).

5. Conclusion

Results presented in this case study provided evidence that *B. cereus* strain MVL2011 was involved in serious food poisoning based on symptoms, microbiological analysis of patient's samples and the presence of *B. cereus* in the implicated food. It has been proposed that *B. cereus* emetic cluster evolved from a panmictic population of diarrheal strains. In this context and according to the results of the present study and the current knowledge on the distribution of virulence traits of *B. cereus*, strain MVL2011 belongs to the sub-population of strains positive for several virulence genes. These characteristics could correlate with the high biological activity of the strain under study.

Acknowledgments

This work was financed by Agencia Nacional de Promoción Científica y Tecnológica, Argentina (ANPCyT, PICT: 0760/08), CONICET (PIP 0708-2009 and PIP 0407-2011), Universidad Nacional de La Plata (X574), and Facultad de Ciencias Exactas (Universidad Nacional de La Plata, Argentina).

References

Al-Abri, S.S., Al-Jardania, A.K., Al-Hosnia, M.S., Kurupb, P.J., Al-Busaidi, S., Beeching, N.J., 2011. A hospital acquired outbreak of *Bacillus cereus* gastroenteritis, Oman. J. Infect. Public Health 4, 180–186.

Beecher, D.J., Wong, A.C.L., 1994. Identification of hemolysin BL-producing *Bacillus cereus* by a discontinuous hemolytic pattern in blood agar. Appl. Environ. Microbiol. 60, 1646–1651.

Bottone, E.J., 2010. *Bacillus cereus*, a volatile human pathogen. Clin. Microbiol. Rev. 23, 382–398.

Cadot, C., Tran, S., Vignaud, M., De Buyser, M., Kolstø, A., Brisabois, A., Nguyen-Thé, C., Lereclus, D., Guinebretière, M., Ramarao, N., 2010. InhA1, NprA, and HlyII as candidates for markers to differentiate pathogenic from nonpathogenic *Bacillus cereus* strains. J. Clin. Microbiol. 48, 1358–1365.

Castiaux, V., N'guessan, E., Swiecicka, I., Delbrassinne, L., Dierick, K., Mahillon, J., 2014. Diversity of pulsed-field gel electrophoresis patterns of cereulide-producing isolates of *Bacillus cereus* and *Bacillus weihenstephanensis*. FEMS Microbiol. Lett. 353 (2), 124–131. http://dx.doi.org/10.1111/1574-6968.12423.

Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M., Mahillon, J., 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. J. Clin. Microbiol. 43, 4277–4279.

Ehling-Schulz, M., Guinebretiere, M.H., Monthán, A., Berge, O., Fricker, M., Svensson, B., 2006. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. FEMS Microbiol. 260, 232–240.

Ehling-Schulz, M., Vukov, N., Schulz, A., Shaheen, R., Andersson, M., Märtlbauer, E., Scherer, S., 2005a. Identification and partial characterization of the nonribosomal peptide synthetase gene responsible for cereulide production in emetic *Bacillus cereus*. Appl. Environ. Microbiol. 71, 105–113.

Ehling-Schulz, M., Svensson, S., Guinebretiere, M., Lindback, T., Andersson, M., Schulz, A., Fricker, M., Christiansson, A., Granum, P.E., Martlbauer, E., Nguyen-Thé, C., Salkinoja-Salonen, M., Scherer, S., 2005b. Emetic toxin formation of *Bacillus cereus* restricted to a single evolutionary lineage of closely related strains. Microbiology 151, 183–197.

Fagerlund, A., Ween, O., Lund, T., Hardy, S.P., Granum, P.E., 2004. Genetic and functional analysis of the *cytK* family of genes in *Bacillus cereus*. Microbiology 150, 2689–2697.

Finlay, W.J.J., Logan, N.A., Sutherland, A.D., 1999. Semiautomated metabolic staining assay for *Bacillus cereus* emetic toxin. Appl. Environ. Microbiol. 65, 1811–1812.

Fogh, J.M., Orfeo, T., 1977. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 59, 221–226.

Ghelardi, E., Celandroni, F., Salvetti, S., Barsotti, C., Baggiani, A., Senesi, S., 2002. Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food-poisoning outbreaks. FEMS Microbiol. Lett. 208, 129–134.

Guinebretiere, M.H., Broussolle, V., Nguyen-Thé, C., 2002. Enterotoxigenic profiles of food-poisoning and food-borne *Bacillus cereus* Strains. J. Clin. Microbiol. 40, 3053–3056.

Kim, Jung-Beom, Kim, Jai-Moung, Kim, So-Yeong, Kim, Jong-Hyun, Park, Yong-Bae, Choi, Na-Jung, Oh, Deog-Hwan, 2010. Comparison of enterotoxin production and phenotypic characteristics between emetic and enterotoxic *Bacillus cereus*. J. Food Prot. 73, 1219–1224.

Kramer, J.M., Gilbert, R.J., 1992. *Bacillus cereus* gastroenteritis. In: Tu, A.T. (Ed.), Food Poisoning. Handbook of Natural Toxins, vol. 7. Marcel Dekker, Inc, New York.

Lancette, G.A., Harmon, S.M., 1980. Enumeration and confirmation of Bacillus *cereus* in foods: collaborative study. J. Assoc. Off. Anal. Chem. 63, 581–586.

López, A.C., Alippi, A.M., 2007. Phenotypic and genotypic diversity of *Bacillus* 208 *cereus* isolates recovered from honey. Int. J. Food Microbiol. 117, 175–184.

López, A.C., Minnaard, J., Perez, P.F., Alippi, A.M., 2013. *In vitro* interaction between *Bacillus megaterium* strains and Caco-2 cells. Int. Microbiol. 16, 27–33. http://dx.doi.org/10.2436/20.1501.01.177.

Mahler, H., Pasi, A., Kraner, J.M., Schulte, P., Scoging, A.C., Bar, W., Krahenbuhl, S., 1997. Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*. N. Engl. J. Med. 336, 1142–1148.

Messelhäusser, U., Frenzel, E., Blöchinger, C., Zucker, R., Kämpf, P., Ehling-Schulz, M., 2014. Emetic Bacillus cereus are more volatile than thought: recent foodborne outbreaks and prevalence studies in Bavaria (2007-2013). Biomed. Res. Int. 2014, 465603. http://dx.doi.org/10.1155/2014/465603.

Minnaard, J., Rolny, I.S., Perez, P.F., 2013. Interaction *between Bacillus cereus* and cultured human enterocytes: effect of calcium, cell differentiation, and bacterial extracellular factors. J. Food Prot. 76, 820–826.

Minnaard, J., Humen, M., Pérez, P.F., 2001. Effect of *Bacillus cereus* exocellular factors on human intestinal epithelial cell. J. Food Prot. 64, 1535–1541.

Minnaard, J., Lievin-Le Moal, V., Coconnier, M., Servin, A., Pérez, P.F., 2004. Disassembly of F-Actin cytoskeleton after interaction of *Bacillus cereus* with fully differentiates human intestinal caco-2 cells. Infect. Immun. 72, 3106–3112.

Minnaard, J., Delfederico, L., Vasseur, V., Hollmann, A., Rolny, I., Semorile, L., Perez, P.F., 2007. Virulence of *Bacillus cereus*: a multivariate analysis. Int. J. Food Microbiol. 116, 197–206.

Naranjo, M., Denayer, S., Botteldoorn, N., Delbrassinne, L., Veys, J., Waegenaere, J., Sirtaine, N., Driesen, R.B., Sipido, K.R., Mahillon, J., Dierick, K., 2011. Sudden death of a young adult associated with *Bacillus cereus* food poisoning. J. Clin. Microbiol. 12, 4379–4381.

Rahmati, T., Labbe, R., 2008. Levels and toxigenicity of *Bacillus cereus* and *Clostridium perfringens* from retail seafood. J. Food Prot. 71, 1178–1185.

Saito, M., Takahashi, N., Ueda, S., Kuwabara, Y., Komiyama, M., Koike, Y., Yada, Y., Honma, Y., Momoi, M.Y., 2010. Cytokine profile in a premature infant with systemic *Bacillus cereus* infection. Pediatr. Int. 52, e34–e36. http://dx.doi.org/10.1111/j.1442-200X.2009.02987.x.

Shiota, M., Saitou, K., Mizumoto, H., Matsusaka, M., Agata, N., Nakayama, M., Kage, M., Tatsumi, S., Okamoto, A., Yamaguchi, S., Ohta, M., Hata, D., 2010. Rapid detoxification of cereulide in *Bacillus cereus* food poisoning. Pediatrics 125, 951–958.

Stenfors Arnesen, L.P., Fagerlund, A., Granum, P.E., 2008. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Rev. 32, 579–606.

Stark, T., Marxen, S., Rütschle, A., Lücking, G., Scherer, S., Ehling-Schulz, M., Hofmann, T., 2013. Mass spectrometric profiling of *Bacillus cereus* strains and quantitation of the emetic toxin cereulide by means of stable isotope dilution analysis and HEp-2 bioassay. Anal. Bioanal. Chem. 405, 191–201. http://dx.doi.org/10.1007/s00216-012-6485-6.