Research Article

Emetic Bacillus cereus Are More Volatile Than Thought: Recent Foodborne Outbreaks and Prevalence Studies in Bavaria (2007–2013)

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Several Bacillus cereus strains possess the genetic fittings to produce two different types of toxins, the heat-stable cereulide or different heat-labile proteins with enterotoxigenic potential. Unlike the diarrheal toxins, cereulide is (pre-)formed in food and can cause foodborne intoxications shortly after ingestion of contaminated food. Based on the widely self-limiting character of cereulide intoxications and rarely performed differential diagnostic in routine laboratories, the real incidence is largely unknown. Therefore, during a 7-year period about 4.300 food samples linked to foodborne illness with a preliminary report of vomiting as well as food analysed in the context of monitoring programs were investigated to determine the prevalence of emetic B. cereus in food environments. In addition, a lux-based real-time monitoring system was employed to assess the significance of the detection of emetic strains in different food matrices and to determine the actual risk of cereulide toxin production in different types of food. This comprehensive study showed that emetic strains are much more volatile than previously thought. Our survey highlights the importance and need of novel strategies to move from the currently taxonomic-driven diagnostic to more risk orientated diagnostics to improve food and consumer safety.

1. Introduction

Cereulide, an emesis-inducing toxin produced by a fairly homogenous group of B. cereus strains called “emetic B. cereus,” is a small heat-stable cyclic peptide [1]. The emetic poisoning caused by cereulide is usually characterized by vomiting starting after 0.5 hour to six hours after consumption of the contaminated food. Intoxications proceed mostly with mild symptoms and last normally not more than one day, but severe cases requiring hospitalization are increasingly reported (for review see Ehling-Schulz et al., 2004 [2], and Ehling-Schulz et al., 2011 [3]).

Because of the short period of illness, the emetic syndrome caused by B. cereus is presumably underreported [4]. In addition, the symptoms of an emetic intoxication caused by B. cereus parallel the symptoms caused by S. aureus enterotoxins, bearing the risk of misdiagnosis of the disease. In the year 2011 the European Food Safety Authority (EFSA) reported an increase of 122.2% in the number of foodborne intoxications and toxicoinfections caused by B. cereus in Europe. The overall reporting rate was 0.04 cases per 100.000 inhabitants [5]. Even if intoxication with the emetic toxin cereulide in most cases produces only mild symptoms, consistently also fatal cases are reported [6–8].

Although B. cereus is an ubiquitous spore former, emetic strains are rarely found in the environment and their natural niches and entrance points into the food production and processing are largely unknown [4]. So far, mostly high-carb
food matrices, such as rice and pasta, as well as milk and dairy products, have been investigated for the presence of emetic strains of _B. cereus_ [9–13], whereas other food matrices have rarely been included in the analyses. To improve HACCP-based concepts and prevent foodborne intoxications caused by emetic _B. cereus_, information on the general prevalence of emetic strains in foods of different origin is of utmost importance and data on the risk of toxin formation in different food categories are required.

This study therefore aimed to (i) investigate the prevalence of emetic _B. cereus_ strains in a wide range of food matrices, covering foods from plant as well as animal origin, to identify potential contamination sources, and to (ii) facilitate hazard identification by exploring the potential of diverse food matrices for the risk of cereulide toxin production. In this context, a perennial survey from 2007 to 2013 was carried out, including food samples connected to emesis-related foodborne illnesses as well as samples not related to foodborne outbreaks. By using an in situ bioassay indicative of cereulide production levels, a general scheme for categorizing foods with respect to their risk of cereulide production was generated.

### 2. Material and Methods

#### 2.1. Sample Material

Between the years 2007 and 2013 3,564 food samples from Bavaria were analysed for the presence of emetic _B. cereus_ strains in the context of foodborne illness or outbreaks where the consumers showed symptoms of vomiting. The majority of samples were taken from the household of the diseased consumers and from restaurants, canteens, and catering companies. Additionally, the presence of emetic _B. cereus_ strains in different food matrices (n = 742) was investigated in the scope of different monitoring programs. Food categories for the monitoring were chosen from both food of animal origin and food of plant origin. All samples were examined before their expiry date.

#### 2.2. Microbiological Detection of _B. cereus_ and Identification of the Cereulide Synthetase Gene ces

Emetic _B. cereus_ strains were detected with qualitative and quantitative methods (for details see Ehling-Schulz et al., 2011 [3]). The quantitative detection was done weighting 10 g of sample material into 90 mL of tryptone-peptone-glucose-yeast (TPGY) broth and incubating at 30°C under aerobic conditions. After 24 h of cultivation 1 mL of the enrichment broth was taken for the molecular detection of the _ces_ gene, which encode the nonribosomal synthetase responsible for the production of the peptide toxin cereulide. For detection of _ces_, a previously described probe-based diagnostic real-time-PCR assay was used [9, 15].

The quantitative detection of presumptive _B. cereus_ was carried out using standard reference culture methods recommended by the International Organisation of Standardization (ISO) and the U.S. Food and Drug Administration (FDA). Samples were investigated using spiral plate count method on the Mossel agar [16, 17] or with a 3-tube 3-dilution most probable number (MPN) method [18–21]. Presumptive _B. cereus_ colonies were further differentiated by the detection of the _ces_ gene either by real-time-PCR as described above or by using a conventional PCR system according to Ehling-Schulz et al., 2004 [22]. Depending on the results of these reactions the number of colony-forming units per gram (cfu/g) or MPN of emetic _B. cereus_ cells per gram of sample was calculated following the standard methods recommended by FDA and ISO [16, 17, 21].

#### 2.3. Bioassay-Based Risk Categorization of Foods

Analysis of the potential of food matrices to support cereulide production was performed by artificial contamination of 30 g portions with the bioluminescent _B. cereus lux_ reporter strain F4810/72(pMDX[ _P_. _luxABCDE_]) and an IVIS camera system as described earlier [23, 24]. Foods were provided by diverse manufacturers or were obtained from local consumer markets. In the case of powders and freeze-dried products (e.g., infant formulas and instant potato powder) or raw materials (e.g., rice and pasta) foods were prepared according to the manufacturers’ instructions thereby simulating common household conditions. The contents of preportioned packaging units (e.g., single-sliced cheese or biscuit snacks) were combined and blended for 3 min with a stomacher to obtain homogenous testing matrices. Dry foods, such as dates, apricots, cocoa powder, and herbal salt, were additionally soaked with sterile water or pasteurized milk (1.5% fat content) as indicated. Matrices were filled into Petri dishes and inoculated to a final reporter strain cell count of 10^5 CFU per gram. After an incubation step for 24 hours at 24°C, the luciferase signal intensities were quantified with a photon-counting intensified-charge-coupled-device (ICCD) camera (model 2400-32; Hamamatsu Photonics) and are shown as false-color renderings that were superimposed on gray-scale images of the respective food sample.

### 3. Results and Discussion

This study was designed to get a comprehensive overview of the prevalence of emetic _B. cereus_ strains in both food samples from supposed foodborne intoxications and food samples from general food monitoring programs. These data should provide a profound basis for a better risk assessment concerning the emetic syndrome caused by cereulide producing emetic _B. cereus_ strains. In addition, the influence of food matrix properties on cereulide production was evaluated using a previously established _lux_ reporter system [24].

#### 3.1. Prevalence of Emetic _B. cereus_ in Foods Linked to Foodborne Intoxications and in Nonfood Intoxication Associated Food Samples

Because most studies hitherto targeted only a very limited range of food matrices, such as rice and pasta (e.g., [25–27]), and samples were collected from very specific sites or during very short sampling periods (see e.g., [13]), prevalence data covering samples from different years and diverse food matrices are still missing. However, in the context of preventive consumer protection policy and for a comprehensive risk assessment, data about the prevalence of emetic strains in different food categories from a perennial
Bacillus cereus

To gain a deeper insight into food associated natural niches of emetic B. cereus and potential contamination sources, 742 food samples of animal and plant origin were investigated for the presence of emetic B. cereus strains within different monitoring programs (Figure 2). For food of animal origin, samples were grouped in categories that have been reported in the context of foodborne illness, for example, ready-to-eat meat products, cheese, and cream. For food of plant origin, food matrices were investigated that could be possible contamination sources for ready-to-eat food, such as herbs, spices, and dried mushrooms or fresh foods, such as lettuce, fruits, and vegetables. Emetic strains were most frequently found in pasta filata cheese obtained from retail level (13%), in dried mushrooms (8%), and in herbal teas (8%). The detection rates in these matrices were even higher than in uncooked rice and pasta (6%), whereas also 78 samples were investigated. Overall, 10% of presumptive B. cereus strains, isolated in the context of monitoring programs, possess the ces gene and therefore the ability to produce cereulide toxin. These prevalence rates are slightly higher than the ones reported from previous studies (e.g., [25, 27]). One explanation might be that emetic B. cereus strains are easily overlooked in routine diagnostic since they frequently show an atypical phenotype and might, in addition, be outcompeted on nonselective agar media often used in microbial diagnostics [32]. The food category investigated could also significantly influence the percentage of emetic isolates detected. For instance, as our study showed (in food categories for which more than 50 samples were investigated) the percentage of emetic strains isolated from different food matrices varied between 10% (dried mushrooms) and 17% (pasta filata cheese) (see Figure 2).

However, not only the presence of strains but also the potential of food matrices to support cereulide synthesis should be considered for an accurate risk assessment, since unavoidable low-level contaminations with the spore formers might lead to intoxications or even large-scale outbreaks in
Table 1: Examples for potentially foodborne diseases caused by emetic *B. cereus* in Bavaria between the years 2007 and 2013.

| Year | Diseased persons | Place | Food matrix | Level of emetic *B. cereus* (cfu/g) |
|------|------------------|-------|-------------|------------------------------------|
| 2007 | Several students after a cooking lesson at school | School kitchen | Hard cheese | <100 (only positive using a qualitative detection method, but detection of 2 μg cereulid/g) |
| 2007 | One adult | Restaurant | Cooked pasta | 3.8 \times 10^7 |
| 2008 | Several students | School canteen | Paprika filled with meat and rice | <100 (only positive using a qualitative detection method) |
| 2009 | One adult | Household | Cooked potatoes | <100 (only positive using a qualitative detection method) |
| 2010 | One adult | Restaurant | Cooked pasta with oysters | <100 (only positive using a qualitative detection method) |
| 2010 | Several adults | Canteen | Pouard breast in tomato sauce | <100 (only positive using a qualitative detection method) |
| 2010 | Several adults | Catering | Chana masala (cooked chickpea) with baked potatoes in curry sauce and cooked rice | Cooked rice: 2.8 \times 10^4 (1 μg cereulid/g) Cooked chickpea: <10 (only positive using a qualitative detection method, but detection of 0.3 μg cereulid/g); see also Ehling-Schulz and Messelhaeusser, 2012 [14] |
| 2011 | Several children (1 to 3 years old) | Nursery school | Cooked pasta with tomato sauce | 6.8 \times 10^6 |
| 2011 | Two adults | Restaurants | Cooked pork meat with potatoes | 1.0 \times 10^2 |
| 2011 | One adult | Household | Cured and smoked meat | 1.0 \times 10^2 |
| 2012 | Several students | Canteen | Raspberry quark | 1.4 \times 10^2 |
| 2012 | One adult | Household | cooked mushrooms | 1.9 \times 10^2 |
| 2013 | Several adults | Catering at a wedding | Vitello tonnato | 6.1 \times 10^2 |

*Currently, no officially validated method for the quantitative detection of cereulide in food matrices is available; therefore quantitative data on cereulide toxin are only shown for selected samples. However, recently a European initiative has been started to establish appropriate ISO methods (CEN/TC 275/WG 6).*

Figure 2: Presumptive and emetic *B. cereus* in different food matrices investigated in the context of monitoring programs.

3.2 Broad-Scale Risk Categorization of Food Matrices concerning Cereulide Synthesis. Although the EFSA stressed the necessity of identifying categories of foods that may pose a risk for human health with respect to cereulide contamination [4], a comprehensive evaluation of food matrices
Table 2: Bioassay-based categorization of 70 retail foods according to their potential for supporting cereulide production. Bioluminescence intensity produced by the cereulide synthesis reporter strain F4810/72(pMDX[P1/luxABCDE]) was measured after 24 hours of incubation at 24°C. Representative images are shown in Figure 3 and Figures S1–S3. Threshold values established for risk categorization are listed in Table S1.

| Low-risk foods | Risk foods | High-risk foods |
|----------------|------------|-----------------|
| Dried apricots | Reconstituted milk powder (organic) | Cereal-based reconstituted infant food (fruits flavour) |
| Dried apricots rehydrated with water | Cocoa powder with milk | Cereal-based reconstituted infant food (whole grain/apple flavour) |
| Cheese slices with Suisse flavour | Chocolate mouse | Cereal-based reconstituted infant food (fruit flavour) |
| Cheese slices with mozzarella flavour | Mashed potatoes (powder reconstituted with water) | Cheesecakes with vanilla flavour |
| Mashed with water | Milk powder with tapioca flour | Quark |
| Mashed with water | Soy milk | Soy milk based dessert with vanilla flavour |
| Cheese slices with Suisse flavour | Soy milk | Soymilk-based dessert with vanilla flavour |
| Mashed potatoes (made from cooked potatoes) | Mashed potatoes (powder reconstituted with water) | Mashed potatoes (powder reconstituted with water) |
| Mashed potatoes (made from cooked potatoes) | Mashed potatoes (powder reconstituted with water) | Mashed potatoes (powder reconstituted with water) |
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Figure 3: Scheme for abiotic factors influencing the activity of the ces NRPS promoter driving the synthesis of cereulide. The parameters were deduced from the examination of 70 foods and food ingredient using an emetic lux reporter strain [10]. The arrow denotes an increasing toxin formation capability with respect to the food composition. Examples of typical food matrices for each category are shown.

was hampered due to laborious, time-consuming, and error-prone methods to quantify cereulide amounts in foodstuffs. Recently, a SIDA-based method allowing the quantitative detection of cereulide has been developed [33]. However, alternative high-throughput methods to estimate the risk of toxin production in diverse food matrices are needed. The lux-based reporter system for real-time monitoring of toxin gene expression described by Dommel et al. [24] might represent an interesting tool in the latter context. We previously showed that cereulide production in model food matrices is proportional to the intensity of the bioluminescence signals emitted by the engineered B. cereus reporter strain [23, 24].

In this study, we employed the lux reporter system for the analysis of a total of 70 retail products in order to decipher abiotic and nutritional factors, either promoting or suppressing toxin synthesis. Luciferase signals were quantified with a software-assisted region-of-interest (ROI) analysis and foods were categorized into three main classes regarding
their toxin formation capability: high-risk, risk, and low-risk foods (Table 2, Figures S1–S3 in Supplementary Material available online at http://dx.doi.org/10.1155/2014/465603). Derived mean ROI values of each risk category and the corresponding determined threshold values are listed in Table S1. The bioassay revealed that 44% of the foods could be categorized as high-risk foods, while the remaining 20% and 36% were categorized as risk or low-risk foods, respectively (Table 2). Products classified as being insensitive were dairy based, displayed a low pH value (e.g., cream cheese and unsweetened quark), had a high fat content like chocolate and nut spread, and/or were characterized by low water availability or high osmolarity (e.g., dried fruits and 10% herbal salt solution). Earlier studies showed that growth of B. cereus was suppressed in foods with pH values below 5.0 [34–36], which is in line with our low-risk classification of matrices that had pH values around 4.3 to 4.8, such as the whey drinks. The combination of neutral pH values and medium $a_w$ values with high amounts of fat and cocoa was found to be indicative of the group of products being at medium risk of toxin synthesis (Table 2 and Figure S2). Likewise, proteinaceous foodstuff containing high fat amounts, such as minced beef or milk powder-based processed cheeses, fell in the same category. This is in agreement with a previous study [37] showing that cereulide was produced in small quantities in artificially contaminated meat products. The same study also supports our results concerning the pasteurized milk: usually, only low to medium cereulide levels are produced under stationary conditions at room temperature [30, 37]. Additionally, dairy products dulcified with glucose or fructose (quark desserts, cream-filled soft biscuits) fell in the intermediate class in terms of the risk for cereulide production. It was shown previously that glucose had a stimulating effect on cereulide synthesis [4]. The group of high-risk products comprised farinaceous foods, as well as powdered products that were reconstituted with water or milk (Table 2, Figure S3). Dairy- and cereal-based infant food formulas, which were additionally enriched with vitamins or trace elements, promoted exceptional high $ces$ promoter activities. The latter indicates that a combination of readily available saccharides, vitamins, and macronutrients in a pH neutral environment may stimulate toxin formation. Indeed, cereulide was detected in high levels in farinaceous matrices or systems containing high amounts of $K^+$ ions and vitamins [10, 38].

A summary of food characteristics commonly observed in the three categories is provided in Figure 3. This generalized scheme allows a basic preevaluation of foods and their ingredients concerning their capability to support cereulide formation and should facilitate hazard identification in terms of HACCP concepts.

4. Conclusion

Overall, our results indicate that emetic B. cereus strains occur more frequently and in a much broader diversity of foods than noticed so far. In addition, the lux-based real-time monitoring assay turned out to be a valuable tool for assessing the actual risk of cereulide toxin production in different types of food, allowing us to set up a general scheme for the categorizing of foods with respect to their cereulide production risk. Our survey of presumptive emetic B. cereus foodborne outbreaks also showed that the risk of an emetic syndrome caused by the B. cereus cereulide toxin is not restricted to high-carb foods, such as pasta and rice. Much more attention must be paid to other foods, especially the ones supporting cereulide production, as shown by the lux reporter assay.

Conflict of Interests

The authors have declared no conflict of interests.

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References

[1] N. Agata, M. Ohta, M. Mori, and M. Isobe, “A novel dodecadecapeptide, cereulide, is an emetic toxin of Bacillus cereus,” FEMS Microbiology Letters, vol. 129, no. 1, pp. 17–20, 1995.

[2] M. Ehling-Schulz, M. Fricker, and S. Scherer, “Bacillus cereus, the causative agent of an emetic type of food-borne illness,” Molecular Nutrition & Food Research, vol. 48, no. 7, pp. 479–487, 2004.

[3] M. Ehling-Schulz, U. Messelhäusser, and P. E. Granum, “Bacillus cereus in milk and dairy production,” in Rapid Detection, Characterization and Enumeration of Food-Borne Pathogens, J. Hoorfar, Ed., pp. 275–289, ASM Press, Washington, DC, USA, 2011.

[4] European Food Safety Authority (EFSA), “Opinion of the scientific panel on biological hazards of Bacillus cereus and other Bacillus spp. in foodstuff,” The EFSA Journal, vol. 175, pp. 1–48, 2005.

[5] European Food Safety Authority (EFSA) and European Center of Disease Control (ECDC), “The European Union Summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011,” The EFSA Journal, vol. 11, no. 4, Article ID 3129, 2013.

[6] K. Dierick, E. van Coillie, I. Swiecicka et al., “Fatal family outbreak of Bacillus cereus-associated food poisoning,” Journal of Clinical Microbiology, vol. 43, no. 8, pp. 4277–4279, 2005.

[7] K. M. Pósfay-Barbe, J. Schrenzel, J. Frey et al., “Food poisoning as a cause of acute liver failure,” Pediatric Infectious Disease Journal, vol. 27, no. 9, pp. 846–847, 2008.

[8] M. Naranjo, S. Denayer, N. Botteldoorn et al., “Sudden death of a young adult associated with Bacillus cereus food poisoning,” Journal of Clinical Microbiology, vol. 49, no. 12, pp. 4379–4381, 2011.

[9] U. Messelhäuser, P. Kämpf, M. Fricker et al., “Prevalence of emetic Bacillus cereus in different ice creams in Bavaria,” Journal of Food Protection, vol. 73, no. 2, pp. 395–399, 2010.

[10] R. Shaheen, M. A. Andersson, C. Apetroaei et al., “Potential of selected infant food formulas for production of Bacillus...
cereus emetic toxin, cereulide,” International Journal of Food Microbiology, vol. 107, no. 3, pp. 287–294, 2006.

[11] B. Svensson, A. Monthan, R. Shaheen, M. A. Andersson, M. Salkinoja-Salonen, and A. Christiansson, “Occurrence of emetic toxin producing Bacillus cereus in the dairy production chain,” International Dairy Journal, vol. 16, no. 7, pp. 740–749, 2006.

[12] C. Ankolekar, T. Rahmati, and R. G. Labbé, “Detection of toxigenic Bacillus cereus and Bacillus thuringiensis spores in U.S. rice,” International Journal of Food Microbiology, vol. 128, no. 3, pp. 460–466, 2009.

[13] L. Delbrassinne, M. Andjelkovic, K. Dierick, S. Denayer, J. Mahillon, and J. van Loco, “Prevalence and levels of Bacillus cereus emetic toxin in rice dishes randomly collected from restaurants and comparison with the levels measured in a recent foodborne outbreak,” Foodborne Pathogens and Disease, vol. 9, no. 9, pp. 809–814, 2012.

[14] M. Ehling-Schulz and U. Messelhäusser, “One pathogen but two different types of food borne outbreaks, Bacillus cereus in catering facilities in Germany,” in Case Studies in Food Safety and Quality Management: Lessons from Real-Life Situations, J. Hoorfar, Ed., pp. 63–70, Woodhead, Cambridge, UK, 2012.

[15] M. Fricker, U. Messelhäusser, U. Busch, S. Scherer, and M. Ehling-Schulz, “Diagnostic real-time PCR assays for the detection of emetic Bacillus cereus strains in foods and recent foodborne outbreaks,” Applied and Environmental Microbiology, vol. 73, no. 6, pp. 1892–1898, 2007.

[16] L. Maturin and J. T. Peeler, “Aerobic plate count,” in Bacteriological Analytical Manual, chapter 3, U.S. Food and Drug Administration, Silver Spring, Md, USA, 2001.

[17] International Organization of Standardization (ISO), “Microbiology of food and animal feeding stuffs—horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)—part 1: technique using Baird-Parker agar medium,” ISO 6888-1:1999, International Organization of Standardization (ISO), Geneva, Switzerland, 1999.

[18] International Organization of Standardization (ISO), “Microbiology of food and animal feeding stuffs—horizontal method for the determination of low numbers of presumptive Bacillus cereus—most probable number technique and detection method;” EN ISO 21871:2006, International Organization of Standardization (ISO), Geneva, Switzerland, 2006.

[19] International Organization of Standardization (ISO), “Milk and milk products—preparation of test samples and dilutions for microbiological examination,” EN ISO 8261:2001, International Organization of Standardization (ISO), Geneva, Switzerland, 2001.

[20] International Organization of Standardization (ISO), “Microbiology of food and animal feeding stuffs—preparation of test samples, initial suspension and decimal dilutions for microbiological examination—parts 1–5;” EN ISO 6887-4:2004, International Organization of Standardization (ISO), Geneva, Switzerland, 2004.

[21] International Organization of Standardization (ISO), “Microbiology of food and animal feeding stuffs—general requirements and guidance for microbiological examinations;” PrEN ISO 7218:2005, International Organization of Standardization (ISO), Geneva, Switzerland, 2005.

[22] M. Ehling-Schulz, M. Fricker, and S. Scherer, “Identification of emetic toxin producing Bacillus cereus strains by a novel molecular assay,” FEMS Microbiology Letters, vol. 232, no. 2, pp. 189–195, 2004.

[23] E. Frenzel, T. Letzel, S. Scherer, and M. Ehling-Schulz, “Inhibition of cereulide toxin synthesis by emetic Bacillus cereus via long-chain polyphosphates,” Applied and Environmental Microbiology, vol. 77, no. 4, pp. 1475–1482, 2011.

[24] M. Dommel, E. Frenzel, B. Straßer, C. Blöchinger, S. Scherer, and M. Ehling-Schulz, “Identification of the main promoter directing cereulide biosynthesis in emetic Bacillus cereus and its application for real-time monitoring of ees gene expression in foods,” Applied and Environmental Microbiology, vol. 76, no. 4, pp. 1232–1240, 2010.

[25] S. Samapundo, M. Heyndrickx, R. Xhaferi, and F. Devlieghere, “Incidence, diversity and toxin gene characteristics of Bacillus cereus group strains isolated from food products marketed in Belgium,” International Journal of Food Microbiology, vol. 150, no. 1, pp. 34–41, 2011.

[26] L. I. Ouoba, L. Thorsen, and A. H. Varnam, “Enterotoxins and emetic toxins production by Bacillus cereus and other species of Bacillus isolated from Sumbalba and Bikalga, African alkaline fermented food condiments,” International Journal of Food Microbiology, vol. 124, no. 3, pp. 224–230, 2008.

[27] L. M. Wijnands, J. B. Dufrenne, F. M. Rombouts, P. H. In’t Veld, and F. M. van Leusden, “Prevalence of potentially pathogenic Bacillus cereus in food commodities in the Netherlands,” Journal of Food Protection, vol. 69, no. 11, pp. 2587–2594, 2006.

[28] A. Doménech-Sánchez, E. Laso, M. J. Pérez, and C. I. Berrocal, “Emetic disease caused by Bacillus cereus after consumption of tuna fish in a beach club,” Foodborne Pathogens and Disease, vol. 8, no. 7, pp. 835–837, 2011.

[29] M. Dommel, G. Lücking, S. Scherer, and M. Ehling-Schulz, “Transcriptional kinetic analyses of cereulide synthetase genes with respect to growth, sporulation and emetic toxin production in Bacillus cereus,” Food Microbiology, vol. 28, no. 2, pp. 284–290, 2011.

[30] A. Rajkovíc, M. Uyttendaele, S.-A. Ombregt, E. Jaaskelainen, M. Salkinoja-Salonen, and J. Debevere, “Influence of type of food on the kinetics and overall production of Bacillus cereus emetic toxin,” Journal of Food Protection, vol. 69, no. 4, pp. 847–852, 2006.

[31] M. Ehling-Schulz and U. Messelhäusser, “Bacillus next generation” diagnostics: moving from detection toward subtyping and risk-related strain profiling,” Frontiers in Microbiology, vol. 4, article 32, 2013.

[32] M. Fricker, R. Reissbrodt, and M. Ehling-Schulz, “Evaluation of standard and new chromogenic selective plating media for isolation and identification of Bacillus cereus,” International Journal of Food Microbiology, vol. 121, no. 1, pp. 27–34, 2008.

[33] T. Bauer, T. Stark, T. Hofmann, and M. Ehling-Schulz, “Development of a stable isotope dilution analysis for the quantification of the Bacillus cereus toxic cereulide in foods,” Journal of Agricultural and Food Chemistry, vol. 58, no. 3, pp. 1420–1428, 2010.

[34] M. Valero, P. S. Fernandez, and M. C. Salmeron, “Influence of pH and temperature on growth of Bacillus cereus in vegetable substrates,” International Journal of Food Microbiology, vol. 82, no. 1, pp. 71–79, 2003.

[35] D. Lindsay, V. S. Brözel, J. F. Mostert, and A. Holy, “Physiology of dairy-associated Bacillus spp. over a wide pH range,” International Journal of Food Microbiology, vol. 54, no. 1-2, pp. 49–62, 2000.
[36] N. Agata, M. Ohta, M. Mori, and K. Shibayama, “Growth conditions of and emetic toxin production by Bacillus cereus in a defined medium with amino acids,” Microbiology and Immunology, vol. 43, no. 1, pp. 15–18, 1999.

[37] N. Agata, M. Ohta, and K. Yokoyama, “Production of Bacillus cereus emetic toxin (cereulide) in various foods,” International Journal of Food Microbiology, vol. 73, no. 1, pp. 23–27, 2002.

[38] C. Apetroaie-Constantin, R. Shaheen, L. Andrup, L. Smidt, H. Rita, and M. Salkinoja-Salonen, “Environment driven cereulide production by emetic strains of Bacillus cereus,” International Journal of Food Microbiology, vol. 127, no. 1-2, pp. 60–67, 2008.