Research Note

Prevalence of Emetic Bacillus cereus in Different Ice Creams in Bavaria

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ABSTRACT

In this study, 809 samples of ice cream from different sources were investigated by using cultural methods for the presence of presumptive Bacillus cereus. Isolates from culture-positive samples were examined with a real-time PCR assay targeting a region of the cereulide synthetase gene (ces) that is highly specific for emetic B. cereus strains. The samples were collected from ice cream parlors and restaurants that produced their own ice cream and from international commercial ice cream companies in different regions of Bavaria during the summer of 2008. Presumptive B. cereus was found in 508 (62.7%) ice cream samples investigated, and 24 (4.7%) of the isolates had the genetic background for cereulide toxin production. The level of emetic B. cereus in the positive samples ranged from 0.1 to 20 CFU/g of ice cream.

In Germany, the incidence of food poisoning caused by the emetic Bacillus cereus toxin cereulide is largely unknown. Most cases of foodborne B. cereus intoxication are self-limiting with mild and temporary symptoms, and the affected people do not usually seek medical treatment. Attending doctors in Germany have a legal obligation (5) to report the suspicion or diagnosis of B. cereus intoxication; however, these cases often pass unnoticed. In 2007, five foodborne outbreaks caused by diarrheic and emetic toxins of Bacillus spp. were reported in Germany, with 72 human cases; however, no hospitalizations or deaths occurred (13). Nevertheless, in the last few years the possible severity of the emetic syndrome was reported in cases from Switzerland and Belgium, where foodborne B. cereus intoxications caused hospitalization and death in both children and adults (9, 24, 29).

During the last few summers, possible cases of foodborne illness were noticed in Bavaria after consumption of ice cream from ice cream parlors and restaurants, which produce their own ice cream, and after consumption of ice cream produced by international companies. The children and adults had symptoms typical of a bacterial intoxication normally caused by the enterotoxins of Staphylococcus aureus or emetic B. cereus, i.e., emesis 1 or 2 h after the consumption of the suspected ice cream. Testing of the samples for the presence of foodborne pathogens, especially S. aureus and its enterotoxins, yielded negative results in most cases. However, the samples were not routinely investigated for the presence of emetic B. cereus or cereulide toxin. To our knowledge, in other countries there are few available data on the presence of emetic B. cereus strains in ice cream (25) and no data on confirmed cases of foodborne illness caused by the cereulide toxin in ice cream.

For development of a well-grounded risk assessment and an effective consumer protection policy, it is important to have information about the prevalence of emetic B. cereus strains in different kinds of food. The prevalence of emetic B. cereus has been reported in ready-to-eat food in Denmark (31), in rice in the United States (3), and in different soils, animal feces, and vegetable samples in the United Kingdom (2). In Germany, only a few reports exist about the detection of emetic B. cereus strains and cereulide toxin in outbreak situations (15) associated with common foods such as rice or pasta (26) or in food supply facilities of the German Federal Armed Forces (23).

The aim of our study was to investigate the prevalence of emetic B. cereus strains in different ice creams. Classical microbiological methods were used in combination with a validated real-time PCR assay targeting a region of the cereulide synthetase gene (ces) that is highly specific for emetic B. cereus strains (15).

MATERIALS AND METHODS

Material. A total of 809 ice cream samples, 800 from ice cream parlors and restaurants producing their own ice cream and 9 from international companies in different regions of Bavaria, were investigated during the summer of 2008 for typical hygiene parameters (incidence of Enterobacteriaceae and S. aureus and presence of Salmonella) and for the presence of emetic B. cereus. An overview of the examined ice cream flavors is presented in Figure 1. In the German guidelines for ice cream, “milk ice
B. cereus gene with real-time PCR assay. The MPN of emetic B. cereus, Salmonella (18) was used for the detection of the emetic gene cluster. The heterologous Internal Amplification Control (IAC) System based on the pUC 19 plasmid (Fermentas, St. Leon-Rot, Germany) was used with a modified primer and probe system as described previously (26). The pUC 19 plasmid was used without any modifications and with primers (IAC_2-fw: 5'-CGT AAT AGC GAA G-3' and probe (IAC_2-re: 5'-GAG CGT AAT AGC GAA G-3') and probe (IAC_2-P: HEX-GAG AAA ATA CCG CAT CAG GC-TAMRA) specific for the pMB1 replicon of the plasmid.

The real-time PCR assay was performed as described previously (15, 26). One suspect colony was suspended in 1.5 ml of 0.9% NaCl and heated for 10 min at 95°C, and 5 μl of the suspension was used with 20 μl of a commercial MasterMix (Agilent Technologies, Santa Clara, CA) and 1 fg of the pUC 19 plasmid. For the PCR positive control, 5 μl of ces-positive DNA was used, and for the no-template control, 5 μl of 0.9% NaCl was used instead of the colony material.

RESULTS

Hygiene parameters and Salmonella. None of the 809 investigated ice cream samples were positive for S. aureus or Salmonella. For 681 samples, the quantitative results for the detection of Enterobacteriaceae were below 100 CFU/g; in 35 samples, we detected contamination at more than 1,000 CFU/g (Fig. 2).

Qualitative analysis. In 508 (62.7%) of 809 ice cream samples, presumptive B. cereus strains were found after enrichment and plating. The isolates from 24 samples were positive for the ces gene based on the results of the real-time method was used in accordance with standard reference culture methods recommended by the ISO (20) and the U.S. Food and Drug Administration Bacteriological Analytical Manual (7). A 10-g sample of material was weighed into 90 ml of sterile peptone diluent, 0.1% (1:10 dilution) and decimal dilutions were prepared, and 1-ml portions were transferred to three tubes with TPGY broth for each dilution for at least three consecutive dilutions. The tubes were incubated at 30 ± 2°C under aerobic conditions for 24 ± 2 h. After the incubation period, one loopful of every tube was plated on Mossel agar and incubated as described above. Every suspect colony on Mossel agar was examined for the presence of the ces gene with real-time PCR assay. The MPN of emetic B. cereus was calculated base on the real-time PCR results (7).

Investigation of hygiene parameters and Salmonella.

Every ice cream sample was examined for the level of Enterobacteriaceae (19) and the presence of S. aureus (17) in 10 g of ice cream and the presence of Salmonella (18) in 25 g of ice cream, in accordance with standard reference culture methods recommended by the International Organization for Standardization (ISO).

Qualitative detection of B. cereus. For the qualitative detection of emetic B. cereus, 10 g of sample material was transferred to 90 ml of TPGY (tryptone, glucose, yeast) broth (Merck, Darmstadt, Germany), gently mixed, and incubated at 30 ± 2°C under aerobic conditions. After 24 h of incubation, one loopful of the enrichment broth was plated on Mossel (mannitol, egg yolk, polymyxin) agar (Oxoid, Basingstoke, UK) and incubated at 30 ± 2°C under aerobic conditions. Plates were read after 24 and 48 h. One to three different colonies with morphology typical of B. cereus on Mossel agar were examined for the presence of the ces gene using a real-time PCR assay (15, 26).

Quantitative detection of B. cereus. For quantitative detection of emetic B. cereus, the most-probable-number (MPN)
PCR assay, indicating the genetic potential to produce the cereulide toxin. One emetic B. cereus isolate originated from fruit ice cream (Fig. 1B), and the other 23 isolates were from different types of milk ice cream (Fig. 1A). Most of the ice cream samples were collected in restaurants and ice cream parlors, and 22 of the positive samples came from these sources. These ice cream types contained pasteurized milk or water (one sample) and either fresh ingredients, e.g., fresh fruit or nuts, or different dried ice cream mixtures. Two ice cream samples that were positive for emetic B. cereus were commercial products from different ice cream companies. One sample was from vanilla ice cream covered with a strawberry icing, and the other sample was from a mixture of vanilla ice cream, pear ice cream, and strawberry sauce.

Quantitative analysis. The samples positive for emetic B. cereus after enrichment were also investigated with an MPN colony count technique in combination with the real-time PCR assay for detection of the ces gene. The results of the quantitative analysis are shown in Table 1. Most samples were positive for emetic B. cereus only after enrichment (calculated level of emetic B. cereus at 0.1 to 1 CFU/g), and the most contaminated sample contained 20 CFU/g.

**DISCUSSION**

Spores and vegetative cells of the B. cereus group are ubiquitous in the environment and therefore are commonly found in different kinds of unprocessed food. However, emetic B. cereus strains seem to be rare in the environment (2) and in different foods other than rice and pasta, such as fermented food (28), ready-to-eat food (31), and vegetables (2). Rice and pasta are the foods typically associated with emetic B. cereus-related foodborne outbreaks (15) and are therefore investigated regularly for the presence of this pathogen or the cereulide toxin.

Comprehensive studies of the prevalence of emetic B. cereus strains mainly in foods other than rice and pasta have not been reported in Germany until now. Samples collected during foodborne intoxication investigations are routinely evaluated for the presence of S. aureus or its enterotoxins but rarely evaluated for the presence of emetic B. cereus. However, the emetic cereulide toxin and the Staphylococcus enterotoxins can cause nearly the same symptoms, which may be why causative agents of many foodborne intoxications remain unknown. In Bavaria, possible cases of foodborne illness, contracted after consumption of ice cream, that manifest the symptoms typical of a bacterial intoxication are observed every year without any indication of the presence of S. aureus enterotoxin. Therefore, the aim of our study was to use routine diagnostic methods to check ice cream samples from different sources for the prevalence of B. cereus strains with the genetic potential to produce the cereulide toxin. Presumptive B. cereus isolates were found in 62.7% of the ice cream samples evaluated. Most of these samples (800 of 809 samples) were collected from ice cream parlors and restaurants, which could be why the prevalence of emetic B. cereus found in this study is much higher than that described in one of the few other studies of ice cream samples, in which commercially produced ice cream was the focus. In that study, Masud (25) found presumptive B. cereus in only 4.0% of different commercially prepared ice cream samples in Pakistan.

The detection of emetic B. cereus in the ice cream samples was not correlated with the hygienic status of the samples. In all ice cream portions positive for emetic B. cereus, the level of Enterobacteriaceae, the indicator for adequate production hygiene, was below 100 CFU/g, the limit the European Commission (12) set in Regulation (EC) No 2073/2005 for ice cream at the end of the production process. Therefore, adequate hygienic during the production does not preclude the presence of emetic B. cereus or eliminate this microorganism from the end product and both homemade and commercially produced ice cream samples were positive for the presence of emetic B. cereus. In our qualitative and quantitative investigations, the different dried ice cream mixtures used were not contaminated with emetic B. cereus strains. However, because representative sampling of larger lots of dried ice cream mixtures is very difficult, we cannot exclude the possibility of an entry of emetic B. cereus through the use of contaminated mixtures. Another possibility for the origin of the emetic B. cereus strains is the use of pasteurized milk in all of the samples investigated. B. cereus spores can survive the pasteurization process (27), and there are some reports of emetic B. cereus strains in raw milk (33), milk products (10, 11), and dried infant formula (32), production of which also includes a pasteurization step. Therefore, pasteurized milk is a possible vehicle for the entry of emetic B. cereus into ice cream.

The results of our study indicate that emetic B. cereus intoxication can be caused by contaminated ice cream, but adequate risk assessment is very complex. Thorsen et al. (35) found psychrotolerant emetic Bacillus weihenstephan-
nensis strains, highlighting the possibility that emetic strains can grow at refrigerator temperature. However, Carlin et al. (8) and others assumed, based on their results, that emetic strains do not represent a special risk in refrigerated food (21). Cereulide production at 8°C is minimal (14, 16), and at 5°C there is no detectable cereulide production (34).

The level of emetic B. cereus found in samples associated with foodborne outbreaks normally ranged between 10⁴ and 10⁶ CFU/g (15). Nevertheless, in some outbreaks, lower B. cereus numbers have been reported (22). Therefore, the levels of emetic B. cereus detected in ice cream in this study generally might be too low to produce a sufficient amount of cereulide toxin to cause human intoxication, apart from the possible presence of strains producing unusually high amounts of cereulide (6). The highest risk for foodborne emetic B. cereus intoxication caused by ice cream is associated with the use of contaminated ingredients, e.g., milk containing cereulide (1) or the generation of cereulide toxin because of inadequate temperature management during the production process. During the ice cream freezing process, the number of vegetative cells of B. cereus might be reduced to a very low or undetectable level, but the cereulide survives the freezing process without losing toxicity. Therefore, the detection of emetic B. cereus strains in ice cream is an indication of possible cereulide production and the possible presence of cereulide toxin in the sample. Because of its high heat and acid stability (30), cereulide toxins formed in the raw material or in an early food production step will not be eliminated by normal food processing.

Further investigations, e.g., of the ingredients of ice cream, are necessary to identify the contamination points associated with emetic B. cereus intoxication from the consumption of ice cream.

REFERENCES


