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Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food

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Abstract

Among 48,901 samples of ready-to-eat food products at the Danish retail market, 0.5% had counts of *Bacillus cereus*-like bacteria above 10^4 cfu g^{-1}. The high counts were most frequently found in starchy, cooked products, but also in fresh cucumbers and tomatoes. Forty randomly selected strains had at least one gene or component involved in human diarrhoeal disease, while emetic toxin was related to only one *B. cereus* strain. A new observation was that 31 out of the 40 randomly selected *B. cereus*-like strains could be classified as *Bacillus thuringiensis* due to crystal production and/or content of *cry* genes. Thus, a large proportion of the *B. cereus*-like organisms present in food may belong to *B. thuringiensis*.

Keywords: *Bacillus cereus*; *Bacillus thuringiensis*; Ready-to-eat food; Emetic toxin; Enterotoxins; Insecticidal toxins

1. Introduction

Bacillus cereus-like organisms can readily be isolated from various food products [1]. The organisms are common in nature, and due to their resistant endospores they may survive different stresses during food production, e.g. drying and heat treatment. The group of *B. cereus*-like organisms comprises, besides *B. cereus* sensu stricto, the insect pathogen *Bacillus thuringiensis*, the human pathogen *Bacillus anthracis*, and the rhizoid *Bacillus mycoides*. Recently, *Bacillus pseudomycoides* [2] and the psychrotolerant *Bacillus weihenstephanensis* [3] have also been included into this group. *B. cereus* and *B. thuringiensis* are closely related and genomic studies have proposed that they should be merged into a single species [4]. However, the name *B. thuringiensis* is retained for those strains that produce crystalline parasporal inclusions.

B. cereus is a well-known food borne pathogen causing two types of illness: the emetic and the diarrhoeal syndrome. The former is due to a small-molecular weight cyclic toxin, cereulide [5], while the diarrhoeal syndrome results from the production of enterotoxins [6]. Cereulide is produced in the food, whereas the enterotoxins are believed to be produced in the intestine after ingestion of *B. cereus*-like organisms [7]. The two most well-characterised enterotoxins are haemolysin BL (HBL) and the non-haemolytic enterotoxin (NHE). Both are three-component toxins requiring expression

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of all three components for virulence [8,9]. These two enterotoxins typically give relatively mild and short-lived diarrhoeal syndrome. A third enterotoxin, the single-component cytotoxin K (CytK) has so far only been reported to be involved in a single case of severe food poisoning outbreak including the death of three persons [10].

B. thuringiensis is used worldwide as a biological insecticide due to the production of crystal (*cry*) proteins (δ-endotoxins) with highly specific activity against certain insects [11]. For example, strains of *B. thuringiensis* subspecies *kurstaki* producing the crystal protein Cry 1, are toxic for insect species within *Lepidoptera*, strains of *B. thuringiensis* subsp. *israelensis* producing Cry 11 are toxic for species within *Diptera*, and strains of *B. thuringiensis* subsp. *tenebrionis* producing Cry 3 are toxic for species within *Coleoptera* [12]. More than 100 different δ-endotoxins have been characterised, the majority with insecticidal activity. Besides the Cry proteins other insecticidal proteins unrelated to Cry are also produced by some strains of *B. thuringiensis*, e.g. the cytolytic (Cyt) proteins. These toxins are thought to act in combination with Cry proteins to cause cytolysis in insects, however, Cyt proteins are in contrast to the δ-endotoxins not specific against insects, but are also haemolytic and cytolytic against many mammalian cell lines [13].

Insecticides based on different *B. thuringiensis* strains producing different δ-endotoxins are commercially available as powdered or granulated formulations containing a mixture of endospores and crystal proteins. The products are sprayed onto crops such as cabbage, wine grapes, tomatoes, cucumbers, and peppers. Approximately 50% of the bio-pesticides on the Danish market are *B. thuringiensis*-based, and *B. thuringiensis*-containing products constitute 90% of microbial bio-insecticides on the world market [14].

After the commercialization of *B. thuringiensis*-based insecticides, studies have shown that *B. thuringiensis* (including commercial strains used for insect control) like *B. cereus* produces enterotoxins responsible for human diarrhoea [15]. *B. thuringiensis* has, however, only in one case been described to be implicated in food borne disease [16]. Despite the pathogenic characteristics of *B. thuringiensis*, the presence of this bacterial species in food and food borne disease is not well described, probably because methods for detection and enumeration of *B. cereus*-like organisms in food and clinical settings do not distinguish between *B. cereus* and *B. thuringiensis*. Given the taxonomic similarity of *B. cereus* and *B. thuringiensis* and the introduction of high numbers of *B. thuringiensis* spores onto vegetables treated with *B. thuringiensis*-based insecticides, we speculate that a proportion of *B. cereus*-like organisms present in ready-to-eat food are *B. thuringiensis* and consequently, that some of the food borne diseases diagnosed as *B. cereus* infections are actually caused by *B. thuringiensis*.

The aims of the present study were to determine the occurrence of *B. cereus*-like organisms in ready-to-eat food at retail level in Denmark, to estimate the relative occurrence of *B. thuringiensis* in randomly selected food samples, to evaluate the potential of *B. cereus* and *B. thuringiensis* in food borne disease based on their production of emetic and diarrhoeal toxins, and finally to examine the antibiotic resistance of *B. cereus*-like organisms in food in order to evaluate if antibiotic resistance could be used for species differentiation.

2. Materials and methods

2.1. Sampling

Samples of ready-to-eat food products were randomly collected nationwide from local retail establishments (greengrocers, butchers, supermarkets, restaurants, etc.) by the Regional Veterinary and Food Authorities according to standard procedures as a part of the authorities' routine control of the microbiological quality of food. The random sampling independent of food conditions such as shelf-life and storage temperature was carried out to estimate the variety in the microbial quality of food ready for consumption and thereby the variety in human exposure from these products. After sampling the food samples were kept at 0–5 °C until analysis within 24 h.

2.2. Enumeration and confirmation of Bacillus cereus-like strains

The number of *B. cereus*-like organisms was estimated according to a Nordic standard procedure [17] using plate spreading of known amounts of sample on blood agar and/or on *B. cereus* selective agar (Oxoid, Basingstoke, UK). This method detects the mesophilic species *B. cereus* sensu stricto and *B. thuringiensis*, but also the psychrotolerant *B. weihenstephanensis*. In this study, we have not included methods to differentiate between *B. weihenstephanensis* and *B. cereus* sensu stricto. In the present study, we only mention *B. cereus*, although we are aware of the fact that the strains may belong both to *B. cereus* sensu stricto and *B. weihenstephanensis*. A number of 40 strains from different food sources, containing both high and low concentrations of *B. cereus*-like organisms, were randomly selected for further characterization. It was confirmed that the strains belonged to the *B. cereus* group by carrying out biochemical tests comprising fermentation of nine different carbohydrates and cleavage of several enzyme substrates linked to fluorophores using the Sensititre system for identification of Gram-positive bacteria (Trek Diagnostic Systems Ltd., East Grinstead, UK),. Three commercially available *B. thuringiensis*-based insecticides, Dipel®, Vectobac® and Bactimos® were also included in the investigation.

2.3. Detection of crystal proteins and selected insecticide toxin genes

The isolates were streaked onto Agar Starch Medium (BD Bioscience, Erembodegem, Belgium) and inspected by phase-contrast microscopy for intracellular crystals, which are characteristic for *B. thuringiensis* after growth for 2–3 days at 30 °C.

Furthermore, PCR analyses were carried out to detect various groups of insecticide toxin genes from *B. thuringiensis*. Five general primer sets were employed. The primer sets for detecting *cry1*, *cry3*, *cry11* and *cyta* were previously described [18]. An additional primer set was designed to detect genes for the insecticidal toxin Cry4 (Cry4a: 5′-CAGGTACCGGTGGAATGAATTATA-3′ and Cry4b: 5′-GCTCTAGAGACTTCTACTTTAGTA) generating a fragment of 1932 bp. Total genomic DNA was isolated by the method of Boe and co-workers [19]. All PCR amplifications were performed in a Programmable Thermal Controller PTC-100 (MJ Research, Bio-Rad, Waltham, MA, USA). One PCR comprised 24 μl PCR SUPERMIX (Gibco BRL, Invitrogen, Taastrup, Denmark), 20 μM primer, and 1 μg genomic DNA. The PCR conditions were the following: an initial denaturation step for 5 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 52 °C, and 3 min at 72 °C, and a final extension at 72 °C for 5 min.

2.4. Detection of genes and components for enterotoxins and emetic toxin

The gene for the B component of HBL (*hblA*) was amplified using the HblA primers described by Wencheng [20]. The recently published CK primers [21] and EM1 primers [22] were used for detection of genes coding for CytK and the emetic toxin, respectively. DNA was extracted by boiling a bacterial colony for 10 min in TE-buffer. After centrifugation, the supernatant was used in a PCR consisting of one Ready-To-Go PCR bead (Amersham Pharmacia Biotech, Buckinghamshire, UK), 10 pmol of each primer and 5 μl of DNA. PCR was performed in a Peltier Thermal Cycler PTC-225 (MJ Research, Bio-Rad, Waltham, MA, USA) with the following conditions: an initial denaturation step at 94 °C for 10 min, followed by 30 cycles at 92 °C for 40 s, 55 °C for 40 s, 72 °C for 90 s, and a final extension at 72 °C for 7 min.

Enterotoxin production was tested using the *B. cereus* Enterotoxin reverse Passive Agglutination test from Oxoid (BCET-RPLA) (Oxoid, Basingstoke, UK) and the *B. cereus* Diarrhoeal Enterotoxin Visual Immuno Assay (TECRA BDE-VIA) (Tecra Diagnostics, Reading, UK). The BCET-RPLA kit detects the L_2 component of HBL whereas the TECRA BDE-VIA kit detects the 45 kDa protein of the NHE complex. Overnight cultures originating from single colonies were diluted 1:20 in fresh BHI broth medium (Oxoid, Basingstoke, UK) and incubated with aeration at 30 °C for further 12–14 h. The supernatant was isolated by centrifugation (2000*g*, 20 min, 4 °C) and analysed in accordance with the manufacturers' instructions.

2.5. Susceptibility to antimicrobials

The antimicrobial susceptibility was determined by broth dilution testing from Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK). The microtitre wells were inoculated according to NCCLS guidelines [23] and incubated aerobically at 37 °C for 18–24 h. The MIC-value was defined as the lowest concentration of antimicrobial that produced no visible growth. Isolates were tested with the following antibiotics and testing ranges: chloramphenicol (1–64 μg ml^{-1}), ciprofloxacin (0.03–8 μg ml^{-1}), gentamicin (0.5–32 μg ml^{-1}), streptomycin (2–128 μg ml^{-1}), penicillin (2–128 μg ml^{-1}), erythromycin (1–32 μg ml^{-1}), vancomycin (2–32 μg ml^{-1}) and tetracycline (0.5–32 μg ml^{-1}). For interpretation of the MIC results the following breakpoints were chosen: ciprofloxacin $>= 4$ μg ml^{-1}, erythomycin $>= 8$ μg ml^{-1}, gentamicin, vancomycin and tetracycline $>= 16$ μg ml^{-1}, chloramphenicol, streptomycin, and penicillin $>= 32$ μg ml^{-1}.

3. Results

3.1. Occurrence of B. cereus-like organisms in ready-to-eat food

In the routine surveillance of ready-to-eat foods for sale at the retail market in Denmark, a total of 48,901 randomly collected samples with unknown preparation and storage conditions were tested for the occurrence of *B. cereus*-like organisms at the point of sampling in the period 2000–2003 (Table 1). The products analysed were fresh fruits and vegetables, heat-treated products such as ready-prepared dishes, sauces, meat, pasta, and rice, and products with both fresh and heat-treated ingredients, e.g. sandwiches, pasta salad, vegetable/meat/fish mayonnaise, and desserts including ice cream and cream-cakes. The desserts analysed contained typically heat-treated starchy ingredients and/or milk and milk products. Enumeration of *B. cereus*-like organisms showed that 98.7% of the products had counts below 10^3 cfu g^{-1}, 0.7% were in the range 10^3–10^4 cfu g^{-1}, and 0.5% of the samples had counts above 10^4 cfu g^{-1} (Table 1). The high counts were most frequently found in fresh cucumbers and tomatoes, heat-treated rice, cake custard, and in desserts with milk and rice. The latter is a Danish speciality called 'ris a la mande', which is made from rice boiled in milk added almonds and whipped cream.

Table 1
The occurrence of *B. cereus*-like organisms isolated from retail ready-to-eat food in Denmark in the period 2000–2003

Food category	Number of samples investigated	Percentage of samples containing *B. cereus*-like organisms within the range		
	N	<1000 cfu g^{-1}[a]	1000–10,000 cfu g^{-1}[a]	>10,000 cfu g^{-1}[a]
Fresh food				
Lettuce	131	97.7	2.3	0
Root vegetables	41	100.0	0	0
Cucumbers, tomatoes	38	97.4	0	2.6
Sprouts	40	100.0	0	0
Other vegetables	367	95.9	3.8	0.3
Berries	57	100.0	0	0
Fruits	317	97.2	2.8	0
Sum	991	97.2	2.6	0.2
Heat-treated food				
Ready-prepared dishes	14,393	99.0	0.7	0.4
Sauces	4288	98.8	0.7	0.4
Soups	1723	98.6	0.7	0.7
Pâté, liver paste, etc.	529	99.8	0.2	0
Vegetables	428	98.1	0.9	0.9
Sausages	1666	99.1	0.6	0.3
Meat for open sandwiches	4,215	99.7	0.3	0.1
Bread	53	100.0	0	0
Pasta	2216	98.7	1.0	0.3
Rice	1070	97.6	1.1	1.3
Sum	30,581	99.0	0.6	0.4
A combination of raw and heat treated food				
Open sandwiches	419	99.0	1.0	0
Pasta salad	593	98.5	1.2	0.3
Dressings	696	99.0	0.9	0.1
Vegetable/meat/fish mayonnaise	1748	98.8	1.1	0.1
Desserts with milk and flour	2350	97.5	1.5	1.0
Desserts with milk and rice	223	95.1	1.8	3.1
Cake custard	1601	97.5	1.3	1.2
Ice-cream with milk products	4751	99.3	0.5	0.2
Cream-cakes, etc.	4948	97.8	1.5	0.8
Sum	13,873	98.3	1.1	0.6
Total sum	48,901	98.7	0.7	0.5

[a] According to the Danish guideline for evaluation of *B. cereus-like* organisms in ready-to-eat food <1000 cfu g^{-1} is satisfactory, 1000–10,000 cfu g^{-1} is not satisfactory, and >10,000 cfu g^{-1} is not acceptable [25].

3.2. Detection of crystals and crystal protein genes

The ability of 40 randomly selected strains to produce intracellular crystals, together with their content of selected *cry* genes, is presented in Table 2. Twenty-eight of the strains produced visible crystals and were therefore classified as *B. thuringiensis*. Of these 28 strains, 10 were positive for *cry1* and two positive for *cry11*. Furthermore, one strain was positive for *cry11* and two positive for *cyta* without a visible crystal protein giving a total of 31 *B. thuringiensis* strains. None were found to harbour genes for production of Cry3 and Cry4. Nine strains contained no visible crystal protein and were negative in PCR for the *cry* genes tested.

3.3. Distribution of genes and components involved in human disease

The production of selected protein components, and the presence of selected genes coding for proteins involved in the diarrhoeal and emetic syndrome, is listed in Table 2. The 45-kDa protein of the NHE complex was produced by all 40 strains, 36 strains also produced the L_2 component of the HBL complex or had genes coding for the B component of HBL, and 27 strains had genes coding for CytK. These results show that all strains examined had genes or components for toxins involved in human diarrhoeal disease. Genes for the emetic toxin were only found in one strain identified as *B. cereus*.

Table 2
Production of insecticidal toxins, enterotoxins and emetic toxin by *B. cereus*-like organisms isolated from ready-to-eat food

Strain	Source	Insecticidal proteins			Enterotoxins					Emetic toxin
		cry1 (PCR)	*cry11* (PCR)	*cyta* (PCR)	Crystal visible	*hblA* (PCR)	HBL (Oxoid[a])	NHE (Tecra[b])	*cytK* (PCR)	(PCR)
9939	Pasta	−	−	−	+	+	+	+	+	−
9942	Pasta	−	−	−	+	+	+	+	+	−
9943	Pasta	−	−	−	+	+	+	+	+	−
9972	Pasta	−	−	−	+	+	+	+	+	−
10368	Baby maize	−	−	−	+	+	+	+	+	−
10617	Honey	−	−	−	+	+	+	+	+	−
10752	Pasta	−	−	−	+	+	+	+	+	−
10787	Spinach	−	−	−	+	+	+	+	+	−
11129	Soft ice	−	−	−	+	+	+	+	+	−
9999	Raw sausage	+	−	−	+	+	+	+	+	−
10587	Pasta	+	−	−	+	+	+	+	+	−
990003	Red pepper	+	−	−	+	+	+	+	+	−
990004	Cauliflower stowage	+	−	−	+	+	+	+	+	−
990005	Red pepper	+	−	−	+	+	+	+	+	−
Dipel	Commercial biopesticide	+	−	−	+	+	+	+	+	−
Bactimos	Commercial biopesticide	−	+	+	+	+	+	+	+	−
Vectobac	Commercial biopesticide	−	+	+	+	+	+	+	+	−
10480	Pasta	−	−	−	+	+	+	+	−	−
10484	Parsley	−	−	−	+	+	+[c]	+	−	−
10569	Salad	−	−	−	+	+	+	+	−	−
10786	Parsley	−	−	−	+	+	+	+	−	−
11486	Dill	−	−	−	+	+	+	+	−	−
9945	Pasta	−	−	+	+	+	+	+	+	−
10469	Spinach stowage	−	−	+	+	+	+	+	+	−
9902	Bread	+	−	+	+	+	+	+	+	−
10326	Leek	+	−	+	+	+	+	+	+	−
903	Broccoli	−	−	−	−	+	+	+	+	−
9941	Pasta	−	−	−	−	+	+	+	+	−
9944	Pasta	−	−	−	−	+	+	+	+	−
10557	Pasta	−	−	−	−	−	−	+	+	−
11488	Mashed potatoes	−	−	−	−	−	−	+	+	−
10570	Mashed potatoes	−	−	−	−	−	−	+	−	−
11128	Aubergine	−	−	−	−	−	−	+	−	−
9900	Pasta salad	−	−	+	−	−	−	+	−	−
9937	Pasta	−	−	+	−	+	+	+	+	−
9946	Kebab	−	−	−	+	−	+	+	+	−
10290	Salad	+	+	+	+	−	+	+	+	−
10329	Pasta	−	−	−	−	−	−	+	−	+
10584	Carrots	−	−	−	+	−	+[c]	+	−	−
10616	Honey	+	−	−	+	+	+	+	−	−
11280	Pasta	−	−	−	−	+	+	+	−	−
11294	Strawberry tart	−	+	−	−	−	−	+	−	−
11302	Fig spread	+	+	+	+	+	+	+	+	−
Total[d]		10/40	3/40	8/40	28/40	30/40	36/40	40/40	27/40	1/40

The strains are grouped according to their geno-and phenotypic profiles.
[a] +, activity at 30 and 37 °C; −, no activity at 30 and 37 °C.
[b] +, OD value between 0.2 or higher than that obtained for the positive control at 30 and 37 °C; −, OD lower than or equal to 0.2 at 30 and 37 °C.
[c] Strains 10484 and 10584 were positive at 30 °C, but negative at 37 °C (the strains grew poorly at 37 °C).
[d] The number of positive food isolates/the number of food isolates tested.

3.4. Geno- and phenotypic profiles

Grouping of the 40 randomly selected strains based on their geno- and phenotypic characteristics as regards crystal proteins, enterotoxins and emetic toxin (Table 2) showed that these characteristics were independent on the food source of the various strains. By comparing the profiles of the food isolates with the profiles of the commercial biopesticides it was observed that five strains isolated from sausage, pasta, red pepper (×2), and cauliflower stowage had profiles similar to the commercial Dipel® strain.

3.5. Susceptibility to antimicrobials

Apart from intrinsic penicillin resistance found in 36 strains – caused by the production of β-lactamase by *B. cereus*-like organisms [24], the strains were sensitive to

Table 3
Distribution of Minimum Inhibitory Concentration (MIC) and occurrence of resistance to eight antimicrobial agents among 40 randomly selected isolates of *B. cereus*-like organisms isolated from ready-to-eat food

Antimicrobial agent	MIC (μg/ml)	% Resistant		
	Range	MIC_{50}	MIC_{90}	$n = 40$
Chloramphenicol	<=1–4	2	2	0
Ciprofloxacin	<=0.03–0.25	0.12	0.12	0
Gentamicin	<=0.5–2	<=0.5	<=0.5	0
Streptomycin	<=2–8	2	4	0
Tetracycline	<=0.5–8	<=0.5	4	0
Penicillin	8–>128	>128	>128	90
Erythromycin	<=1–4	1	2	0
Vancomycin	<=2–2	2	2	0

all the antimicrobials tested (Table 3). Two strains classified as *B. cereus* (10570, 11128) and two strains classified as *B. thuringiensis* (10584, 11294) were sensitive to penicillin as well.

4. Discussion

This study has shown that *B. cereus*-like organisms can be isolated from many different ready-to-eat food products for sale at retail. In most of the food samples studied, the counts of these organisms were below 10^3 cfu g^{-1} (satisfactory according to the Danish guidelines) [25]. In 0.5% of the samples, though, the counts were above 10^4 cfu g^{-1} (unacceptably high according to the Danish guidelines). As expected, the high counts were mainly found in heat-treated, starchy products, where growth of the organisms might have occurred as a consequence of improper cooling of the products after heat-treatment. Similar high counts of *B. cereus* in pasta and rice products have been reported in a Dutch study [26] and in a study of ready-to-eat food in Taiwan [27]. The present finding is in good agreement with the fact that the majority of food borne outbreaks of *B. cereus*-originated illness are caused by cooked, starchy products [1]. Due to high toxin production in such products [28], the emetic syndrome is also related to these food types.

Valero and co-workers [29] observed that cucumbers among several vegetables analysed presented high counts of *B. cereus*. Likewise, the present study found high counts of *B. cereus*-like organisms in cucumbers and tomatoes (>10^4 cfu g^{-1}). These counts are most likely not due to growth of the organisms, but may be natural contaminants or residues of *B. thuringiensis* insecticides. Of the forty isolates further characterized, five isolates from different food categories grouped together with the commercial Dipel® strain according to content of *cry* genes and enterotoxin genes and proteins. However, further studies are needed to clarify the genetic relationship of the isolated strains to commercial *B. thuringiensis* strains.

Other food categories, which have occasionally been reported to present high numbers of *B. cereus*-like organisms (above 10^4 cfu g^{-1}), are spices [26] and milk [30,31]. These products were not included in our study but are subjects of investigations presently being conducted in our institute.

All forty strains tested had at least one gene or component of HBL and NHE. A high occurrence of protein components and/or genes involved in diarrhoeal disease has previously been described for *B. cereus* from food [29,32,33] and *B. thuringiensis* [34,35]. In addition, genes for the cytotoxin CytK were abundantly found among the isolates, as also reported in another study [21]. The importance of this frequent occurrence of CytK in *B. cereus*-like organisms is unknown though, as the role of CytK in food borne disease is not yet fully understood. Recent research has identified a new variant of *cytK*, designated as *cytK-2*, with the original *cytK* being *cytK-1* [36]. This new variant has only 89% identity at amino acid sequence level to the original CytK, and was shown to have a lower toxicity than CytK-1 against mammalian cell lines. Furthermore, it has recently been shown that in the clinical strain responsible for the death of three persons [10], *cytK* was more strongly transcribed [37]. The high CytK expression may account of the high virulence of this strain. However, in our study we used primers that do not differentiate between these two types of CytK. Genes for the emetic toxin were only found in one strain of *B. cereus* isolated from pasta. The production of emetic toxin is believed to be restricted to a certain group of *B. cereus* [38,39], and in agreement with the findings in an earlier study, the mentioned strain did not possess genes or protein products for HBL [38].

Within a given strain, the presence of toxic components or genes encoding them does not necessarily lead to food borne disease following ingestion. Therefore, the exact influence of *B. cereus* and *B. thuringiensis* on human disease cannot be estimated from the present results. Similarly, such estimates are not possible from Danish surveillance data on human gastrointestinal diseases, since individual cases caused by *B. cereus*-like organisms are not registered. Nonetheless, outbreaks have been described in Denmark [40] as well as in other countries. In the Netherlands and in England and Wales, *B. cereus* has been reported to be the causative organism of approximately 2% of the outbreaks of known origin [41]. In France, the reported frequency of *B. cereus* outbreaks was 4–5% [42], and in the United States, 1–2% of the outbreaks have been attributed to *B. cereus* [43].

Except from penicillin, all 40 isolates were susceptible to the antibiotics tested. Hence, potential infections caused by the *B. cereus*-like organisms deriving from the present study may be treated with antimicrobial agents. Antimicrobial resistance among *B. cereus* has

previously been reported in milk [44] and dairy products [45]. Phenotypic characterisation of the isolates based on antibiotic resistance profiles was not possible in this investigation, though other studies have shown that the resistance patterns of different *Bacillus* spp., in part, are species related [46–48].

The majority of the strains isolated from the food samples belonged to *B. thuringiensis* due to the presence of intracellular crystals and/or genes for selected *cry* genes. We believe this to be the first larger study with the specific aim to identify *B. thuringiensis* in food, since earlier studies did not distinguish between *B. thuringiensis* and *B. cereus* sensu stricto. Previously, *B. thuringiensis* has been isolated from grapes [49,50], farm bulk tank and creamery silo milk [51], and in a small sample of pasta, bread and milk [52]. These observations indicate that *B. thuringiensis* could actually be responsible for many of the food borne outbreaks previously attributed to *B. cereus sensu stricto*.

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