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OCCURRENCE AND TOXIN GENE PROFILE OF *BACILLUS CEREUS* IN DAIRY PRODUCTS

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ABSTRACT

Bacillus cereus, a bacteria frequently associated with food spoilage, is responsible for 2 different foodborne illness in humans: a diarrheal disease, associated with cytotoxin K, hemolysin BL and a non-hemolytic enterotoxin, and an emetic syndrome, associated with the cereulide toxin.
The aim of this study is to investigate the occurrence of *B. cereus* in 515 hard and soft cheeses collected in southern Italy. The strains were molecularly characterized for the presence of *hbl*A, *hbl*C, *hbl*D, *nhe*A, *nhe*B, *nhe*C, *cyt*K, and *entFM* genes, related to the production of enterotoxins (trimeric hemolysin BL, trimeric non-hemolytic enterotoxin, cytotoxin K and enterotoxin FM). One hundred thirty eight samples (26.8%) were found contaminated by *B. cereus*. One hundred of the contaminated samples (72.5%) showed a level of *B. cereus* contamination $\leq 10^3$ CFU/g, whereas 9 (6.5%) samples showed a contamination $>10^5$ CFU/g. Seven different molecular profiles were found among the 138 strains of *B. cereus* isolated. The most common profile was *nhe*ABC-*ent*FM, found in 42 strains, followed by the *hbl*ACD-*nhe*ABC-*cyt*K-*ent*FM, found in 33 strains, the *nhe*ABC-*cyt*K-*ent*FM, found in 24 strains, the *hbl*ACD-*nhe*ABC-*ent*FM, found in 11 strains, the *hbl*ACD-*nhe*AC-*ent*FM, found in 15 strains, and the *nhe*C-*entFM*, found in 12 strains. Eleven strains did not harbored any of these genes.
In 7.2% (37/515) of samples, characterized by a bacterial contamination $>10^3$ CFU/g, we found *B. cereus* strains harboring genes encoding for at least one complete enterotoxin.

Keywords: Concentration, enterotoxin genes, PCR, toxigenic profile, diarrheal disease

INTRODUCTION

The *Bacillus cereus* group comprises 7 closely related species of Gram positive spore-forming aerobic bacteria. In particular, *B. cereus sensu stricto* has been associated with food poisoning, *B. thuringiensis* (an insect pathogen the toxin of which proved to be an alternative to synthetic insecticides), *B. anthracis* (the agent of anthrax) and the other clinically less important species *B. mycoides*, *B. pseudomycoides* and *B. weihenstephanensis*. Two further species, named *B. cytotoxicus* and *B. toyonensis*, have been recently proposed (**Guinebretiére *et al.*, 2013; Lapidus *et al.*, 2008; Oren and Garrity, 2014**).
B. cereus sensu stricto is responsible of 2 different types of food poisoning: diarrhoeal syndrome and the emetic syndrome. The diarrhoeal syndrome is caused by several heat-labile toxins including hemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe), cytotoxin K (CytK), *Bacillus cereus* enterotoxin T (BceT) and enterotoxin FM (EntFM) (**Beecher and Wong 1997; Lund and Granum, 1997; Schoeni and Wong, 2005; Tran *et al.*, 2010**), all of them produced during the vegetative bacterial growth in the small intestine. The emetic syndrome is caused by the production of the heat-stable peptide toxin cereulide (Ces) (**Ehling-Schulz *et al.*, 2015**), that could be produced by cells growing in foodstuff. Hbl and Nhe are both tripartite toxins requiring the combined action of all components for the onset of the toxic action, while CytK and EntFM are monomeric proteins. The BceT toxic activity is still under debate, for it has been classified as enterotoxin on the basis of genetic and structural relations with other bacterial enterotoxins, rather than on the basis of clinical evidence (**Agata *et al.*, 1995**).
Though *B. cereus* poisoning is not usually a serious illness, some severe, and even lethal, cases have been reported (**EFSA and ECDC, 2013, 2014; Lund *et al.*, 2000**). Emetic syndrome, characterized by nausea and vomiting, occurs few hours after the consumption of a contaminated meal, while diarrhoeal syndrome characterized by abdominal pain, watery or bloody diarrhoea and, occasionally, nausea and emesis, has an average incubation time ranging from 6 to 24 h. The duration of the emetic syndrome is normally 12–24 h (**Kramer and Gilbert, 1989**). The infective dose of the diarrhoeal syndrome seems to be at least 10^4-10^5 CFU/g (**EFSA, 2005; Granum and Lund, 1997**). However, considering that bacterial concentrations lower than 10^4 CFU/g have been found able to trigger the onset the poisoning syndrome, any food containing between 10^3 CFU/g and 10^4 CFU/g of *B. cereus* cannot be considered completely safe for consumption (**Gilbert and Kramer, 1986**). Almost all kind of food has been implicated in *B. cereus* foodborne poisoning, although the cases reported are mainly related to consumption of rice, vegetables, mixed food or buffet meals and fresh fruit (**EFSA, 2016; EFSA and ECDC, 2013; Ombui *et al.*, 2008**). Due to its widespread environmental diffusion and its resistant endospores, *B. cereus* can also contaminate food during manufacturing, and survive the cooking temperature (**Majed *et al.*, 2016**).
The presence of *B. cereus* in milk and in dairy product is well reported (**Ahmed *et al.*, 1983; Bonerba *et al.*, 2010; De Santis *et al.*, 2008; Johnston and Bruce, 1982; Larsen and Jørgensen, 1997; Molva *et al.*, 2009**). It is mainly due to heat resistance of its endospores and their ability to survive in milk and in milk powder, making dairies products possible causes of *B. cereus* food poisoning (**EFSA and ECDC, 2014; Kumari and Sarkar, 2014; Wang *et al.*, 2009; Zhang *et al.*, 2016**). In Europe, from 2010 to 2012, *Bacillus* outbreaks increased significantly (99 outbreaks in 2010; 220 outbreaks in 2011; 259 outbreaks in 2012); the reported rate in the EU was 0.04 and 0.05 per 100,000 inhabitants in 2011 and 2012, respectively (**EFSA and ECDC, 2013, 2014**). In Italy, notifications and reports of the *B. cereus* poisoning are scant (**EFSA and ECDC, 2013; Martinelli *et al.*, 2013; Zicari *et al.*, 2011**). The illness course, generally self-limiting and usually of not severe symptomatology, lead to underestimate its diffusion.
The distinguishing features among the species comprised in the *B. cereus* group are defined by genes located on plasmids or in the chromosome (**Van der Auwera *et al.*, 2007**). The identification based on classical biochemical tools does not easily distinguish between the species in the *B. cereus* **group (Bavykin *et al.*, 2004; Stenfors *et al.*, 2008)**. Considering that genes encoding for toxins associated with diarrhoeal disease are present in most *Bacillus* species, excepted *B. anthracis* (**Mignot *et al.*, 2001**), it is probable that the poisoning caused by species other than *B. cereus sensu stricto*, are still under-reported. This is why,

from a clinical standpoint, is preferable to characterize the isolates according to their virulence profiles, rather than to identify the species through to traditional biochemical tests **(Cho *et al.*, 2015; Granum *et al.*, 1999)**.
The aim of this study is to evaluate the occurrence of *B. cereus* group in cheese and dairy products in southern Italy and to assess the risk of diarrhoeal food poisoning by means of the PCR based detection of genes related to *B. cereus* virulence.

MATERIAL AND METHODS

Samples

The study was carried out on 515 samples of dairy products collected from dairies, supermarkets and company canteens in southern Italy from January 2014 to June 2015. The samples were stored at 4 °C and analyzed within 24 h from sampling. The samples were classified, according to their water activity (Aw), into hard cheeses (Aw 0.79-0.93) and soft cheeses (Aw >0.96). The first group included seasoned cheeses and salted ricotta, while the latter included soft stretched curd cheeses, fresh cheeses and fresh ricotta (Table 1). The Aw measurements were carried out using the AquaLab 4TE (Decagon Devices, Pullman, WA, USA) in accordance with the manufacturer's instructions.

Table 1 Occurrence and enumeration of *B. cereus* in the dairy products.

Dairy products	Samples analyzed	Positive samples*	*B. cereus* CFU/g				
			$\leq 10^3$	$>10^3$-10^4	$>10^4$-10^5	$>10^5$-10^6	$>10^6$
Soft stretched curd cheeses (Sc)	331	81 (24.5%)	51	18	6	3	3
Fresh cheeses (Sc)	59	18 (30.5%)	18	0	0	0	0
Fresh ricotta (Sc)	33	11 (33.3%)	7	4	0	0	0
Salted ricotta (Hc)	52	14 (26.9%)	11	1	0	1	1
Seasoned cheeses (Hc)	40	14 (35%)	13	0	0	0	1
Total	**515**	**138 (26.8%)**	**100**	**23**	**6**	**4**	**5**

Sc: Soft cheese (Aw >0.96), Hc: Hard cheese (0.79≤Aw≤0.93), * Detection limit: 100 CFU/g

Enumeration and identification of *B. cereus*

The enumeration of viable *B. cereus* were performed according to **UNI EN ISO 7932:2005**. In brief, 10 g of samples were homogenized in buffered peptone solution (1:10 W/V) and then diluted up to 10^{-4}; 0.1 ml of each dilution was transferred in Mannitol Egg Yolk Polymyxin Agar (MYP) (Oxoid, Basingstoke, UK) and incubated at 30 °C for 48 h. Suspected colonies were counted and tested in order to evaluate their hemolytic activity on trypticase soy agar with 5% sheep blood (TSA+SB) (Biolife, Milano, Italy). Biochemical confirmatory assessment of the isolates were carried out by means of the automated system VITEK 2 (bioMerieux, Marcy l'Étoile, France), according to the manufacturer's instructions.

Molecular assays

B. cereus genes related to the production of enterotoxins responsible for the diarrheal syndrome and the molecular markers specific for *B. thuringiensis* were investigated according to the authors reported in Table 2. From each positive samples, one colony, grown on TSA+SB and biochemically identified as *B. cereus*, was molecularly characterized. The colonies were transferred in nutrient broth (Biolife) and after 4 hours of incubation (30 °C) 1 mL of culture broth was centrifuged for 3 min at 4,000 g. The pellet was washed with 500 µl of sterile DNase-free water, centrifuged a second time, re-suspended in 100 µl of sterile MilliQ water, and heated (98±1 °C for 10 min). Suspension was centrifuged again (10,000 g for 5 min) and the supernatant was collected and stored at −20 °C until use. The PCR products were visualized by means of gel electrophoresis on 2.5% (w/v) agarose under UV light.
The isolates were analyzed for the presence of *cry* genes (*cry* 1 and *cry* 9) related to the insecticidal activity of *B. thuringiensis*. The assay was performed by a modified **Bravo *et al.* (1998)** and **Rosas-Garcia *et al.* (2008)** protocol. In brief, for *cry* 1 detection, the annealing step was carried out at 54 °C instead of 52 °C, whereas *cry* 9 detection was carried out through a SYBR green assay by means the iQ SyBr Green SuperMix (Bio-Rad laboratories, Hercules, CA, USA) using 0.2 µM of each primer and 3 µL DNA extract (neat and diluted 1/10) in a final volume of 25 µL. Thermocycling was done in a CFX96 deep well real time PCR detection System (Bio-Rad), with an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 sec, and 54 °C for 45 sec. Fluorescence was detected during the annealing/extension step at each PCR cycle. The presence of *cry* 9 was investigated using both agarose gel electrophoresis and melt-curve analysis.
Bacillus cereus ATCC 11778 PK/5 and *B. thuringiensis* subsp. *aizawaii* strain GC-91, were used as positive controls.

Table 2 Primers sequence utilized to identify molecular targets in *B. cereus* isolates

Target gene	Primer sequence (5'-3')	Product size (bp)	Reference
*hbl*C	F-CCTATCAATACTCTCGCAA R-TTTCCTTTGTTATACGCTGC	695	**Ngamwongsatit *et al.*, 2008**
*hbl*D	F-GAAACAGGGTCTCATATTCT R-CTGCATCTTTATGAATATCA	1018	**Ngamwongsatit *et al.*, 2008**
*hbl*A	F-GCAAAATCTATGAATGCCTA R-GCATCTTGTTCGTAATGTTTT	884	**Ngamwongsatit *et al.*, 2008**
*nhe*A	F-TAAGGAGGGGCAAACAGAAG R-TGAATGCGAAGAGCTGCTTC	759	**Ngamwongsatit *et al.*, 2008**
*nhe*B	F-CAAGCTCCAGTTCATGCGG R-GATCCCATTGTGTACCATTG	935	**Ngamwongsatit *et al.*, 2008**
*nhe*C	F-ACATCCTTTTGCAGCAGAAC R-CCACCAGCAATGACCATATC	618	**Ngamwongsatit *et al.*, 2008**
*cyt*K	F-CGACGTCACAAGTTGTAACA R-CGTGTGTAAATACCCCAGTT	565	**Ngamwongsatit *et al.*, 2008**
*ent*FM	F-GTTCGTTCAGGTGCTGGTTAC R-AGCTGGGCCTGTACGTACTT	486	**Ngamwongsatit *et al.*, 2008**
cry 1	F- CTGGATTTACAGGTGGGGATAT R-TGAGTCGCTTCGCATATTTGACT	543-593	**Bravo et *al.*, 1998**
cry 9	F-CGGTGTTACTATTAGCGAGGGCGG R-GTTTGAGCCGCTTCACAGCAAATCC	351-354*	**Rosas-Garcia et *al.*, 2008**

*****Melting temperature of the *cry* 9 amplicon 81.5 ±0.5 °C

Statistical analysis

The statistical analysis was carried out by means of the EpiInfo™ 7 software package (Centers for Diseases Control and Prevention, GA, USA). A two-tailed chi-square ($\chi 2$) test and the Odds Ratio (OR) test were performed. P values <0.05 and an OR values >1 (95% CI) were considered as indicators of statistically significant difference among data.

RESULTS

Microbiological assays

Five hundred and fifteen dairy products were analyzed and 138 (26.8%) of them were found contaminated by *B. cereus* strains (Table 1). The occurrence was higher in hard cheeses (30.4%) than in soft cheeses (26%). In particular, the higher frequency of contamination was observed in the seasoned cheeses (35%), fresh ricotta (33.3%), and fresh cheeses (30.5%); whereas lower ones were observed in salted (seasoned) ricotta (26.9%) and soft stretched curd cheeses (24.5%) (Table 1). The positive samples were classified in 5 groups on the basis of their *B. cereus* concentration (Table 1): a) $\leq 10^3$ CFU/g (72.5%); b) $>10^3$-10^4 CFU/g (16.7%); c) $>10^4$-10^5 CFU/g (4.3%); d) $>10^5$-10^6 CFU/g (2.9%); e) $>10^6$ CFU/g (3.6%).

Molecular assays

Eleven out of the 138 isolates (8%) were lacking of all genes responsible for enterotoxins production; the genes of more common detection were *nheC* and *entFM* (127/138, 92%), followed by *nheA* (125/138, 90.6%), *nheB* (110/138, 79.7%), and the *hbl* components (*hblACD*) (59/138, 42.8%). *CytK* was of less common detection (57/138, 41.3%) (Table 3).

Table 3 Occurrence of enterotoxin genes in *B. cereus* strains.

Toxin	Gene	Positive strains	
		No.	%
Hemolysin BL	*hblA*	59	42.8
	hblC	59	42.8
	hblD	59	42.8
Non-hemolytic enterotoxin	*nheA*	125	90.6
	nheB	110	79.7
	nheC	127	92
Cytotoxin K	*CytK*	57	41.3
Enterotoxin FM	*EntFM*	127	92

The complete trimeric toxins Hbl and Nhe were detected in 42.8% and 79.7% of the isolates, respectively. The 3 genes of the toxin Hbl were always jointly detected in the 59 positive strains and were lacking in the remaining 79 strains, whereas the 3 *Nhe* genes were differently linked (Table 4). The *nheC* gene was present alone in 2 isolates, while in 15 strains it was associated with *nheA*, which was never been detected alone. Finally, in agreement with the results of **Ngamwongsatit *et al.* (2008)**, *nheB* was always detected together with both other 2 genes (*nheAC*). Forty-four isolates out of 138 (31.9%) harbored the *hbl*ACD and *nhe*ABC. In addition, *EntFM* and *nhe*C genes were always detected jointly (127/138, 92%). All the 57 isolates positive for *cytK* gene were also positive for *entFM* and *nheC*, also (P<0.004).

The results of the molecular assays allowed to identify, in addition to the virulence profiles described by **Ngamwongsatit *et al.* (2008)**, 2 new profiles presumably responsible for enteric disorders: *hbl*ACD-*nhe*AC-*ent*FM and *nhe*C-*ent*FM (Table 4).

Table 4 Molecular profiles in *B. cereus* stains from dairy products.

Molecular profile	Group	Strains	
		No.	%
*nhe*ABC-*entFM*	I*	42	30.4
*hbl*ACD-*nhe*ABC-*cytK-entFM*	II*	33	23.9
*nhe*ABC-*cytK-entFM*	III*	24	17.4
*hbl*ACD-*nhe*ABC-*entFM*	IV*	11	8
*hbl*ACD-*nheAC-entFM*	V	15	10.9
*nhe*C-*entFM*	VI	2	1.4
no gene coding for toxins detected	VII	11	8

* Molecular profile previously described (**Ngamwongsatit *et al.*, 2008**)

Due to the prevalent detection of enterotoxigenic *B. thuringiensis* strains (**Molva *et al.*, 2009; Stenfors *et al.*, 2008**), we devoted a particular attention to the identification of the *B. cereus* group species: the *cry*1 and *cry*9 genes, which are the molecular fingerprint of *B. thuringiensis*, were never found in our 138 isolates.

DISCUSSION

B. cereus is a common soil inhabitant, often present in different food and foodstuff, including milk and dairy products (**Larsen and Jørgensen 1997; Molva *et al.*, 2009; Wang *et al.*, 2009; Zhang *et al.*, 2016**). Despite the widely acknowledged occurrence in food and foodstuff, reports on the occurrence of *B. cereus* in cheese and in other dairy products are scant. **Bonerba *et al.* (2010)** analyzing catered food found 11.1% of the mozzarella-cheese samples positive for *B. cereus*, with a contamination always lower than 10^6 CFU/g. Data available from studies carried out in different countries show that the *B. cereus* contamination of dairy products in China (**Wong *et al.*, 1988**) ranged from 52% (icecream) to 2% (pasteurized milk). In Turkish cheese the prevalence of both *B. cereus* and *B. thuringiensis* (**Molva *et al.*, 2009**) was found to be 12%, while *B. cereus* and *B. thuringiensis* were detected in 6% and 22% of the analyzed samples, respectively. In Italy **De Santis *et al.* (2008)** reported the pathogenicity profile of *B. cereus* isolated from Italian ricotta-cheese, pointing out that the 3 genes of the *nhe* operon were the most commonly detected (*nheB* 56%, *nheA* 66.7%, *nheC* 71.2%), while the 3 genes of the toxin Hbl were less common (*hblA* 28.8%, *hblC* 31.8%, *hblD* 47%).

Although our study highlighted a relevant occurrence (26.8%) of *B. cereus* in the analyzed dairy products, the contamination of the 89.1% of positive samples did not exceed 10^4 CFU/g. Among the samples showing a contamination $\geq 10^4$ CFU/g (10.9%), 3.6% harbored more than 10^6 *B. cereus* CFU/g (Table 1).

The absence of contamination by *B. thuringiensis* in our samples is worth noting, given its wide use in modern agriculture as pest suppressant and its frequent detection in cheese (**Molva *et al.*, 2009**). It should be stressed that commercial biochemical identification systems, widely used by most authors to identify *B. cereus* in food, are unable to adequately discriminate between *B. thuringiensis* and *B. cereus* (**Bavykin *et al.*, 2004; Stenfors *et al.*, 2008**). This is why, we focused our study on the assessment of the virulence toxin gene profile of the isolates, founding 7 different molecular profiles among our 138 isolates (Table 4). Six of those gene profiles were often associated to virulence. The most common profile was found in 42 isolates (30.4%), that showed positivity for *nheABC* and *entFM* genes (Group I). Other profiles were: a) strains harboring all genes investigated (23.9%, 33/138) (Group II); b) strains positive for *nheABC*, *cytK* and *entFM* genes (24/138, 17.4%) (Group III); c) strains positive for all genes codifying the 2 tripartite toxins Hbl and Nhe and for *entFM* gene (11/138, 8%) (Group IV); d) strains positive for *nheAC*, *hblACD*, and *entFM* genes (15/138, 10.9%) (Group V); e) strains harboring only *nheC* and *entFM* genes (Group VI) (2/138, 1.4%). In addition, 11 strains were lacking all these 8 genes (Group VII). The first 4 profiles were previously described by **Ngamwongsatit *et al.* (2008)** (Table 4); the Group V is reported for the first time in this work as cause of possible food poisoning.

Isolates which were positives for all virulence genes considered (Group II), or at least for one of the 2 operons *hbl* and *nhe* (Group I, III, IV and V), are probably the most dangerous for human. The strains harboring *cytK* gene (Group III), because of the necrotizing activity of the *cytK* toxin (Lund et al., 2000), should be considered of highly toxic. The Group VI, including 2 strains (1.4%), harboring only one component of nhe complex (*nheC*) and the *entFM* gene, involved in bacterial shape, motility, adhesion to epithelial cells, biofilm formation, and vacuolization of macrophages (**Tran *et al.*, 2010**), appear to be less virulent (Table 4). Therefore, 127 of the 138 isolates (92%) analyzed in our study should be regarded as a potential source of enteric disorders of different severity.

In agreement with **Bonerba *et al.* (2010)**, we found that the *nhe* complex, observed in 79.9% (110/138) of our isolates, was significantly more frequent than the *hbl* complex, which was found in the 42.8% (59/138) of the isolates (χ^2=6.48; P <0.01). In addition, we found that all components of Hbl tripartite toxin were always simultaneously present in the positive strains. This was not the case for the *nhe* complex. It is interesting to highlight that the positivity regarding the *nheB* genes always matched with the presence of the genes encoding for the other 2 components of the Nhe toxin (*nheAC*). This finding, combined with the high occurrence of this gene among our isolates, suggests the protein NheB as suitable target for a new assay for the toxigenic characterization of *B. cereus* isolates, as its presence is always associated whit the proteins NheA and NheC, which make the toxin active.

Interestingly, *B. cereus* strains belonging to Groups IV, V and VI were mainly detected in samples with bacterial contamination $\leq 10^3$ CFU/g (26/28, 92.9%); instead, the percentage of detection of *B. cereus* strains Groups I, II and III was higher in samples with *B. cereus* contamination $>10^3$ CFU/g (35/99; 35.4%) (P<0.005; OR=6.5). **Gilbert and Kramer (1986)** observed that food with a contamination of *B. cereus* cells around 10^3 CFU/g represents a potential risk for human health, because *B. cereus* poisoning is not only related to the level of food contamination, being the number of sporulated cells withstanding the stomach acid barrier and the strains virulence profile are of great importance too (**Rowan and Anderson, 1997**). These results are of the higher interest as doses $\leq 10^3$ *B. cereus* CFU/g have been found in food that caused enteric diseases (**Gilbert and Kramer, 1986**). In our study, most of the contaminated dairy products showed a concentration of *B. cereus* $\leq 10^3$ CFU/g, while 27.5% (38/138) of contaminated samples showed a concentration $>10^3$ CFU/g. In particular, 6 samples exceeded 10^4, 4 10^5 and 5 10^6 *B. cereus* CFU/g.

The detection of 37 strains carrying toxin genes, in samples characterized by a bacterial contamination $>10^3$ CFU/g, it is worth to note. These strains were classified in this work as Group I (17 strains), Group II (11 strains), Group III (7 strains), and Group VI (2 strains). Strains belonging to Groups II and III, are probably the most dangerous because of the presence of genes coding for the complete toxins.

CONCLUSION

EFSA reports (**EFSA, 2013, 2014**) highlight a steady increase of *B. cereus* outbreaks from 2010 to 2012 and points out that 5.3% of these outbreaks are related to cheese consumption. Moreover, food making, as well as the use of milk powder in dairy food production, increase the risk of poisoning, especially in case of not pasteurized raw material (**Rowan and Anderson, 1997**).

The results of this study support the EFSA findings about the *B. cereus* contamination of dairy products. In addition, 6.5% of positive samples fall in the area of high risk of food poisoning ($>10^5$ CFU/g) as indicated by EFSA (**EFSA, 2016**). We found that, even in low contaminated dairy products (around 10^3 *B. cereus* cells/g), the isolates express all the 6 virulence profiles identified in this work. So, a contamination around 10^3 CFU/g cannot be considered completely safe, as reported by the EFSA Panel of Biological Hazards (**EFSA, 2016**). The number of *B. cereus* cells per g of food, as well as the plain biochemical identification of strains, are not adequate tools for screening food poisoning risks. In conclusion, in order to protect human health, it would be necessary to screen strains harboring virulence genes alongside the analysis of the concentration of *B. cereus* in food and foodstuff.

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