



# Molecular characterization and toxigenic profiles of *Bacillus cereus* isolates from foodstuff and food poisoning outbreaks in Brazil

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## Abstract

*Bacillus cereus sensu stricto* (*s.s.*) is a well-known foodborne pathogen that produces a range of enterotoxins and is able to cause two different types of foodborne illnesses—the emetic and the diarrheal syndromes. In this study, 54 *B. cereus s.s.* strains isolated from foodstuff and foods involved in food poisoning outbreaks were characterized according to the presence of toxin-encoding genes, virulence-encoding genes, and *panC* typing. Most isolates were assigned to *panC* groups IV (61.1%) and III (25.9%), but members of groups II and V could also be found. Investigation of specific alleles revealed high numbers of isolates carrying toxin and other virulence genes including *nheA* (100%), *nheB* (100%), *hblA* (79.6%), *hblC* (79.6%), *hblD* (74.1%), *cytK-2* (61.1%), *clo* (100%), *pc-plc* (75.9%), *sph* (68.5%), *pi-plc* (66.6%), *hlyIII* (62.9%), and *hlyII* (24.1%). All isolates were negative for *ces* and *cytK-1*. In summary, we detected various enterotoxin and other virulence factor genes associated with diarrheal syndrome in strains analyzed, implicated or not with food poisoning. Furthermore, the most isolates analyzed belong to high-risk phylogenetic groups' *panC* types III and IV. Our study provides a convenient molecular scheme for characterization of *B. cereus s.s.* strains responsible for food poisoning outbreaks in order to improve the monitoring and investigation and assess emerging clusters and diversity of strains.

**Keywords** *Bacillus cereus sensu stricto* (*s.s.*) · Food poisoning · *panC* group · Toxin-encoding genes · Virulence-encoding genes

## Introduction

*Bacillus cereus sensu lato* (*s.l.*) is a group of Gram-positive spore-forming bacteria with close phylogenetic characteristics [1]. The group comprises several genetically closely related species, with *B. cereus sensu stricto* (*s.s.*) as well as *B. anthracis*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, *B. weihenstephanensis*, *B. cytotoxicus*, and *B. toyonensis* as the most prominent members [2].

Despite their phylogenetic similarity, these species show great ecological diversity [3, 4]. Among the species in the group, three are well described in the literature due to their pathogenic characteristics, *B. anthracis*, *B. cereus s.s.*, and the entomopathogen *B. thuringiensis* [3, 5, 6].

*B. cereus s.s.* is among the concerning species in the food industry since its spores are heat resistant [7] and chemical resistant [8]. In addition, some strains are able to produce toxins which are frequently associated with food poisoning outbreaks [5]. There are two forms of foodborne illness caused by *B. cereus s.s.*: the emetic syndrome, associated with cereulide production, and the diarrheal syndrome, associated with enterotoxins [9].

The emetic syndrome is characterized by vomiting and nausea, usually 30 min to 6 h after ingestion, and can be confused with food poisoning outbreaks caused by *Staphylococcus aureus*. Diarrheal syndrome is characterized by abdominal cramps and watery diarrhea within 8 to 16 h after ingestion of contaminated foods. These diarrheal symptoms

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and incubation periods can be easily confused with those caused by *Clostridium perfringens* food poisoning [5, 9].

Cereulide is a small (< 15 kDa), heat resistant, acid stable, and proteolysis-resistant peptide that is pre-formed by cells in the food matrix [10, 11]. The enterotoxins produced by *B. cereus* s.s. that are recognized as playing a major role in the diarrheal disease are hemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe), and cytotoxin K (CytK) [6]. Additional virulence factors including cereolysin O (Clo), hemolysin II (HlyII), hemolysin III (HlyIII), phosphatidylinositol phospholipase (Pi-plc), phosphatidylcholine phospholipase (Pc-plc), and sphingomyelinase (Sph or SMase) have been described and might also contribute to enteropathogenicity [12–14].

Based in cytotoxic activity levels of culture filtrates and toxin distribution, the ability of *B. cereus* strains to cause food poisoning has been suggested to vary depending on phylogenetic affiliation with *panC* groups I to VII rather than species affiliation. An online tool (<https://www.tools.symprvius.org/Bcereus/english.php>) to assign strains to the different phylogenetic groups has been established [15].

According to the Brazilian Ministry of Health, between 2013 and 2022, *B. cereus* was reported as the fourth causative agent of foodborne diseases in Brazil [16]. Food poisoning outbreaks are usually associated with failures in preservation of foods through exposure to inadequate times and temperatures, thus allowing these microorganisms to multiply to reach significant levels (above  $10^5$  CFU/g) [5]. Severe outbreaks are often investigated by the Reference Laboratories that are part of the Sanitary and Epidemiological Services in each Brazilian state [17]. Nevertheless, there are only few studies that have investigated the presence of genes encoding virulence factors in *B. cereus* isolated food origin in Brazil [19–22]. A better knowledge of the characteristics of these strains will help to establish information for microbiological risk evaluation.

In Minas Gerais, Brazil, the Health Surveillance of the State Secretariat—VISA/MG—developed the Food Quality Monitoring Program—PROGVISA, created and implemented in 2000. PROGVISA is the largest food monitoring project ever deployed in the country. Through the program, the Board of Food Surveillance can sample and analyze the foods produced in Minas Gerais or in other states of the federation but that are exposed to consumption by the local population [18]. In that state, the Central Public Health Laboratory (LACEN-MG/Fundação Ezequiel Dias) routinely analyzes food samples from the Food Quality Monitoring Program (Progvisa). Strains of *B. cereus* have been isolated from these samples.

The objective of the present work was to evaluate the presence of toxins and other virulence genes and determine the population structure by *panC* typing in *B. cereus*

strains isolated from foodstuff and food implicated in food poisoning outbreaks.

## Materials and methods

### Bacterial strains

Fifty-four *B. cereus* strains isolated from foodstuff and food implicated in food poisoning cases reported in Minas Gerais/Brazil were characterized. The strains were previously isolated between 2004 and 2016 from foods matrices commercialized in the State of Minas Gerais, Brazil ( $n=21$ ); Tucupi (cassava fermented beverage) ( $n=9$ ) in 2016; and foods associated with 19 cases of the food poisoning outbreaks reported in the State of Minas Gerais, Brazil, between 2005 and 2017 ( $n=24$ ). All isolates were deposited in the Coleção de Bactérias do Gênero *Bacillus* e Gêneros Correlatos (CCGB) at FIOCRUZ (Rio de Janeiro, Brazil). Background isolation data for the strains is summarized in Table S1.

### Epidemiological information on the isolation context for strains associated with the poisoning outbreaks

The epidemiological data related to each food poisoning outbreak were mainly collected through interviews or questionnaires by local health authorities. Collected data included a record of the type of suspected food, preparation location, date, symptoms, and incubation periods. Foods suspected in each outbreak of food poisoning were sent by local health authorities to the Central Public Health Laboratory (LACEN-MG/Fundação Ezequiel Dias). For each food poisoning outbreak, all bacterial strains from suspected food were isolated by plating leftovers on selective culture media allowing the discrimination of *B. cereus* from other bacterial pathogens (*S. aureus*, *C. perfringens*, etc.).

### Isolation and quantification of *B. cereus*

Isolation of *B. cereus* from food samples was previously performed as described in the *Bacteriological Analytical Manual* [23]. A 25-g sample of food was homogenized in 225 mL of sterile peptone water. After homogenization, 1 mL was transferred to tubes containing 9 mL of sterile peptone water and prepared serial dilutions. 0.1-ml aliquots of each serial dilution were surface plated onto MYP (mannitol-egg yolk with polymyxin) agar plates that were incubated for 18–24 h at 30 °C. Characteristic pink colonies showing lecithinase activity were initially identified as *B. cereus* s.l. The level of *B. cereus* s.l. in each sample was

calculated based on the arithmetic mean of CFU (colony forming units) values of the plates.

### Phenotypic characterization

Each *B. cereus* strains were characterized by physiological and biochemical tests as described in the *Bacteriological Analytical Manual* [23]. Catalase test, anaerobic glucose metabolism, nitrate reduction, Voges-Proskauer (VP), tyrosine decomposition and lysozyme resistance were used for phenotyping. Additionally, rhizoid growth, hemolytic activity, crystal detection at optical microscopy, motility tests, growth at 6 °C and at 43 °C were performed. For quality control purposes, *B. cereus* ATCC 10876 and *B. thuringiensis* ATCC 33679 were used.

### Molecular characterization: *panC* typing

Strains were inoculated in BHI broth and incubated at 30 °C overnight. Genomic DNA was extracted using INSTAGENE MATRIX (BIO-RAD, Brazil) according to the manufacturer's recommendations. Genotyping approach targeting *panC* was carried out according to Guinebretière et al. [15]. PCR products were purified using QIAquick PCR Purification (QIAGEN®, Germany), following the manufacturer's recommendations. PCR products were directly sequenced in both directions using BigDye™ Terminator 3.1 Cycle Sequencing Kit, as described by the manufacturer (Thermo Fisher Scientific, Waltham, MA, USA). *panC* sequencing was performed at platform PDTIS/FIOCRUZ-RJ and sequence data was aggregated in Symprevius. (<https://www.tools.symprevius.org/Bcereus/english.php>).

### Phylogenetic analysis based on *panC* sequencing

Phylogenetic analysis based on partial sequencing of the *panC* gene was performed. Firstly, consensus sequences were compared using the multiple alignment program CLUSTALW. Subsequently, the phylogenetic tree was constructed using the neighbor-joining (NJ) method based on the Tamura 3-parameter model. MEGA 7.0 software was used to generate a graphic representation of the tree resulted from the aligned sequences. The publicly available *panC* sequences of the following reference strains were included in phylogenetic analysis: *B. pseudomycooides* DSM 12442 (*panC* type I); *B. wiedmannii* bv. *thuringiensis* FCC41 (*panC* type II); *B. cereus* CC-1 (*panC* type III); *B. cereus* WPySW2 (*panC* type IV); *B. toyonensis* P18 (*panC* type V); *B. weihenstephanensis* WSBC10204 (*panC* type VI); *B. cytotoxicus* NVH 391–98 (*panC* type VII).

### Detection of toxins and other virulence genes by PCR

Detection of the NHE complex (*nheA* and *nheB*) was performed as previously described [15]. The HBL complex (*hblA*, *hblC*, and *hblD*) and the cereulide peptide synthetase gene (*ces*) were amplified using a multiplex PCR as described elsewhere [24]. For differentiation of *cytK-1* and *cytK-2* variants, a duplex PCR approach was used [25]. Additional virulence genes (*pi-plc*, *pc-plc*, *sph*, *hlyll*, and *hlylll*) [26] and (*clo*) [27] were amplified as previously described. Positive controls used for PCR assays were *B. cereus* strain CCGB1539 for *ces*, NVH 391/98 for *cytK1*, and CCGB406 for the other genes.

### Statistical analysis

To evaluate the association between the presence of toxin-encoding genes, virulence-encoding genes, and *panC* groups, Fisher's exact test was used at 5% significance level. All statistical analyses were performed in GraphPad QuickCalcs software (<https://www.graphpad.com/quickcalcs/contingency1.cfm>).

## Results

### Data of the food poisoning outbreak

Our study characterized 24 *B. cereus* strains isolated from food samples analyzed during microbiological investigations of 19 cases of the food poisoning outbreaks reported in the State of Minas Gerais, Brazil, between 2005 and 2017, where no other pathogenic bacteria were detected in the food samples analyzed. The incubation period (time between ingestion of contaminated food and symptom onset) varied from 8 to 16 h. The predominant symptom of the 19 outbreak cases was diarrhea. The main foods involved in the outbreaks were mixed foods. The main place of occurrence of the outbreaks was the residences.

About the contamination level, populations between  $10^3$  and  $10^7$  CFU/g of *B. cereus* were found in the incriminated foods. Of the 24 foods described, 12 had *B. cereus* level above  $10^5$  CFU/g, a limit that, according to the literature, is capable of causing disease. For the other incriminated foods that had *B. cereus* level below  $10^5$ , other factors such as non-uniform distribution of microorganisms in the foods or loss of viability of the vegetative cells during the transport of the samples were considered by the research group in view of other factors such as clinical and epidemiological.

## Quantification of *B. cereus* in food matrices analyzed under the PROGVISA scope and Tucupi (cassava fermented beverage)

Our study characterized also 30 food-related *B. cereus* strains isolated from foods samples not related to food poisoning outbreaks. About the contamination level, populations between  $10^2$  and  $10^4$  CFU/g of *B. cereus* were found among the 21 food samples monitored by PROGVISA. Among the 9 food samples of the Tucupi, the contamination level varied between  $10^2$  and  $10^4$  CFU/g of the *B. cereus*.

### Phenotypic identification of *Bacillus cereus* s.s.

All 54 isolates were confirmed phenotypically as *B. cereus* s.s. by using standard biochemical and physiological methods. For more information, see supplementary material (Table S1).

### Phylogenetic affiliation to *panC* groups and genetic diversity analysis

The *panC* typing confirmed that most isolates were identified as belonging to group IV ( $n = 33$ ). Of the other 21 isolates, 14 were assigned to group III, 5 were assigned to group V, and 2 were assigned to groups II (Table 1). None of the isolates analyzed were assigned to groups I, VI, and VII.

A neighbor-joining phylogenetic tree (Fig. 1) was constructed to depict the similarity of *panC* nucleotide sequences among the isolates. The isolates in the present study formed clusters with the respective reference strains *B. wiedmannii* bv. *thuringiensis* FCC41 (*panC* type II), *B. cereus* CC-1 (*panC* type III), *B. cereus* WPySW2 (*panC* type IV), and *B. toyonensis* P18 (*panC* type V). The clusters in the tree were consistent with *panC* typing results, except for the strains CCGB-1744, CCGB-1785, CCGB-1750, CCGB1740, and CCGB1741. These strains, classified as group III by *panC* type, clustered with the strains belonging to group II.

**Table 1** Distribution of *B. cereus* s.s. isolates in *panC* phylogenetic groups according to the source of isolation

Group	Number of isolates	
	Food poisoning–related strains	Non-outbreak strains
II	1 (4.2%)	1 (3.3%)
III	7 (29.1%)	7 (23.3%)
IV	15 (62.5%)	18 (60.0%)
V	1 (4.2%)	4 (13.3%)
Total	24 (100%)	30 (100%)

## Toxicogenic and virulence profiles of the strains

Distribution of six enterotoxin genes (*nheA*, *nheB*, *hblA*, *hblC*, *hblD*, and *cytK*) and emetic toxin synthetase encoding gene (*ces*) among the isolates was determined. *nheAB* complex was found in all isolates. *hblA*, *hblC* and *hblD* were detected in 74.1%, 74.1%, and 68.5% of the strains, respectively. The *hblACD* complex was detected in 68.5% of the strains. In three isolates (5.5%), *hblD* was not detected. *cytK-2* was detected in 61.1% of the isolates. All isolates were negative for *ces* and *cytK-1* genes. Among other virulence genes studied, *clo* was found in 100% of the isolates, *pc-plc* in 75.9%, *sph* in 68.5%, *pi-plc* in 66.6%, and *hlyIII* in 62.9% of the isolates. Less common, *hlyII* was detected in 24.1% of the isolates (Fig. 2).

Isolates were assigned to toxicogenic profiles, according to the presence or absence of toxin encoding genes, as following: profile I (51.8%)—*nheAB*, *hblACD*, *cytK-2*, profile II (24.1%; *nheAB*, *hblACD*), profile III (9.25%; *nheAB*, *cytK-2*), profile IV (9.25%; *nheAB*), and profile V (5.5%; *nheAB*, *hblAC*) (Table 2 and 3).

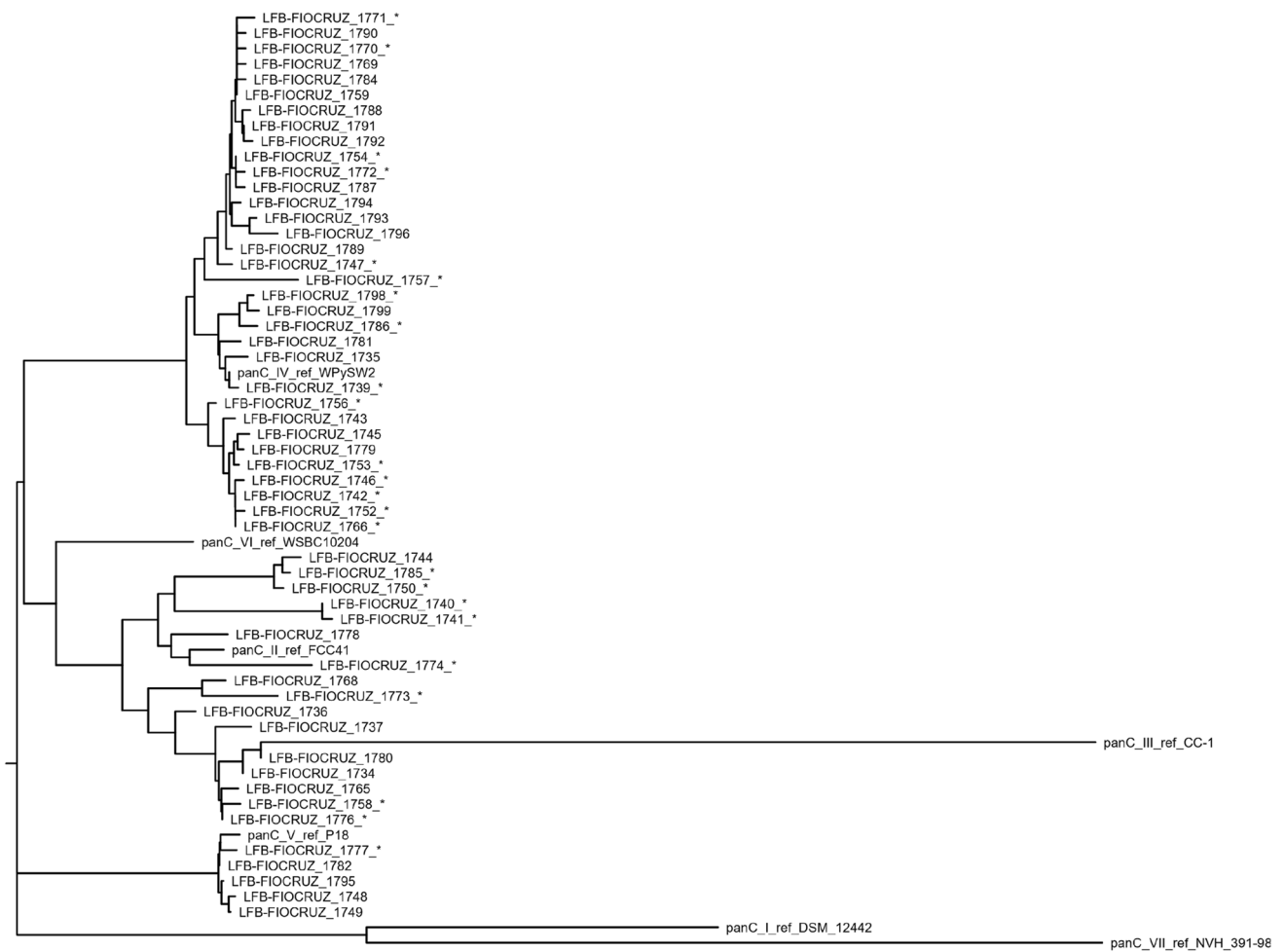
In addition, the analysis of the occurrence of virulence-encoding genes allowed the classification of the strains into 20 virulence profiles. The predominant profile, profile 2, included 18 (33.3%) strains that were positive for genes *clo*, *hlyIII*, *pi-plc*, *pc-plc*, and *sph*. Virulence profile 1, including strains harboring *clo*, *hlyII*, *hlyIII*, *pi-plc*, *pc-plc*, and *sph* genes, was the second most frequent, being detected in 3 (5.5%) isolates (Tables 2 and 3).

The strains included in *panC* group IV had a significantly higher prevalence of *cytK-2* compared with those included in groups III and V ( $p < 0.05$ ). In addition, prevalence of *pi-plc* and *pc-plc* was significantly higher ( $p < 0.05$ ) in isolates belonging to group IV compared with those belonging to group V. No statistically significant differences were found in the prevalence of toxin-encoding genes, virulence-encoding genes, toxicogenic profile, or virulence profile between isolates related and were not related to food poisoning cases (Fig. 3).

## Discussion

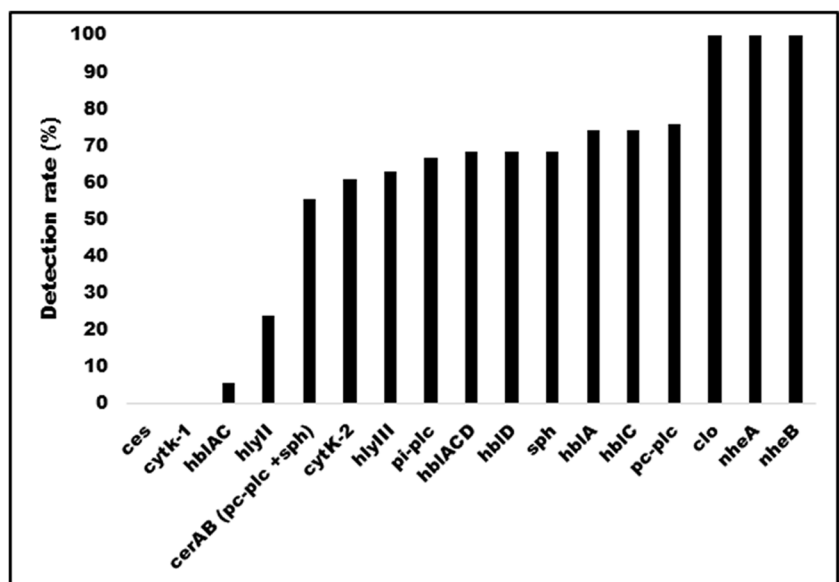
In the present study, 54 strains of *B. cereus* s.s were evaluated for the presence of toxin-encoding genes and virulence-encoding genes and affiliated to phylogenetic groups determined by the *panC* sequencing.

*panC* typing is used to allocate isolates into phylogenetic groups (I to VII) that are indicative of the food poisoning risk posed by the isolate [4, 15]. In this study, the most isolates were affiliated to *panC* types III and IV. Strains of these groups can be highly cytotoxic and present a high risk associated with the ability to cause food poisoning [15]. Other



**Fig. 1** Phylogenetic tree of *B. cereus* *panC* sequences. The tree was constructed using the neighbor-joining (NJ) method based on the Tamura 3-parameter model. The neighbor-joining consensus tree used 1000 bootstrap replicates

**Fig. 2** Overall detection rate of toxin and virulence genes for isolates



**Table 2** Distribution of toxigenic and virulence profiles among *B. cereus s.s* isolated in different foodstuff not associated with food poisoning outbreaks

Source of isolation (n)*	panC group (n)	Toxigenic profile (n)	Virulence profile (n)	
Other foodstuff not related to food poisoning (30)	II (1)	III (1) <i>nheAB</i> , <i>cytk-2</i>	14 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i>	
	III (7)	I (1) <i>nheAB</i> , <i>hblACD</i> , <i>cytk-2</i>	3 (1) <i>clo</i> , <i>hlyII</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i>	
		II (3) <i>nheAB</i> , <i>hblACD</i>	2 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 17 (2) <i>clo</i> , <i>pc-plc</i> , <i>sph</i>	
		IV (3) <i>nheAB</i>	6 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> 15 (2) <i>clo</i> , <i>hlyIII</i> , <i>pc-plc</i>	
	IV (18)	I (14) <i>nheAB</i> , <i>hblACD</i> , <i>cytk-2</i>	1 (2) <i>clo</i> , <i>hlyII</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 2 (6) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 5 (1) <i>clo</i> , <i>hlyII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 11 (1) <i>clo</i> , <i>hlyIII</i> , <i>sph</i> 12 (2) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>sph</i> 13 (1) <i>clo</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 17 (1) <i>clo</i> , <i>pc-plc</i> , <i>sph</i>	
		II (2) <i>nheAB</i> , <i>hblACD</i>	19 (1) <i>clo</i> 20 (1) <i>clo</i> , <i>pc-plc</i>	
		III (1) <i>nheAB</i> , <i>cytk-2</i>	2 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i>	
		IV (1) <i>nheAB</i>	14 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i>	
		V (4)	II (2) <i>nheAB</i> , <i>hblACD</i>	18 (2) <i>clo</i> , <i>sph</i>
			IV (1) <i>nheAB</i>	19 (1) <i>clo</i>
V (1) <i>nheAB</i> , <i>hblAC</i>			2 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i>	

\*n Number of isolates

**Table 3** Distribution of toxigenic and virulence profiles among *B. cereus s.s* isolated in different foodstuff associated with food poisoning outbreaks

Source of isolation (n)*	panC group (n)	Toxigenic profile (n)	Virulence profile (n)
Foodstuff related to food poisoning Outbreak (24)	II (1)	I (1) <i>nheAB</i> , <i>hblACD</i> , <i>cytk-2</i>	2 (1) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i>
	III (7)	I (3) <i>nheAB</i> , <i>hblACD</i> , <i>cytk-2</i>	2 (2) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 3 (1) <i>clo</i> , <i>hlyII</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i>
		II (3) <i>nheAB</i> , <i>hblACD</i>	8 (1) <i>clo</i> , <i>hlyII</i> , <i>hlyIII</i> , <i>pc-plc</i>
		V (1) <i>nheAB</i> , <i>hblAC</i>	10 (1) <i>clo</i> , <i>hlyII</i> , <i>pc-plc</i> , <i>sph</i> 16 (1) <i>clo</i> , <i>hlyII</i> , <i>pc-plc</i> 7 (1) <i>hlyII</i> , <i>hlyIII</i> , <i>pi-plc</i>
		IV (15)	I (9) <i>nheAB</i> , <i>hblACD</i> , <i>cytk-2</i>
	IV (15)	II (3) <i>nheAB</i> , <i>hblACD</i>	2 (3) <i>clo</i> , <i>hlyIII</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i>
		III (2) <i>nheAB</i> , <i>cytk-2</i>	13 (1) <i>clo</i> , <i>pi-plc</i> , <i>pc-plc</i> , <i>sph</i> 15 (1) <i>clo</i> , <i>hlyIII</i> , <i>pc-plc</i>
		V (1) <i>nheAB</i> , <i>hblAC</i>	17 (1) <i>clo</i> , <i>pc-plc</i> , <i>sph</i>
	V (1)	III (1) <i>nheAB</i> , <i>cytk-2</i>	19 (1) <i>clo</i>

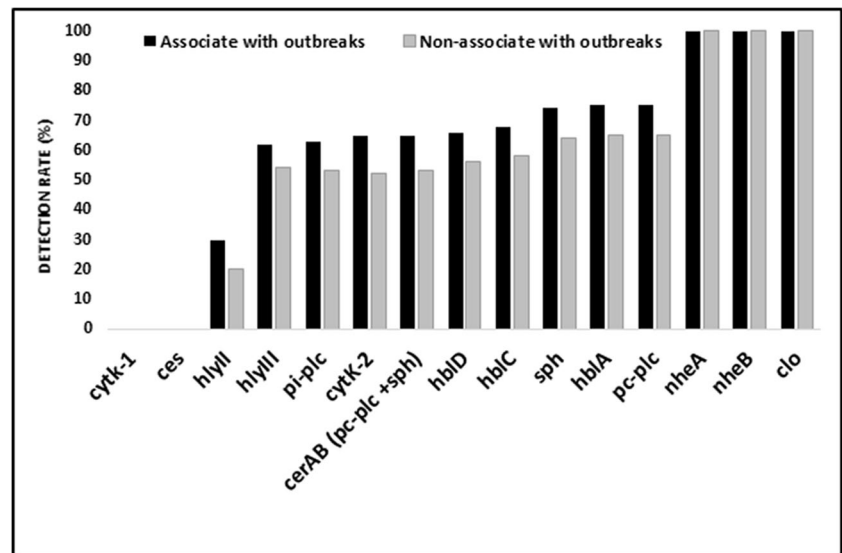
\*n Number of isolates

studies conducted in different countries have reported *panC* groups IV and III as the most prevalent among the isolates involved in food poisoning outbreaks [28, 29]. However, in the present study, *panC* groups IV and III were also the most prevalent among isolates not involved in outbreaks (Table 1).

Studies conducted in Brazil have also reported these two phylogenetic groups as the most prevalent among isolates not involved in outbreaks [20, 21].

PCR results showed a significant toxigenic potential for the isolates tested. *nheA* and *nheB* genes were

**Fig. 3** Toxigenic and virulence genes distribution for isolates associated with food poisoning outbreaks and those not related to food poisoning cases



detected in 100% of the isolates, consistent with previous publications reporting that *nhe* genes are present in most *B. cereus s.s.* [2, 9, 15]. The *hblACD* prevalence in the isolates analyzed in this study was higher than reported in other Brazilian studies [19, 20]. The prevalence of *cytK-2* was slightly higher than those found in other studies in Brazil [20, 30]. As expected for *B. cereus s.s.*, all *cytK*-positive isolates exhibited the *cytK2* variant. It is known that the occurrence of *cytK-1* is rare and is associated with strains belonging to *panC* group VII, which comprises the thermotolerant *B. cytotoxicus* [2, 31]. *B. cereus* group strains assigned to *panC* IV have been shown to frequently harbor *cytK-2* [32, 33], which is consistent with the findings in this study.

In three isolates, only two (*hblA* and *hblC*) out of the three HBL complex genes were found. The polymorphism among HBL genes is the most likely explanation for the failure to amplify all genes in those isolates [34]. Other publication also reported a lack of PCR amplification of one or two components of the HBL complex [26]. An alternative would be to evaluate the presence of the *hblD* using whole genome sequencing [33].

PCR results suggest that none of the *B. cereus s.s.* isolates analyzed in the present study carried the *ces* gene. These results are consistent with previous publications confirming that *ces* gene is rare in *B. cereus s.s.* and is usually associated with some strains belonging to *panC* group III [15] and that *ces*-positive strains have been rarely isolated from food samples [2, 11, 19, 34].

Except for the *hlyII* gene, which showed limited distribution, a high prevalence of hemolysins and phospholipases encoding genes was detected in the *B. cereus s.s.* strains evaluated. Other studies have reported similar results [20, 26]. These virulence factors are important in

extra-gastrointestinal infections and are related to the ability to cause hemolysis, cell lysis, and membrane pore formation [27, 35]. In cases of food poisoning, investigating the presence of virulence genes is important since the symptoms are probably caused by the combined action of multiple toxins and degradation enzymes, including collagenases, phospholipases, and hemolysins [12, 26]. For example, phospholipase C enhances BL hemolysin activity by synergism [35]. In addition, the cytolytic unit cereolysin AB (*CerAB*), formed by phospholipases PC-PLC and SPH, has been associated with the diarrheal syndrome, acting on the permeability and viability of intestinal epithelial cells [12, 36].

Although the strains studied have the potential to produce diarrheal toxins since they harbor the required genes, studies have determined that toxin expression may not always occur as it is modulated by several factors including growth substrate composition and intrinsic bacterial factors [37]. An alternative for a better evaluation of the enterotoxigenic potential of the strains studied would be to evaluate the production of enterotoxins in media that mimic small bowel conditions [38]. Recently, one virulence assessment scheme for enteropathogenic *B. cereus*, including the most important steps of a foodborne infection—survival of the stomach passage, adhesion to epithelial cells, germination, motility, toxin production, and cytotoxicity—was proposed as a methodology to provide a more accurate indication of the risk of enteropathogenic *B. cereus* strains [14]. The application of these methodologies to the strains analyzed in this work could generate additional data and contribute to better evaluate their risk potential.

In summary, we detected various enterotoxin and others virulence factor genes associated with diarrheal syndrome in the strains analyzed. Thus, one may suggest that

*B. cereus s.s.* isolates implicated or not with food poisoning outbreaks do not have a specific genetic profile. Rather, different combinations of genes encoding virulence factors may be present in *B. cereus s.s.* isolates. Furthermore, the most isolates analyzed were affiliated to *panC* types III and IV. This corroborates the results of Chaves et al. and Rossi et al., indicating that most strains isolated in Brazil belong to high-risk phylogenetic groups [20, 21].

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**Data Availability** All data generated or analysed during this study are included in this published article.

## Declarations

**Competing interests** The authors declare no competing interests.

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