



Genetic diversity and the presence of circular plasmids in *Bacillus cereus* isolates of clinical and environmental origin

Tjaša Cerar Kišek¹ · Nežka Pogačnik² · Karmen Godič Torkar²

Received: 21 January 2021 / Revised: 19 March 2021 / Accepted: 22 March 2021 / Published online: 8 April 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

The diversity of 61 *Bacillus cereus* strains isolated from different clinical specimens, food including raw milk and milk products, and water was evaluated. PFGE analysis could discriminate 61 distinct pulsotypes with similarity levels from 25 to 82%, which were divided into 13 clonal complexes. The similarity between clonal complexes was at least 40%. Clinical strains were divided into 10 clonal complexes, while the strains, isolated from milk, food and water were included in 9, 6 and 6 clonal complexes, respectively. Three clonal complexes were dominated by clinical isolates, while they were absent in two complexes. Bacterial isolates from food, being a probable source of alimentary toxoinfection, showed low similarity to isolates from stool specimens. The isolates from both sources were classified together in only 4 out of 13 clonal complexes. The large circular and linear plasmids with the sizes between 50 and 200 kb were detected in 24 (39.3%) and 14 (23%) *B. cereus* strains, respectively. Thirteen (21.3%) strains contained only one plasmid, two plasmids were found in 6 (9.8%) of strains, and three or more plasmids were obtained in 5 (8.2%) of tested strains. The plasmids were confirmed in 30.8% and 40% of isolates from clinical specimens and food and milk samples, respectively. No clear correlation between the PFGE profiles, the source as well as plasmid content among all tested strains was observed.

Keywords *Bacillus cereus* · PFGE · Plasmids · Food · Clinical specimens

Introduction

Bacillus cereus is a ubiquitous Gram-positive, motile, aerobic, non-encapsulated, endospore-forming rod-shaped bacterium (Chen et al. 2003).

The natural environmental reservoir for *B. cereus* consists of decaying organic matter, fresh and marine waters, vegetables and fomites, and the intestinal tract of invertebrates, from which soil and food products may become contaminated, leading to the transient colonisation of the human intestine (Jensen et al. 2003). This bacteria is associated with foodborne poisoning expressed as diarrhoea and emesis, serious wound infections, pneumonia, bacteriemia,

meningitis, endophthalmitis, necrotising fasciitis, osteomyelitis, and endocarditis (Chen et al. 2003). The *B. cereus* spores are thermal resistant and cause post-processing contamination of ready-to-eat food, milk powder, and sausage (Griffiths and Schraft 2017).

Bacillus cereus sensu lato is a group of bacteria displaying close phylogenetic relationships but a high ecological diversity. The three most studied species are *Bacillus anthracis*, *Bacillus cereus* sensu stricto and *Bacillus thuringiensis*. However, the species delineation between *B. thuringiensis* and *B. cereus* sensu stricto has been problematic despite the various approaches and techniques used. It has been suggested that plasmids, especially large toxin-carrying plasmids, play a crucial role in the phenotypical heterogeneity of the *B. cereus* group (Fayad et al. 2019; Patino-Navarrete and Sanchis 2017), which might be one of the reasons that *B. cereus* sensu stricto itself often demonstrated high genetic heterogeneity between the strains (Vassileva et al. 2007; Yang 2017). There were also still no definite conclusions on whether the *B. cereus* emetic strains belonged to a single clone or a diversiform complex (Vassileva et al. 2007; Yang 2017).

Communicated by Erko Stackebrandt.

✉ Karmen Godič Torkar
karmen.torkar@zf.uni-lj.si

¹ Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

² Faculty of Health Sciences, University of Ljubljana, Zdravstvena pot 5, 1000 Ljubljana, Slovenia

Many of the species-specific phenotypes of the *B. cereus* sensu lato group are encoded by plasmid genes. The major virulence factors are located extrachromosomally on large plasmids. For *B. thuringiensis* strains, typical plasmid-encoded crystalline inclusions contain *Cry* and *Cyt* proteins, some of which are toxic against a wide range of insect orders, nematodes and human-cancer cells (Palma et al. 2014). The pXO1 and pXO2 plasmids, found in *B. anthracis* strains, are responsible for producing anthrax exotoxin and the capsule, respectively. The genes for emetic toxins are located at *B. cereus* on plasmids, and they have already been sequenced and analysed (Rasko et al. 2007).

Meanwhile, the diarrhoeal haemolytic, nonhaemolytic enterotoxins and cytotoxin K are encoded chromosomally (Griffiths and Schraft 2017). Some acrySTALLIFEROUS *B. cereus* also carry conjugative plasmids with *Cry* genes native to *B. thuringiensis*, the pXO1 and pXO2-like plasmids were confirmed in some *B. cereus* strains as well (Rasko et al. 2004). Plasmids are vectors for redundant or similar chromosomal genes in the *B. cereus* group (Zheng et al. 2015). Therefore, the presence of these plasmids cannot serve as signatures for species identification (Liu et al. 2015), and it is more useful to consider the *B. cereus* group as a unique species comprised of extremely diverse strains whose properties differ due to plasmid content or because of gene expression associated with key regulatory genes (Helgason et al. 2000; Rasko et al. 2005).

The purpose of our study was to determine the diversity of *B. cereus* strains, obtained from patients' specimens, food and water samples. The number and the length of plasmids that could contribute to the genetic heterogeneity of isolates were also investigated.

Materials and methods

Bacterial isolates

A total of 61 isolates of *B. cereus* collected between 2007 and 2015 were analysed in the study. Twenty-six of them originated from patients hospitalised in different clinical wards in Ljubljana hospitals. Isolates were obtained from various specimens such as, wounds, burns, faeces, other excreta, ear ducts, nose mucosa swabs, etc. Another thirty were isolated from food samples. Their sources were raw milk from individual farms, pasteurised milk, cream, ice-cream, skim milk powder, ultra-high-temperature-treated milk produced by a Slovenian dairy (21 strains), and salad, rice meal, pudding, infant food, selected sauces, dumplings, spices, beefsteaks, etc. prepared in public catering plants (9 strains). Five isolates were obtained from drinking and underground water. Identification of the isolates was carried out by conventional methods including colony morphology,

cell morphological and physiological characteristics and haemolytic activity (ISO 7932 2004).

The isolates were biochemically identified with the API 50CHB and API10 S test systems using the API WEB identification programme Vitek 2.1 (bioMérieux, Marcy-l'Étoile, France) and by multiplex PCR according to Park et al. (2007) and Leski et al. (2009). Total DNA was extracted using the SDS method, followed by purification using the phenol–chloroform–isoamyl alcohol protocol, as previously described (Mäntynen and Lindström 1998; Moore et al. 2004; Sambrook et al. 1989).

Reference strains used for the quality control: *B. cereus* ATCC 14579^T, *B. mycoides* IAM 1190, *B. thuringiensis* ATCC 10,792 (CCM, Brno, Czech Republic), *B. cereus* ATCC 11,778 (Oxoid, Cambridge, UK) and *B. subtilis* BGA (Merck, Darmstadt, Germany).

DNA extraction and PFGE for detection of genetic diversity of *B. cereus* strains

Cell lysis of overnight cultures and genomic DNA in agarose plugs was prepared as described elsewhere (Liu et al. 1997; Sjölund et al. 2005) using 1.5 mg of lysozyme (our modification instead 1 mg) per mL, and 5 U of lysostaphin per mL. A slice of each plug (2.5 mm) was cut out and incubated for 1 h at 37 °C with 25 U of *Sma*I restriction endonuclease (Roche, Switzerland) according to the recommended conditions. PFGE was performed using a CHEF-DR®III, Bio-Rad Technologies, USA) for 30 h at 11 °C, with an electric field of 6 V/cm at an angle of 120°; the pulse time was increased from 5.3 to 34.9 s. A low range Lambda Ladder PFGE Marker 50–1000 kb (BioLabs, New England) was used as the molecular weight marker. Gels were stained with ethidium bromide and photographed under ultraviolet illumination (Liu et al. 1997; Sjölund et al. 2005).

Isolation of large circular and linear plasmids by PFGE

A method for detecting and estimating the sizes of large bacterial plasmids in the presence of genomic DNA by pulsed-field gel electrophoresis (PFGE) was used according to Barton et al. (1995) with a few modifications. The agarose plugs with DNA, prepared in the same way as for studying the genetic diversity between the isolates, were cut into two slices with a sterile glass coverslip. The first one was soaked in the buffer S1 for 1 h at 37 °C, while to the second one appr. 450 U (0.3 µL) of endonuclease S1 in 1 × S1 buffer (100 µL) (Thermo Fisher Scientific, ZDA) was added and incubated for 30 min at room temperature. Digested and undigested slices were applied to wells in 1% agarose gel, prepared in 0.5 × TBE buffer (45 mM Tris-OH [pH 8.0], 45 mM boric acid, 1 mM EDTA), and run in a

CHEF-DR®III apparatus for 24 h at 11 °C, with an electric field of 6 V/cm at an angle of 120°, the pulse time was increased from 1 to 12 s. The presence of linear plasmids was demonstrated in undigested slices, while the circular plasmids were detected in digested slices.

Macrorestriction profile analysis was made in BioNumerics 7.1 (Applied Maths, Saint-Martens, Belgium) using the Dice coefficient, and represented by unweighted pair grouping by mathematical averaging (UPGMA) with 0.5% band tolerance and 0.5% optimisation settings. Images of *B. cereus* ATCC 14579^T were used as a marker to calibrate images' position, with manual correction if necessary.

Statistical analysis

The SPSS software (version 25.0; IBM, USA) was used for statistical analyses. The statistical difference of the PFGE clonal complexes with the source of the strains and the presence of plasmids was calculated using the Kullback $\hat{2}I$ (Likelihood Ratio) and Pearson Chi-square tests. A *p* value lower than 0.05 was considered statistically significant.

Results

Diversity of pulsed-field gel electrophoresis patterns of *B. cereus* strains

Genomic DNA of 61 *B. cereus* strains analysed by PFGE with *Sma*I restriction enzyme yielded 10 to 23 bands of approximately 145.5 kb to 727.5 kb (Fig. 1).

A total of 61 distinct pulsotypes was obtained from the PFGE banding patterns with a similarity level from 25 to 82%, which were divided into 13 clonal complexes. The similarity between clonal complexes was at least 40%, and they contained from 1 (clonal complex no. 2) to 7 pulsotypes (clonal complexes no. 3 and 4) (Fig. 2).

These results showed a high genetic polymorphism existing among isolates with a diversity index of 0.62. Clinical strains were deployed into 10 clonal complexes, while the strains isolated from milk, food and water were included in 9, 6 and 6 clonal complexes, respectively. Three clonal complexes no. 9, 10, 13, were dominated by clinical isolates, while they were absent in complexes no. 5 and 8.

Bacterial isolates from foods, being probable sources of alimentary toxoinfection, showed very low similarity to isolates from faeces and other clinical specimens. The isolates from both sources were classified together in only 4 out of 13 clonal complexes.

Eleven out of 21 strains, obtained from milk and milk products, were isolated from raw milk. They were classified into 6 complexes, and they dominated in the complex no. 3 (4 strains), while the strains from milk products were mostly

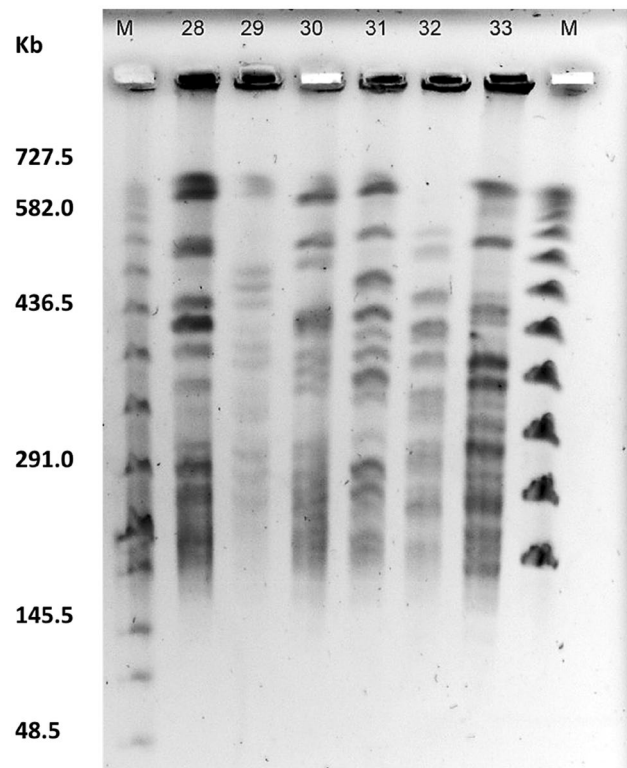


Fig. 1 PFGE of *Sma*I digested genomic DNA from *B. cereus* isolates. Lane M: bacteriophage lambda ladder; Lanes: 28–33: *B. cereus* strains no. 28, 29, 30, 31, 32, 33

present in the fourth complex (4 strains). The standard strain ATCC 14579 was classified into the twelfth clonal complex.

A significant difference was observed in the distribution of the strains of different origin between the clonal complexes (*p* = 0.008).

Characterisation of large plasmids

Large circular and linear plasmids were detected in 24 (39.3%) and 14 (23%) *B. cereus* strains, respectively. Thirteen (21.3%) strains contained only one plasmid, two plasmids were found in six (9.8%) of strains, and three or more plasmids were obtained in five (8.2%) of the tested strains. The sizes of plasmids were between 50 and 200 kb. Ten out of 45 plasmids found had 100 kb, seven plasmids each had the sizes 80 kb and 100 kb, respectively, while only one was 200 kb in size. Plasmids were present in 40% of strains isolated from food and milk, as well as in 30.8% of clinical specimens. Four out of five water isolates also contained one or more plasmids. The exceptions were the fourth and eleventh clonal complexes, where only one and no plasmid-containing strains were classified, respectively. The plasmids were detected in all strains of the second and sixth complex (Table 1). A significant difference was detected between

Fig. 2 PFGE profile of *Sma*I digested DNA of 61 *Bacillus cereus* isolates. Dendrogram of similarities between strains was calculated using the UPGMA algorithm and Dice coefficient. S: clinical specimen; F: food, sampled on suspicion of infection; M: raw milk and milk products; W: water; BcX: *B. cereus* strain number; 1–13: the number of clonal complex

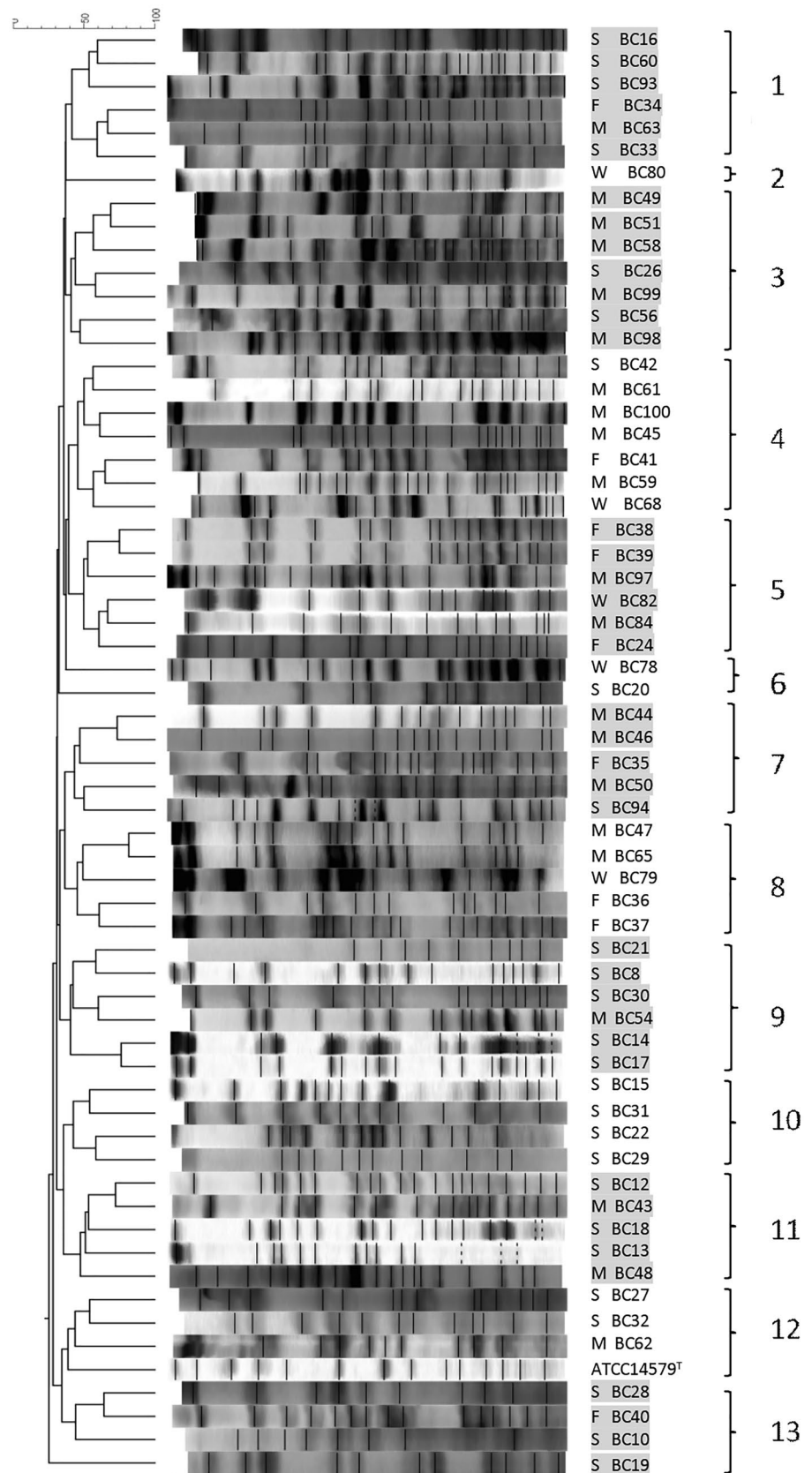


Table 1 Number of *B. cereus* strains from different origins and those containing plasmids, classified in individual clonal complexes

Clonal complex	Origin of the strains				Total no. of pulsotypes	Number of strains (pulsotypes) containing plasmids
	Specimen /feces	Food	Milk and milk products	Water		
1	4/2	1	1	0	6	2
2	0	0	0	1	1	1
3	2/1	0	5	0	7	3
4	1/0	1	4	1	7	1
5	0	3	2	1	6	3
6	1/0	0	0	1	2	2
7	1/1	1	3	0	5	2
8	0	2	2	1	5	3
9	5/2	0	1	0	6	3
10	4/2	0	0	0	4	2
11	3/2	0	2	0	5	0
12	2/1	0	1	0	3	1
13	3/1	1	0	0	4	1
Σ	26/12	9	21	5	61	24

the origin and the linear plasmid content of the isolates ($p=0.006$) as well as between the strains possessing linear and circular plasmids ($p<0.001$).

Furthermore, no significant difference was determined between strains containing plasmids and their distribution in different clonal complexes ($p>0.05$).

Discussion

Comparison of PFGE patterns has been extensively used in epidemiological studies to confirm or to discriminate the sources of disease, but also to evaluate the genetic diversity among a group of closely related strains from the same species (Castiaux et al. 2014). One aspect of this diversity may be explained by the dynamic repertoire of plasmids found in the *B. cereus* group as well as in individual strains of the *B. cereus sensu stricto* (Rasko et al. 2004, 2005, 2007).

We determined the relationships between the *B. cereus* strain, isolated from clinical, food and water sources using PFGE analysis using *Sma*I restriction endonuclease (Akamatsu et al. 2019; Liu et al. 2016; Yavuz et al. 2004). The fingerprints generated by macrorestriction of the DNA comprised approximately 10 to 23 bands of 48.5 to 727.5 kb. The number of bands obtained in our study was quite similar to Yavuz et al. (2004) findings, but they were slightly longer (Fig. 1).

The profiles showed a remarkable polymorphism existing among all strains, which was also reported by Merzougui et al. (2013). These authors also confirmed a visible correlation between PFGE types and the sources of *B. cereus*

food isolates, which cannot be claimed for the strains in a recent study. Each out of 61 tested strains was included in the individual pulsotype, which were classified in 13 clonal complexes. Most complexes contained from four to seven pulsotypes. The second and the sixth complex account only one and two strains, respectively, both contained water isolates. The water isolates were distributed in different clonal complexes. In only 4 (33.3%) out of 12 complexes, in which the strains from food or faecal specimens were classified, were strains from both origins present together. These data show that there was no significant similarity between strains that cause gastrointestinal problems, and those present in food samples, as reported by Liu et al. (2016). It should be emphasised that food and stool samples were not epidemiologically or temporally related, despite the fact that they were obtained in the same period from 2013 to the first half of 2014. The milk and food isolates together were present in 5 (50%) out of 10 complexes, which involved isolates from these two sources and showed only partial aggregation. The strains from raw milk were present mostly in the third and eighth complexes, while the isolates from milk products prevailed in the fourth and eleventh complexes. The source of *B. cereus* strains could be the milking cows themselves, the environment in farms or bulk milk tanks. It could be presumed, that the reason for the presence of *B. cereus* in milk products is more common post-processed contamination from the production line in dairy and not from the raw milk (Fig. 2, Table 1). Milk and clinical isolates were grouped in 7 out of 13 clonal complexes, and we could not classify them into separate groups, as was reported by Helgason et al. (2000), who compared the genetic diversity of

the periodontal *B. cereus* and *B. thuringiensis* isolates to isolates from dairies using PFGE analysis. Cluster analysis revealed two major groups, one cluster included solely isolates from dairies, while the other cluster, included all human isolates as well as the isolates from dairies (Helgason et al. 2000).

The degree in PFGE patterns higher than 70% was yielded between the strains Bc37 (pepper) and Bc38 (risotto) both sampled in the same restaurant, between Bc44 (raw milk) and Bc46 (pasteurised milk), between Bc42 (pasteurised milk) and Bc12 (faeces), between Bc14 (wound) and Bc17 (acoustic duct) as well as between Bc 47 and Bc 65 with the origin in raw bulk milk, transferred in January and July to the same dairy, respectively, which suggested possible connections in origin as well as some deficiencies in cleaning and disinfection of the equipment.

Adesetan et al. (2020) reported, that the RAPD profile of *B. cereus* isolates from some retailed foods showed that all the strains are closely related, with a similarity coefficient of 70%. Tourasse et al. (2011) concluded that isolates from food and dairy-related sources frequently share identical genotypes with strains of diverse environmental origins.

We could not confirm these findings, because we did not include enough environmental samples in the study, but we can assume that the environment (i.e., water) and animals (i.e., raw milk) represent a potential common origin of the pathogenic *B. cereus* strains caused food contamination as well as clinical infections. Therefore, Castiaux et al. (2014) recommended that animals should be a focus of attention in the process of identifying a potential common origin of food contamination by emetic *B. cereus* strains. Chang et al. (2018) determined that the *B. cereus* strains involved in skin infection tended to form a distinct genetic cluster compared to isolates associated with invasive diseases like bacteriemia, which also had unique genetic features. The strains causing the same types of illnesses were also classified in different clonal complexes in our study. For example, the clonal complex no. 10 included only isolates from clinical specimens, but from different sources, such as faeces, haemoculture and nasal mucosa.

Large circular plasmids have been described in a variety of microorganisms. Many of them are responsible for distinctive and significant bacterial traits, including virulence, nitrogen fixation, root nodulation, antibiotic and heavy metal resistance, conjugation and other metabolic transformations. Their closed-circular supercoiled forms move very slowly in pulsed-field gels and relaxed or nicked open-circular forms remain trapped in the sample wells (Barton et al. 1995).

Some authors considered that among the representatives of *B. cereus* sensu lato plasmids are essential for defining only the *B. anthracis*, *B. cereus* sensu stricto and *B. thuringiensis* species (Ehling-Schulz et al. 2006; Vilas-Bóas et al. 2007; Zheng et al. 2015); however, Andrup et al. (2008)

confirmed that *B. mycooides* also harboured large plasmids. *B. anthracis*, *B. thuringiensis*, and the emetic *B. cereus* major virulence factors are located extrachromosomally on large plasmids (Rasko et al. 2005). The plasmids in this group display strain-dependent distribution, with some strains containing no plasmids, whereas others have many (more than 10). Some of these plasmids have small genome size, only 2 kb, whereas others are very large, up to 600 kb (He et al. 2010; Liu et al. 2013; Zheng et al. 2015). Fayad et al. (2019) also confirmed the most striking difference between species resides at the level of their plasmid content.

In contrast, the authors Helgason et al. (2000) and Rasko et al. (2005) claimed, that the plasmid profile of *B. cereus* sensu lato was extremely variable, and no well-defined conserved members have been identified that could delineate the species. These plasmid-based species definitions have resulted in the classification of members of the *B. cereus* group that are not valid when molecular typing is applied and the suggestion that these three species should be regarded as a single species.

With the PFGE method using restriction endonuclease S1 we confirmed only 39.3% of *B. cereus* isolates harbouring one or more plasmids, while Helgason et al. (2000) obtained the plasmids in size 15 to 600 kb in 82% of periodontal and dairy isolates. One large circular plasmid was found in 21.3% of our tested strains, while three and more of them in 8.2% of strains, mostly with the sizes from 50 to 200 kb. Rasko et al. (2007) reported about plasmids in *B. cereus* strains ranging from ~54 to 466 kb including for the *B. anthracis* characteristic pXO1-like plasmids in size from ~181 to 272 kb, while Fayad et al. (2019) studied *B. cereus* sensu stricto strains with an average of two plasmids per strain with the size from 2931–715,614 bp. In present study the maximum size of plasmids was 200 kb, which was probably the consequence of the limited methodology we used. We have to highlight that we could not detect smaller plasmids with the regular gel electrophoresis according to Andrup et al. (2007), because we were not able to perform the gel electrophoresis the predicted time at enough low temperature to obtain useful results.

The problem of chromosomal and large plasmid DNA preparation for PFGE is also the sporulation of *B. cereus* (Liu et al. 2016). However, to prevent this, we pre-incubated the fresh culture for DNA extraction for only 4 h, such that only a small number of spores formed. *B. cereus* cells have cell walls, which is difficult to be lysed, so the time of incubation with lysozyme, lysostaphin, and proteinase K was longer than for some other Gram-positive bacteria (Liu et al. 2016; Samapundo et al. 2011).

B. cereus is well known as an intrinsic β -lactamase producer with chromosomal resistance to penicillins and cephalosporins. Three different β -lactamases, named β -lactamase I, II and III, have been reported in this species (Chen et al.

2003). *B. cereus* serine- β -lactamase I (BCI) and β -lactamase III (BCIII) are group 2A enzymes according to the BJM classification that prefer penicillin substrates and are inhibited by CA. *B. cereus* β -lactamase II (BCII) is a heat-stable, chromosomally mediated metallo- β -lactamase from enzyme group 3a and subclass B1, which hydrolyzes cephalosporins and a broad spectrum of carbapenems, but not monobactams, and is inhibited by EDTA and not by CA (Bush et al. 1995; Palzkill, 2013). This molecular subclass includes IMP, VIM, NDM, DIM, GIM, SIM and SPM (Bush and Jacoby 2010; Sawa et al. 2020). Metallo- β -lactamases can be divided into those encoded by transmissible genes or chromosomally mediated. Despite expectations about intrinsic resistance to carbapenems, clinical studies show that most *B. cereus* isolates from different specimens were susceptible to carbapenems such as imipenem and meropenem, although resistant strains have also been isolated (Ikeda et al. 2015; Kiyomizu et al. 2008; Savini et al. 2009). In our previous study testing the same isolates, *bla*_{VIM} genes were confirmed only in 21.2% of *B. cereus* strains, while *bla*_{IMP}, *bla*_{GIM} and *bla*_{SIM} genes were absent (Torkar and Bedenić 2018). It can be assumed that chromosomally encoded BCII found in all *B. cereus* strains, does not inactivate carbapenems and resistance to these antibiotics is probably a consequence of acquired genes.

Some *B. cereus* isolates, resistant not only to β -lactams, but also to cotrimoxazole, clindamycin, erythromycin and tetracyclines have been identified recently (Bottone 2010; Savini et al. 2009). The genes encoding resistance to some of these antimicrobials, i.e., erythromycin or tetracyclines might be located on plasmids (Barbosa et al. 2014; Rather et al. 2012). In our previous work, the resistance to kanamycin, bacitracin, gentamicin, ciprofloxacin, tetracycline or carbapenems was observed in only a few strains, while the amplicons of the family *bla*_{CTX-M} and *bla*_{TEM} genes were confirmed among 68.2% and 34.8% of the samples, respectively (Godič Torkar and Bedenić 2018). We could not confirm any significant differences between the presence of plasmids, PFGE patterns or the source of studied *B. cereus* isolates and their content of resistance genes ($p > 0.05$). The correlation between the presence of plasmids and *bla*_{CTX-M}, *bla*_{VIM} and *bla*_{TEM} genes as well as tetracycline resistance was not significant.

A number of exotoxins contribute to the pathogenicity of *B. cereus* in both gastrointestinal and other infections. The genes encoding the three most important and well-known enterotoxins non-hemolytic enterotoxin (Nhe), hemolysin BL (Hbl) and cytotoxin K (CytK) are chromosomally encoded and their horizontal transfer is rare (Böhm et al. 2015). While the genes encoding Nhe, Hbl and CytK are present in the majority of isolates (63% to 96%), depending on their origin, the *ces* genes encoding emetic toxin were found in only 5% to 9% of isolates (Bianco et al. 2021;

Owusu-Kwarteng et al. 2017; Gao et al. 2018). The *ces* genes are located on a 208 kb to 272 kb mega-plasmid that has high similarities to pXO1-like plasmids (Ehling-Schulz et al. 2006; Rasko et al. 2007). In our *B. cereus* strains, we were able to detect the plasmids up to 200 kb, so we could not speculate on the relationship between the plasmid-containing strains and their toxicity.

Conclusions

The PFGE profiles showed a remarkable polymorphism existing among all *B. cereus* strains, a visible correlation between profiles and the sources of isolates was not confirmed. Bacterial isolates from foods, being the probable source of alimentary toxoinfection, showed very low similarity to isolates from stool specimens and were not epidemiologically or temporally related. The environmental water isolates were each distributed in different clonal complexes. The distribution of the strains of different origin between the clonal complexes was significantly different. Only 39.3% of *B. cereus* isolates harboured one or more plasmids in sizes of 50 to 200 kb. The strains with the plasmids were spread evenly across all PFGE clonal complexes.

Declarations

Conflict of interests The authors do not have any conflict of interest to declare. No competing financial interests exist.

References

- Adesetan TO, Efuntoye MO, Babalola OO (2020) Genotypic profiling of *Bacillus cereus* recovered from some retail foods in Ogun State, Nigeria, and their phylogenetic relationship. *Int J Microbiol* 2020:1–9. <https://doi.org/10.1155/2020/3750948>
- Akamatsu R, Suzuki M, Okinaka K, Sasahara T, Yamane K, Suzuki S, Fujikura D, Furuta Y, Ohnishi N, Esaki M, Shibayama K, Higashi H (2019) Novel sequence type in *Bacillus cereus* strains associated with nosocomial infections and bacteremia. *Japan Emerg Infect Diseases* 25(5):883–890. <https://doi.org/10.3201/eid2505.171890>
- Andrup L, Klingenberg Barfod K, Jensen GB, Smidt L (2008) Detection of large plasmids from the *Bacillus cereus* group. *Plasmid* 59(2):139–43. <https://doi.org/10.1016/j.plasmid.2007.11.005>
- Barbosa TM, Phelan RW, Leong D, Morrissey JP, Adams C, Dobson ADW, O’Gara F (2014) A novel erythromycin resistance plasmid from *Bacillus* sp. strain HS24, isolated from the marine sponge *Haliclona simulans*. *PLoS ONE* 9(12):e115583. <https://doi.org/10.1371/journal.pone.0115583>
- Barton BM, Harding GP, Zuccarelli AJ (1995) A general method of detecting and sizing large plasmids. *Anal Biochem* 226:235–240. <https://doi.org/10.1006/abio.1995.1220>
- Bianco A, Capozzi L, Miccolupo A et al (2021) Multi-locus sequence typing and virulence profile in *Bacillus cereus* sensu lato strains

- isolated from dairy products. *Ital J Food Saf* 9(4):8401. <https://doi.org/10.4081/ijfs.2020.8401>
- Böhm ME, Huptas C, Krey VM, Scherer S (2015) Massive horizontal gene transfer, strictly vertical inheritance and ancient duplications differentially shape the evolution of *Bacillus cereus* enterotoxin operons *hbl*, *cytK* and *nhe*. *BMC Evol Biol* 15:246. <https://doi.org/10.1186/s12862-015-0529-4>
- Bottone EJ (2010) *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* 23:382–398. <https://doi.org/10.1128/CMR.00073-09>
- Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for β -lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 39(6):1211–1233
- Bush K, Jacoby GA (2010) Updated functional classification of β -lactamases. *Antimicrob Agents Chemother* 54(3):969–976
- Castiaux V, N'guessan E, Swiecicka I, Delbrassinne L, Dierick K, Mahillon J (2014) Diversity of pulsed-field gel electrophoresis patterns of cereulide-producing isolates of *Bacillus cereus* and *Bacillus weihenstephanensis*. *FEMS Microbiol Lett* 353(2):124–131. <https://doi.org/10.1111/1574-6968.12423>
- Chang T, Rosch JW, Gu Z, Hakim H, Hewitt C, Gaur A, Wu G, Hayden RT (2018) Whole-genome characterisation of *Bacillus cereus* associated with specific disease manifestations. *Infect Immun* 86(2):e00574–e017. <https://doi.org/10.1128/IAI.00574-17>
- Chen Y, Succi J, Tenover FC, Koehler TM (2003) β -lactamase genes of the penicillin-susceptible *Bacillus anthracis* Sterne strain. *J Bacteriol* 185(3):823–830. <https://doi.org/10.1128/jb.185.3.823-830.2003>
- Ehling-Schulz M, Fricker M, Grallert H, Rieck P, Wagner M, Scherer S (2006) Cereulide synthetase gene cluster from emetic *Bacillus cereus*: structure and location on a mega virulence plasmid related to *Bacillus anthracis* toxin plasmid pXO1. *BMC Microbiol*. <https://doi.org/10.1186/1471-2180-6-20>
- Fayad N, Kallassy Awad M, Mahillon J (2019) Diversity of *Bacillus cereus* sensu lato mobilome. *BMC Genomics* 20:436. <https://doi.org/10.1186/s12864-019-5764-4>
- Gao T, Ding Y, Wu Q et al (2018) Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of *Bacillus cereus* isolated from pasteurized milk in China. *Front Microbiol* 9:533. <https://doi.org/10.3389/fmicb.2018.00533>
- Gođić Torkar K, Bedenić B (2018) Antimicrobial susceptibility and characterisation of metallo- β -lactamases, extended-spectrum β -lactamases, and carbapenemases of *Bacillus cereus* isolates. *Microb Pathogen* 118:140–145. <https://doi.org/10.1016/j.micpath.2018.03.026>
- Griffiths MW, Schraft H (2017) *Bacillus cereus* food poisoning. In: Christine ER, Dodd T, Aldsworth G, Stein RA (eds) *Foodborne Diseases*, 3rd edn. Elsevier inc., Cambridge, pp 395–405
- He J, Shao X, Zheng H, Li M, Wang J, Zhang Q, Li L, Liu Z, Sun M, Wang S, Yu Z (2010) Complete genome sequence of *Bacillus thuringiensis* mutant strain BMB171. *J Bacteriol* 192(15):4074–4075. <https://doi.org/10.1128/JB.00562-10>
- Helgason E, Økstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolstø AB (2000) Genetic structure of population of *Bacillus cereus* and *Bacillus thuringiensis* isolates associated with periodontitis and other human infections. *J Clin Microbiol* 38(4):1615–1622
- Ikeda M, Yagihara Y, Tatsuno K, Okazaki M, Okugawa S, Moriya K (2015) Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infections. *Ann Clin Microbiol Antimicrob* 14(43):1–7. <https://doi.org/10.1186/s12941-015-0104-2>
- ISO 7932 (2004) Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of presumptive *Bacillus cereus*—Colony-count technique at 30°C. ISO document. International Standard Organization, Belgium, Brussels, p 7
- Jensen GB, Hansen MB, Ellenberg J, Mahillon J (2003) The hidden lifestyles of *Bacillus cereus* and relatives. *Environ Microbiol* 5:631–640. <https://doi.org/10.1046/j.1462-2920.2003.00461.x>
- Kiyomizu K, Yagi T, Yoshida H, Minami R, Tanimura A, Karasuno T, Hiraoka A (2008) Fulminant septicemia of *Bacillus cereus* resistant to carbapenem in a patient with biphenotypic acute leukemia. *J Infect Chemother* 14(5):361–367. <https://doi.org/10.1007/s10156-008-0627-y>
- Leski TA, Caswell CC, Pawlowski M, Klinke DJ, Bujnicki JM, Hart SJ, Lukomski S (2009) Identification and classification of *bcl* genes and proteins of *Bacillus cereus* group organisms and their application in *Bacillus anthracis* detection and fingerprinting. *Appl Environ Microbiol* 75:7163–7172. <https://doi.org/10.1128/AEM.01069-09>
- Liu PY-F, Ke S-C, Chen S-L (1997) Use of pulsed-field gel electrophoresis to investigate a pseudo-outbreak of *Bacillus cereus* in a pediatric unit. *J Clin Microbiol* 35(6):1533–1535
- Liu X, Huang M, Zhang H, Li W, Pang Z, Lin P, Qian H (2016) Application of pulsed-field gel electrophoresis (PFGE) in *Bacillus cereus* typing. *Int J Clin Exp Pathol* 9(9):9497–9502
- Liu XJ, Ruan LF, Lin XY, Zhao CM, Zhong CY, Sun M (2013) Distribution of 2-kb miniplasmid pBMB2062 from *Bacillus thuringiensis kurstaki* YBT-1520 strain in *Bacillus* species. *Ann Microbiol (Paris)* 63:1639–1644. <https://doi.org/10.1007/s13213-013-0627-8>
- Liu Y, Lai Q, Göker M, Meier-Kolthoff JP, Wang M, Sun Y, Wang L, Shao Z (2015) Genomic insights into the taxonomic status of the *Bacillus cereus* group. *Sci Rep* 16(5):14082. <https://doi.org/10.1038/srep14082>
- Mäntynen V, Lindström K (1998) A rapid PCR-Based DNA test for enterotoxic *Bacillus cereus*. *Appl Environ Microbiol* 64(5):1634–1639
- Merzougui S, Lkhider M, Grosset N, Gautier M, Cohen N (2013) Differentiation by molecular typing of *Bacillus cereus* isolates from food in Morocco: PFGE-Eric PCR. *Food Public Health* 3(4):223–227. <https://doi.org/10.5923/j.fph.20130304.06>
- Moore E, Arnscheidt A, Krüger A, Strömpl C, Mau M (2004) Simplified protocols for the preparation of genomic DNA from bacterial cultures. *Molecular Microbial Ecology Manual*, 2nd edn. Kluwer Academic Publishers, Netherlands, pp 3–18
- Owusu-Kwarteng J, Wuni A, Akabanda F et al (2017) Prevalence, virulence factor genes and antibiotic resistance of *Bacillus cereus* sensu lato isolated from dairy farms and traditional dairy products. *BMC Microbiol* 17:65. <https://doi.org/10.1186/s12866-017-0975-9>
- Palma L, Muñoz D, Berry C, Murillo J, Caballero P (2014) *Bacillus thuringiensis* toxins: an overview of their biocidal activity. *Toxins* 6(12):3296–3325. <https://doi.org/10.3390/toxins6123296>
- Park SH, Kim HJ, Kim JH, Kim TW, Kim HY (2007) Simultaneous detection and identification of *Bacillus cereus* group bacteria using multiplex PCR. *J Microbiol Biotechnol* 17(7):1177–1182
- Patino-Navarrete R, Sanchis V (2017) Evolutionary processes and environmental factors underlying the genetic diversity and lifestyles of *Bacillus cereus* group bacteria. *Res Microbiol* 168:309–318. <https://doi.org/10.1016/j.resmic.2016.07.002>
- Palzkill T (2013) Metallo- β -lactamase structure and function. *Ann N Y Acad Sci* 1277:91–104. <https://doi.org/10.1111/j.1749-6632.2012.06796.x>
- Rasko DA, Ravel J, Økstad OA, Helgason E, Cer RZ, Jiang L, Shores KA, Fouts DE, Tourasse NJ, Angiuoli SV, Kolonay J, Nelson WC, Kolstø AB, Fraser CM, Read TD (2004) The genome sequence of *Bacillus cereus* ATCC 10987 reveals metabolic adaptations and a large plasmid related to *Bacillus anthracis* pXO1. *Nucleic Acids Res* 32(3):977–988. <https://doi.org/10.1093/nar/gkh258>
- Rasko DA, Altherr MR, Han CS, Ravel J (2005) Genomics of the *Bacillus cereus* group of organisms. *FEMS Microbiol Rev* 29(2):303–329. <https://doi.org/10.1016/j.fmrre.2004.12.005>

- Rasko DA, Rosovitz MJ, Økstad OA, Fouts DE, Jiang L, Cer RZ, Kolstø AB, Gill SR, Ravel J (2007) Complete sequence analysis of novel plasmids from emetic and periodontal *Bacillus cereus* isolates reveals a common evolutionary history among the *Bacillus cereus*-group plasmids, including *Bacillus anthracis* pXO1. *J Bacteriol* 189(1):52–64. <https://doi.org/10.1128/JB.01313-06>
- Rather MA, Rabinder SA, Singh Gill JP, Mir AQ, Hassan MN (2012) Detection and sequencing of plasmid encoded tetracycline resistance determinants (*tetA* and *tetB*) from food-borne *Bacillus cereus* isolates. *Asian Pacific J Tropical Medic* 5(9):709–712. [https://doi.org/10.1016/S1995-7645\(12\)60111-4](https://doi.org/10.1016/S1995-7645(12)60111-4)
- Samapundo S, Heyndrickx M, Xhaferi R, Devlieghere F (2011) Incidence, diversity and toxin gene characteristics of *Bacillus cereus* group strains isolated from food products marketed in Belgium. *Int J Food Microbiol* 150(1):34–41. <https://doi.org/10.1016/j.ijfoodmicro.2011.07.013>
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory, New York, p 1659
- Savini V, Favaro M, Fontana C, Catavittello C, Balbinot A, Talia M, Febbo F, D'Antonio D (2009) *Bacillus cereus* heteroresistant to carbapenems in a cancer patient. *J Hosp Infect* 71(3):288–290. <https://doi.org/10.1016/j.jhin.2008.11.002>
- Sawa T, Kooguchi K, Moriyama K (2020) Molecular diversity of extended-spectrum β -lactamases and carbapenemases, and antimicrobial resistance. *J Intensive Care* 8(13):1–13. <https://doi.org/10.1186/s40560-020-0429-6>
- Sjölund M, Tano E, Blaser MJ, Andersson DI, Engstrand L (2005) Persistence of resistant *Staphylococcus epidermidis* after single course of clarithromycin. *Emerg Infect Dis* 11(9):1389–1393. <https://doi.org/10.3201/eid1109.050124>
- Tourasse NJ, Helgason E, Klevan A, Sylvestre P, Moya M, Haustant M, Økstad OA, Fouet A, Kolstø A-B (2011) Extended and global phylogenetic view of the *Bacillus cereus* group population by combination of MLST, AFLP, and MLEE genotyping data. *Food Microbiol* 28(2):3236–3244. <https://doi.org/10.1016/j.fm.2010.06.014>
- Vassileva M, Torii K, Oshimoto M, Okamoto A, Agata N, Yamada K, Hasegawa T, Ohta M (2007) A new phylogenetic cluster of cereulide-producing *Bacillus cereus* strains. *J Clin Microbiol* 45(4):1274–1277. <https://doi.org/10.1128/JCM.02224-06>
- Vilas-Bôas GT, Peruca APS, Arantes OMN (2007) Biology and taxonomy of *Bacillus cereus*, *Bacillus anthracis* and *Bacillus thuringiensis*. *Can J Microbiol* 53(6):673–687. <https://doi.org/10.1139/W07-029>
- Yang Y, Gu H, Yu X, Zhan L, Chen J, Luo Y, Zhang Y, Zhang Y, Lu Y, Jiang J, Mei L (2017) Genotypic heterogeneity of emetic toxin producing *Bacillus cereus* isolates from China. *FEMS Microbiol Lett* 364(1):fnw237. <https://doi.org/10.1093/femsle/fnw237>
- Yavuz E, Gunes H, Harsa S, Bulut C, Yenidunya AF (2004) Optimisation of pulsed field gel electrophoresis (PFGE) conditions for thermophilic bacilli. *World J Microbiol Biotechnol* 20(8):871–874. <https://doi.org/10.1007/s11274-004-1004-3>
- Zheng J, Guan Z, Cao S, Peng D, Ruan L, Jiang D, Sun M (2015) Plasmids are vectors for redundant chromosomal genes in the *Bacillus cereus* group. *BMC Genomics* 16(1):6. <https://doi.org/10.1186/s12864-014-1206-5>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.