



## Psychrotrophic properties, toxigenic characteristics, and PFGE profiles of *Bacillus cereus* isolated from different foods and spices

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**ABSTRACT:** *Bacillus cereus* is an aerobic and facultatively anaerobic, spore-forming bacterium, and it is found naturally in soil and poses a risk factor for the contamination of food and foodstuffs including cereals, vegetables, spices, ready-to-eat (RTE) foods, meats, milk, and dairy products. This study determined the prevalence of *B. cereus* in raw poultry meat, raw cow's milk, cheese, spices, and RTE foods in Hatay province. The study also analysed the psychrotrophic properties, toxigenic characteristics, and pulsed-field gel electrophoresis (PFGE) profiles of the isolates. The levels of contamination with *B. cereus* determined for cheese, raw milk, RTE foods, spices, and raw poultry meat were 16.6%, 34.2%, 42.8%, 49%, and 55.5%, respectively. *B. cereus* was isolated from 84 (42%) of the 200 samples analysed and the 84 isolates were verified by PCR analysis targeting the haemolysin gene specific for *B. cereus*. Of the total isolates, 64 (76.1%) were psychrotrophic. The toxin gene profiling of *B. cereus* isolates was determined by amplifying the four genes *nhe*, *hbl*, *cytK*, and *ces*. The *nhe* and *cytK* genes were most frequently detected in the isolates, while the *hbl* and *ces* genes were not found. In addition, a high genetic relationship between the isolates was detected at a 92% similarity level by PFGE analysis. In conclusion, the occurrence of both psychrotrophic and toxigenic *B. cereus* strains in this study indicated a potential risk for food spoilage and food poisoning.

**Key words:** *Bacillus cereus*, food poisoning, PCR, PFGE typing, toxin gene.

## Propriedades psicrotróficas, características toxigênicas e perfis PFGE de *Bacillus cereus* isolado de diferentes alimentos e especiarias

**RESUMO:** *Bacillus cereus* é uma bactéria formadora de esporos aeróbica e facultativamente anaeróbica, encontrada naturalmente no solo e representa um fator de risco para a contaminação de alimentos e alimentos, incluindo cereais, vegetais, especiarias, alimentos prontos para comer (RTE), carnes, leite e laticínios. Este estudo teve como objetivo determinar a prevalência de *B. cereus* em carne crua de aves, leite de vaca cru, queijo, especiarias e alimentos RTE na província de Hatay. O estudo também analisou as propriedades psicrotróficas, características toxigênicas e perfis de PFGE dos isolados. Os níveis de contaminação com *B. cereus* determinados para queijo, leite cru, alimentos RTE, especiarias e carne de frango crua foram de 16,6%, 34,2%, 42,8%, 49% e 55,5%, respectivamente. *B. cereus* foi isolado de 84 (42%) das 200 amostras analisadas e os 84 isolados foram verificados por análise de PCR visando o gene da hemolisina específico para *B. cereus*. Do total de isolados, 64 (76,1%) eram psicrotróficos. O perfil do gene da toxina de isolados de *B. cereus* foi determinado pela amplificação dos quatro genes *nhe*, *hbl*, *cytK* e *ces*. Os genes *nhe* e *cytK* foram detectados com maior frequência nos isolados, enquanto os genes *hbl* e *ces* não foram encontrados. Além disso, uma alta relação genética entre os isolados foi detectada em um nível de similaridade de 92% pela análise de PFGE. Em conclusão, a ocorrência de cepas psicrotróficas e toxigênicas de *B. cereus* neste estudo indica um risco potencial de deterioração e intoxicação alimentar.

**Palavras-chave:** *Bacillus cereus*, intoxicação alimentar, PCR, tipagem PFGE, gene da toxina.

## INTRODUCTION

*Bacillus cereus* is one of the major foodborne pathogenic bacteria that causes two types of food poisoning. The first, emetic syndrome, is characterised by nausea and vomiting and occurs within a few hours of consumption of contaminated food. Emetic syndrome is caused by a toxin called

cereulide, a heat-stable peptide synthesised in the food. The second, diarrhoeal syndrome, is characterised by abdominal pain and diarrhoea. Diarrhoea is associated with some enterotoxins, the haemolysin BL (HBL), the non-haemolytic enterotoxin (NHE), and the cytotoxin K (CYTK). Diarrhoeal toxins are sensitive to heat and are synthesised during the vegetative growth of *Bacillus*

*B. cereus* in the small intestine. These enterotoxins are particularly important in the virulence mechanism of *B. cereus* and are encoded by the three genes *hbl*, *nhe*, and *cytK*, respectively. There are also reports that the enterotoxin genes are more commonly found in *B. cereus* strains than the emetic gene (GRANUM & LUND, 1997; STENFORS ARNESEN et al., 2008; HARIRAM & LABBE, 2015; TEWARI et al., 2015; OWUSU-KWARTENG et al., 2017; FOGELE et al., 2018; GAO et al., 2018; BERTHOLD-PLUTA et al., 2019; SÁNCHEZ-CHICA et al., 2020b). At the same time, *B. cereus* can cause serious extraintestinal diseases such as bacteraemia, meningitis, arthritis, and endocarditis (LIU et al., 2016; YU et al., 2020), that need to be taken into consideration as well as food intoxication.

*B. cereus* is generally known as a mesophilic microorganism, which has optimal growth between 35 and 40 °C. However, increasing numbers of psychrotrophic *B. cereus* strains described can grow at refrigerator temperatures and are responsible from the microbial spoilage of refrigerated foods (GRANUM & LUND, 1997; MC KILLIP, 2000; WIJNANDS et al., 2006; STENFORS ARNESEN et al., 2008; PRETORIUS & BUYS, 2021). Until now, *B. cereus* has been isolated from different kinds of foods and foodstuffs such as meats, milk and dairy products, fish and seafood products, rice, starch, flours, spices and herbs, vegetables, ready-to-eat (RTE) foods, powdered foods (infant formula, powdered milk), and pastry (WIJNANDS et al., 2006; PARK et al., 2009; CADIRCI et al., 2013; HARIRAM & LABBE, 2015; TEWARI et al., 2015; OWUSU-KWARTENG et al., 2017; YIBAR et al., 2017; FOGELE et al., 2018; GAO et al., 2018; GDOURA-BEN AMOR et al., 2018; OSMAN et al., 2018; BERTHOLD-PLUTA et al., 2019; ADAME-GÓMEZ et al., 2020; SÁNCHEZ-CHICA et al., 2020a; SÁNCHEZ-CHICA et al., 2020b). The diarrhoeal disease is usually associated with proteinaceous foods, while the emetic type is more commonly due to the consumption of starch-rich foods (AGATA et al., 2002; STENFORS ARNESEN et al., 2008).

Considering that food safety is an important public health concern and consumption of foods contaminated with *B. cereus* can cause food intoxication, we investigated the prevalence of *B. cereus* in different foods and spices, which are very popular, traditional and often consumed by local people in Hatay province. *B. cereus* isolates were further analysed for their ability to grow at 7 °C,

and the presence of toxin genes. Besides, PFGE analysis was carried out to evaluate the genetic relatedness of isolates.

## MATERIALS AND METHODS

### *Samples*

In Hatay, the southeastern province of Turkey, 45 raw poultry meat, 35 raw cow's milk, 30 cheese samples, 55 spices, and 35 ready-to-eat (RTE) foods were randomly collected from December 2018 to February 2020 and transported to the laboratory under cold chain conditions.

The spice samples were dried, powdered, and unpackaged and included thyme, mint, cumin, black pepper, red pepper, ginger, and sumac. The RTE foods included: Abagannus (a kind of salad prepared from roasted eggplant, roasted paprika, roasted tomato, pomegranate juice, garlic, olive oil, and salt), humus (hummus means chickpea in Arabic, and is made from chickpeas, sesame paste, lemon juice, garlic, salt, cumin, and olive oil), Muhammara (prepared for breakfast from walnut paste, tomato paste, spices, and olive oil), haydari (a kind of appetiser made from strained yogurt, mint, and dill), and olive salad (prepared for breakfast and made from green olives without seeds, parsley, tomatoes, green onions, olive oil, pomegranate juice, and red peppers). The cheese samples included traditional cheeses such as Sürk and Carra that are frequently consumed in Hatay province. They are both produced with spices or herbs. Carra cheese is made by mixing white cheese (manufactured from raw goat's milk or cow's milk) and Cokelek (cheese produced by heat-treating yogurt). Black cumin or thyme is added to the cheese. Sürk cheese is also made from Cokelek and thyme, cumin, mint, cinnamon, black peppers, ginger, and red peppers are used in its production.

### *Reference strain*

Lyophilised *Bacillus cereus* strain was obtained from the National Type Culture Collection Laboratory, General Directorate of Public Health, Turkey, and used as a positive control in microbiological and molecular analysis.

### *Isolation and identification*

The conventional culture method (TALLENT et al., 2012) and PCR technique were used in the isolation and identification of *B. cereus*. For the selective and pre-enrichment stages, 90 mL of Trypticase Soy Broth supplemented with polymyxin B (10 mg/mL) was added to each sample and they were

homogenised. Then, the mixtures were incubated for 24 h at 30 °C. A loopful of each selective broth was taken, plated onto Chromogenic *Bacillus cereus* Agar (Oxoid CM1036) mixed with Brilliance *Bacillus cereus* Selective Supplement (Oxoid SR0230), and incubated at 30 °C under aerobic conditions. After 24–48 hours of incubation, all the suspicious blue/green colonies of *B. cereus* were taken and stored at -20 °C for molecular and toxin gene analysis.

#### Molecular analysis

For the molecular identification, all the presumptive *B. cereus* isolates were confirmed by the PCR technique based on the detection of the haemolysin gene. Genomic DNA was extracted using a GF-1 Nucleic Acid Extraction Kit (Vivantis, Malaysia) from the overnight Brain-Heart Infusion broth (Oxoid) cultures. DNA extraction was done according to the manufacturer's instructions. Specific primer pairs and thermal cycling conditions described by WANG et al. (1997) were used in this study. The PCR amplicons were controlled in gel electrophoresis (1.5%) carried out at 100 V for 50 min (CS-300V, England). The gels were stained with Safe View (Abm G108) and visualised with a UV transilluminator (UVP, USA).

#### Toxin gene analysis

The toxin genes (*hbl*, *nhe*, *cytK*, *ces*) of *B. cereus* isolates were detected with a multiplex PCR. In the present study, *hbl*, *nhe*, *cytK*, and *ces* genes were searched using the following primers: (*hbl*F, 5'-GTAAATTAIGATGAICAATTTTC-3'; *hbl*R, 5'-AGAATAGGCATTCATAGATT-3') (*nhe*F, 5'-AAGCIGCTCTTCGIATTC-3'; *nhe*R, 5'-ITIGTTGAAATAAGCTGTGG-3') (*cytK*F, 5'-ACAGATATCGGICAAAATGC-3'; *cytK*R, 5'-CAAGTIACTTGACCIGTTGC-3') (*ces*F, 5'-GGTGACACATTATCATATAAGGTG-3', *ces*R, 5'-GTAAGCGAACCTGTCTGTAACAACA-3') (Ella Biotech) (EHLING-SCHULZ et al., 2005; EHLING-SCHULZ et al., 2006). The reaction mixture for multiplex PCR and amplification protocol was produced according to EHLING-SCHULZ et al. (2006).

#### Psychrotrophic property analysis

To detect the psychrotrophic properties of *B. cereus*, all isolates were subcultured in Brain-Heart Infusion broth and incubated for 24 h at 30 °C. Then, they were streaked on Tryptone Soya Agar. Plates were placed in a refrigerated incubator and the temperature was set at 7 °C. Plates were checked for

bacterial growth after incubation periods of 24, 48, 72, and 168 hours.

#### Pulsed-field gel electrophoresis (PFGE) analysis

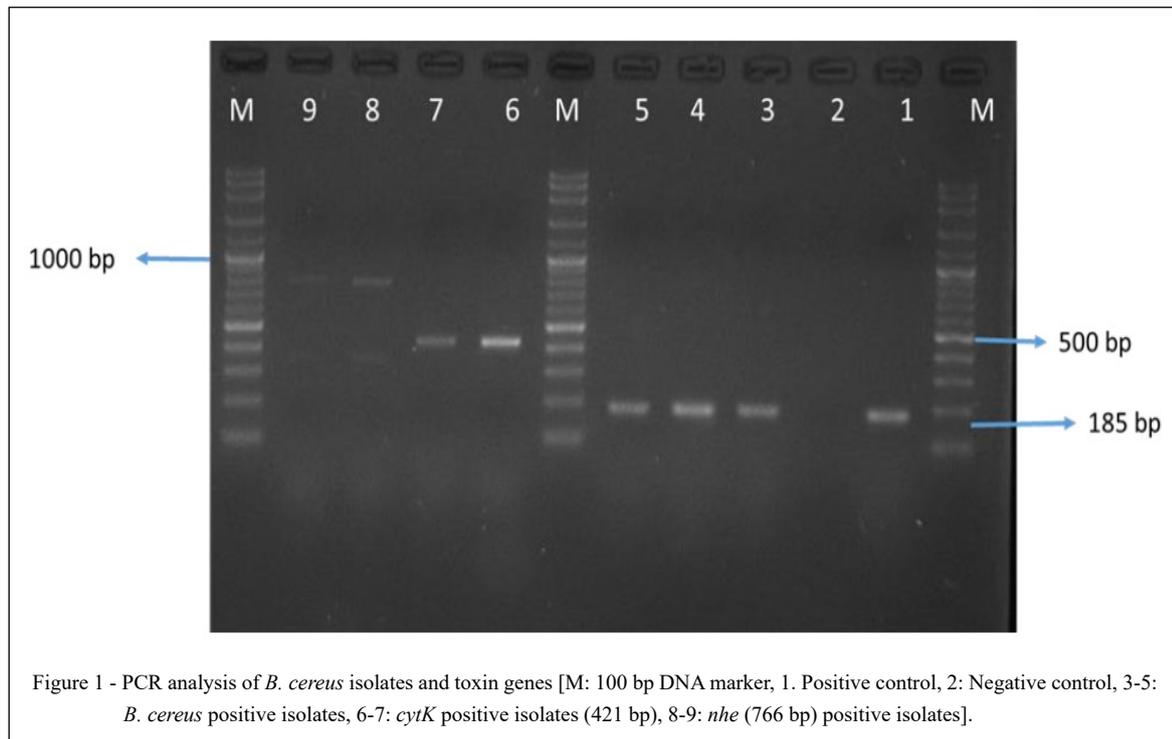
To better understand the genetic relationship between *B. cereus* strains, PFGE was performed according to LIU et al. (2016) using the *Sma*I macrorestriction enzyme. The 84 *B. cereus* isolates were first subjected to a cell lysis process to extract DNA. Then, genomic DNAs of the isolates were digested with *Sma*I (Takara, Japan) restriction enzyme. Following enzymatic digestion, they were subjected to electrophoresis in a CHEF-DR II system (Bio-Rad, Belgium). Then, electrophoretic bands were analysed under UV using a gel documentation system (Gel Doc, Bio-Rad, Belgium). The Bionumerics 5.10 software programme was used for dendrogram analysis, and the dendrogram was created using Dice coefficients. A similarity value was selected as 85% to reveal the genetic relationship of isolates.

## RESULTS AND DISCUSSION

#### Prevalence and psychrotrophic properties of *Bacillus cereus*

In the present study, 84 *B. cereus* isolates were recovered from 200 samples. High prevalence of *B. cereus* isolates was detected in poultry meat (25/45, 55.5%), followed by spices (27/55, 49%), RTE (15/35, 42.8%), raw milk (12/35, 34.2%), and cheese (5/30, 16.6%). Therefore, 84 isolates were molecularly confirmed as *B. cereus* by the amplification of the haemolysin gene (Figure 1). Sixty-four (76.1%) of the isolates had psychrotrophic abilities. All the isolated strains of poultry meat (100%) and eight of 15 (53.3%), eight of 12 (66.6%), 19 of 27 (70.3%), and four of five (80%) isolates recovered from RTE, milk, spices, and cheese, respectively, were able to grow at 7 °C (Table 1, 2 and 3).

The overall prevalence of *B. cereus* was 42% in this study. Compared to our data, different results have previously been reported as 30.9% by TEWARI et al. (2015), 40.4% by OWUSU-KWARTENG et al. (2017), 25.3% by GDOURA-BEN AMOR et al. (2018), 27% by GAO et al. (2018), 29.4% by ADAME-GÓMEZ et al. (2020), and 11% by SÁNCHEZ-CHICA et al. (2020b) in various foods in other countries. When comparing the results with other studies by food type, the *B. cereus* group was isolated from chicken meat at 50% (OSMAN et al., 2018), like this study (55.5%). WIJNANDS et al. (2006) and GDOURA-BEN AMOR et al. (2018) detected *B.*



*cereus* in 3.0% of meat and meat products, and 9.4% of raw poultry meat. Interestingly, GDOURABEN AMOR et al. (2018) and TEWARI et al. (2015) reported high incidence levels in cooked poultry compared to raw poultry samples. They explained this finding by the germination of *B. cereus* spores during inappropriate storage and the inadequate cooking of food.

Given that *B. cereus* is a common soil bacterium, it is often reported in milk. However, large numbers are needed in milk to cause intoxication and at this stage defects in milk are easily observed. In some dairy products, it is more difficult to observe spoilage and they pose a serious risk for *Bacillus cereus* food poisoning (STENFORS ARNESEN et al., 2008). For raw milk and dairy products, the prevalence of *B. cereus* in cheeses reported in this study was very similar to that reported by SÁNCHEZ-CHICA et al. (2020a) (19.6%), whereas WIJNANDS et al. (2006) and YIBAR et al. (2017) reported a lower prevalence (10%) in milk and milk products. Contrary to the above studies, BERTHOLD-PLUTA et al. (2019) detected *B. cereus* contamination in 41.7% of cheese samples.

In the studies of YIBAR et al. (2017), GAO et al. (2018), and BERTHOLD-PLUTA et al. (2019), the prevalence of *B. cereus* in

pasteurised milk was reported as 30%, 27%, and 26%, respectively. This may be considered as higher contamination for pasteurised milk since heat treatment is applied to pasteurised milk. The authors suggested that low/inadequate pasteurisation or post-pasteurisation contamination could be the reason. The heat-stable spores of *B. cereus* are a problem in milk and dairy products as they are not inactivated during the pasteurisation (BENAHMED et al., 2020; PRETORIUS & BUYS, 2021). When comparing raw milk results, a lower incidence of 3.8% was reported by YIBAR et al. (2017). Contrarily, OWUSUKWARTENG et al. (2017) reported a relatively higher prevalence in raw milk (47%), yogurt (35%), and cheese (39%). In addition, GUNDOGAN & AVCI (2014) found that raw milk, cheese, and ice cream samples were contaminated with *B. cereus* at levels of 90%, 70%, and 20%, respectively. *B. cereus* has also been identified in 13% of powdered milk (SÁNCHEZ-CHICA et al., 2020b), and 30% and 11% of infant formulas, respectively, in the studies of BERTHOLD-PLUTA et al. (2019), and SÁNCHEZ-CHICA et al. (2020b), and 7% of dairy desserts (CADIRCI et al., 2013).

Regarding the contamination level in spices, AKSU et al. (2000) and BERTHOLD-PLUTA

Table 1 - Psychrotrophic property, toxin gene, and PFGE profiles of *B. cereus* isolates.

Isolate	-----Source-----	-Psychrotrophic property-	-----Toxin gene-----				-Toxin profile-	---PFGE profile---
			<i>cytK</i>	<i>nhe</i>	<i>hbl</i>	<i>ces</i>		
1	Raw poultry meat	+	+	-	-	-	t <sub>1</sub>	A <sub>1</sub>
2	Raw poultry meat	+	-	+	-	-	t <sub>2</sub>	A <sub>1</sub>
3	Raw poultry meat	+	+	-	-	-	t <sub>1</sub>	C
4	Raw poultry meat	+	+	-	-	-	t <sub>1</sub>	C
5	Raw poultry meat	+	+	-	-	-	t <sub>1</sub>	C
6	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
7	Raw poultry meat	+	-	+	-	-	t <sub>2</sub>	C
8	Raw poultry meat	+	+	-	-	-	t <sub>1</sub>	C
9	Raw poultry meat	+	+	+	-	-	t <sub>3</sub>	A <sub>2</sub>
10	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	A <sub>2</sub>
11	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	A <sub>2</sub>
12	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	A <sub>2</sub>
13	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	A <sub>2</sub>
14	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
15	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
16	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
17	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
18	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
19	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
20	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
21	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
22	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
23	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
24	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
25	Raw poultry meat	+	-	-	-	-	t <sub>4</sub>	C
26	Raw cow milk	-	-	-	-	-	t <sub>4</sub>	C
27	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	C
28	Raw cow milk	-	-	-	-	-	t <sub>4</sub>	B <sub>1</sub>
29	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	B <sub>1</sub>
30	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	B <sub>1</sub>
31	Raw cow milk	-	-	-	-	-	t <sub>4</sub>	C
32	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	C
33	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	C
34	Raw cow milk	-	-	-	-	-	t <sub>4</sub>	C
35	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	C
36	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	C
37	Raw cow milk	+	-	-	-	-	t <sub>4</sub>	C

PFGE: pulsed-field gel electrophoresis; RTE: ready-to-eat; t: toxin profile; t<sub>1</sub>: *cytK*<sup>+</sup>, *nhe*<sup>+</sup>, *hbl*, *ces*<sup>-</sup>; t<sub>2</sub>: *cytK*<sup>-</sup>, *nhe*<sup>+</sup>, *hbl*, *ces*<sup>-</sup>; t<sub>3</sub>: *cytK*<sup>+</sup>, *nhe*<sup>+</sup>, *hbl*, *ces*<sup>-</sup>; t<sub>4</sub>: *cytK*<sup>-</sup>, *nhe*<sup>-</sup>, *hbl*, *ces*<sup>-</sup>.

Table 2 - Psychrotrophic property, toxin gene, and PFGE profiles of *B. cereus* isolates.

Isolate	--Source--	--Psychrotrophic property--	-----Toxin gene-----				--Toxin profile--	--PFGE profile--
			-- <i>cytK</i> --	-- <i>nhe</i> --	-- <i>hbl</i> --	-- <i>ces</i> --		
38	Cheese	-	-	+	-	-	t <sub>2</sub>	C
39	Cheese	+	-	+	-	-	t <sub>2</sub>	C
40	Cheese	+	-	-	-	-	t <sub>4</sub>	C
41	Cheese	+	-	-	-	-	t <sub>4</sub>	C
42	Cheese	+	-	+	-	-	t <sub>2</sub>	C
43	Spices	-	-	-	-	-	t <sub>4</sub>	C
44	Spices	+	-	-	-	-	t <sub>4</sub>	C
45	Spices	+	-	-	-	-	t <sub>4</sub>	C
46	Spices	+	+	+	-	-	t <sub>3</sub>	C
47	Spices	+	+	-	-	-	t <sub>1</sub>	C
48	Spices	+	+	-	-	-	t <sub>1</sub>	B2
49	Spices	+	-	-	-	-	t <sub>4</sub>	C
50	Spices	+	+	+	-	-	t <sub>3</sub>	C
51	Spices	-	+	+	-	-	t <sub>3</sub>	C
52	Spices	-	+	+	-	-	t <sub>3</sub>	C
53	Spices	+	+	+	-	-	t <sub>3</sub>	C
54	Spices	+	-	-	-	-	t <sub>4</sub>	C
55	Spices	+	+	+	-	-	t <sub>3</sub>	C
56	Spices	+	-	-	-	-	t <sub>4</sub>	C
57	Spices	+	+	+	-	-	t <sub>3</sub>	C
58	Spices	-	-	+	-	-	t <sub>2</sub>	C
59	Spices	-	+	-	-	-	t <sub>1</sub>	B1
60	Spices	-	+	-	-	-	t <sub>1</sub>	B1
61	Spices	-	+	+	-	-	t <sub>3</sub>	B1
62	Spices	+	-	+	-	-	t <sub>2</sub>	B1
63	Spices	+	+	+	-	-	t <sub>3</sub>	A2
64	Spices	+	+	+	-	-	t <sub>3</sub>	A2
65	Spices	+	-	-	-	-	t <sub>4</sub>	A2
66	Spices	+	-	+	-	-	t <sub>2</sub>	A2
67	Spices	+	+	+	-	-	t <sub>3</sub>	A2
68	Spices	-	+	+	-	-	t <sub>3</sub>	A2
69	Spices	+	-	+	-	-	t <sub>2</sub>	A2
70	RTE	-	-	-	-	-	t <sub>4</sub>	C
71	RTE	-	+	+	-	-	t <sub>3</sub>	C
72	RTE	+	+	+	-	-	t <sub>3</sub>	C

PFGE: pulsed-field gel electrophoresis; RTE: ready-to-eat; t: toxin profile; t<sub>1</sub>: *cytK*<sup>+</sup>, *nhe*<sup>-</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>; t<sub>2</sub>: *cytK*<sup>-</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>; t<sub>3</sub>: *cytK*<sup>+</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>; t<sub>4</sub>: *cytK*<sup>-</sup>, *nhe*<sup>-</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>.

Table 3 - Psychrotrophic property, toxin gene, and PFGE profiles of *B. cereus* isolates.

Isolate	--Source--	-Psychrotrophic property-	-----Toxin gene-----				-Toxin profile-	-PFGE profile-
			-- <i>cytK</i> --	-- <i>nhe</i> --	-- <i>hbl</i> --	-- <i>ces</i> --		
73	RTE	+	-	-	-	-	t <sub>4</sub>	C
74	RTE	+	-	+	-	-	t <sub>2</sub>	C
75	RTE	+	-	+	-	-	t <sub>2</sub>	C
76	RTE	+	-	-	-	-	t <sub>4</sub>	C
77	RTE	-	-	-	-	-	t <sub>4</sub>	C
78	RTE	-	-	-	-	-	t <sub>4</sub>	C
79	RTE	-	-	-	-	-	t <sub>4</sub>	C
80	RTE	-	-	-	-	-	t <sub>4</sub>	C
81	RTE	+	-	-	-	-	t <sub>4</sub>	C
82	RTE	+	-	-	-	-	t <sub>4</sub>	C
83	RTE	+	-	-	-	-	t <sub>4</sub>	C
84	RTE	-	-	-	-	-	t <sub>4</sub>	C

PFGE: pulsed-field gel electrophoresis; RTE: ready-to-eat; t: toxin profile; t<sub>1</sub>: *cytK*<sup>+</sup>, *nhe*<sup>-</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>; t<sub>2</sub>: *cytK*<sup>-</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>; t<sub>3</sub>: *cytK*<sup>+</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>; t<sub>4</sub>: *cytK*<sup>-</sup>, *nhe*<sup>-</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>.

et al. (2019) reported that the incidence of *B. cereus* in herbs and spices was 63%, with counts ranging from 2.0 to log 3.0 log cfu/g. In our study, the prevalence of *B. cereus* in spices was relatively high (49%) compared to the studies of GDOURA-BEN AMOR et al. (2018) and HARIRAM & LABBE (2015) with 28.8% and 31%, respectively, whereas FOGELE et al. (2018) reported a higher prevalence (76%) in spices. However, WIJNANDS et al. (2006) isolated *B. cereus* from 11.5% of flavourings in the Netherlands. Since *B. cereus* is a spore-forming and common soil microorganism, it could be difficult to eliminate this bacterium from food processing plants and the environment. In another context, PARK et al. (2009) found that 25% of cereal samples were positive for *B. cereus* in Korea. High contamination levels (56.7%) have also been reported in coffee samples by SOUZA & ABRANTES (2011). These results demonstrated that a higher prevalence in spices is possible due to the occurrence of this bacterium in soil and plants (GRANUM & LUND, 1997; STENFORS ARNESEN et al., 2008; BERTHOLD-PLUTA et al., 2019). As spices are often used as aromatic and flavour components in many types of foods (WIJNANDS et al. 2006; HARIRAM & LABBE 2015; FOGELE et al., 2018), this can be a potential risk for the cross-contamination of some food products, such as RTE foods, because they are not subjected to further

heat treatment before consumption (BERTHOLD-PLUTA et al. 2019). In this sense, it is essential to ensure the microbial safety of spices. As other researchers have recommended (AKSU et al. 2000; FOGELE et al., 2018), we suggested sterilisation and the hygienic and adequate packaging of spices.

RTE foods such as Abagannus, humus, haydari, muhammara, and olive salad are very popular and commonly consumed in Hatay province, but they may pose a potential risk for *B. cereus* contamination as they are prepared without any heat treatment and consumed directly. In this study, 15 (42.8%) out of 35 RTE samples were found to contain *B. cereus*, consistent with other studies where *B. cereus* prevalence in RTE foods was reported as 48.2% by WIJNANDS et al. (2006) and 35% by YU et al. (2020). The majority (76.1%) of our isolates were psychrotrophic, which means that they can grow slightly during refrigeration storage and can cause spoilage in refrigerated foods. Unlike our results, WIJNANDS et al. (2006) determined that the minority of their isolates (4.4%) were psychrotrophic using the PCR method while most of them were mesophilic. However, ADAME-GÓMEZ et al. (2020) reported that almost all their isolates (91.3%) were psychrophilic. Also, BERTHOLD-PLUTA et al. (2019) reported that 25% of their isolates could grow at 7 °C. In this respect, the presence of psychrotropic

*B. cereus* strains in various food products cannot be ignored since they have a significant effect especially on the quality and shelf life of refrigerated foods.

#### *Detection of toxin genes by multiplex PCR*

We found that 41.6% of our isolates harboured toxin genes while the remainder had none. The genes *cytK* and *nhe* were commonly detected in the isolates alone or together, but the other enterotoxin encoding gene, *hbl*, and emetic gene (*ces*) were not determined in this study (Table 1, 2 and 3).

According to the presence of toxin genes, four toxin gene profiles ( $t_1$ ; toxin profile 1,  $t_2$ ; toxin profile 2,  $t_3$ ; toxin profile 3, and  $t_4$ ; toxin profile 4) were detected in the study. Nine (10.7%), 11 (13%), 15 (17.8%), and 49 (58.3) isolates belonged to  $t_1$  (*cytK*<sup>+</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>),  $t_2$  (*cytK*<sup>-</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>),  $t_3$  (*cytK*<sup>+</sup>, *nhe*<sup>+</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>), and  $t_4$  (*cytK*<sup>-</sup>, *nhe*<sup>-</sup>, *hbl*<sup>-</sup>, *ces*<sup>-</sup>), respectively. The most prevalent toxin profile among the enterotoxigenic isolates was  $t_3$ , including both *cytK* and *nhe* genes.

When reviewing studies performed in Turkey and elsewhere (PARK et al., 2009; CADIRCI et al. 2013; HARIRAM & LABBE, 2015; TEWARI et al., 2015; OWUSU-KWARTENG et al., 2017; WALKER YORK MOORE et al., 2017; YIBAR et al., 2017; FOGELE et al., 2018; OSMAN et al., 2018; ADAME-GÓMEZ et al., 2020; SÁNCHEZ-CHICA et al., 2020a; SÁNCHEZ-CHICA et al., 2020b; YU et al., 2020), enterotoxin genes were more frequently found than the emetic gene, indicating that diarrhoeal strains have a widespread distribution, consistent with this study. However, we did not identify any emetic strain since all the isolates lacked the *ces* gene, like the findings of HARIRAM & LABBE (2015), FOGELE et al. (2018), ADAME-GÓMEZ et al. (2020), SÁNCHEZ-CHICA et al. (2020a), and SÁNCHEZ-CHICA et al. (2020b).

On the contrary, in the studies of BERTHOLD-PLUTA et al. (2019), GAO et al. (2018), OWUSU-KWARTENG et al. (2017), and YU et al. (2020), the *ces* gene was detected but it was the least frequently observed toxin gene, reported in only 9%, 7%, 5%, and 1.2% of *B. cereus* strains, respectively. Also, WIJNANDS et al. (2006) found the gene responsible for cereulide-like toxin production in 8.2% of their isolates, not alone but in combination with the presence of *cytK*, *nhe*, and *hbl* genes. As mentioned previously, food matrices affect the growth and toxin production of *B. cereus* and emetic syndrome usually occurs from starchy food products (MCKILLIP, 2000; AGATA et al., 2002; WALKER YORK MOORE et al., 2017). Interestingly, the *B. cereus* strains

carrying the emetic toxin gene or having the ability to produce cereulide-like toxin have been isolated from raw milk and milk products (OWUSU-KWARTENG et al., 2017), pasteurised milk (GAO et al., 2018), cooked meat (YU et al., 2020), and fish and fish products (WIJNANDS et al., 2006). However, according to AGATA et al. (2002), emetic toxin production was extremely low in such foods compared to bacterial growth.

#### *Analysis of genetic relationship by PFGE*

Using PFGE, three major patterns (A, B, C) were obtained with the macrorestriction enzyme *SmaI* among the 84 *B. cereus* strains investigated. PFGE patterns A and B were both divided into two sub-groups: A<sub>1</sub> and A<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub>, whereas PFGE pattern C generated only one group. PFGE patterns B, A, and C consisted of eight (9.5%), 14 (16.6%), and 62 (73.8%) *B. cereus* strains, respectively. To compare clonality, a high genetic relationship between the isolates was detected at the 92% similarity level (Figure 2).

Molecular-based techniques such as PFGE, multi-locus sequence typing (MLST), and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) are widely used for bacterial typing, revealing the genetic relationship or genetic diversity. When we analysed our isolates by PFGE, we observed a high genetic relationship between them, and three major PFGE pulsotype profiles were obtained. Unlike our study, LIU et al. (2016), GAO et al. (2018), PARK et al. (2009), and SÁNCHEZ-CHICA et al. (2020a) reported high numbers of pulsotypes using PFGE and ERIC-PCR typing, indicating that there was high genetic diversity among their isolates. GDOURABEN AMOR et al. (2018) found that 33.3% of their isolates were divided into 27 different clusters while the remainder were clustered individually. In another study, large genetic diversity was observed in Colombia among 46 *B. cereus* isolates recovered from powdered food samples that were grouped in seven clusters (SÁNCHEZ-CHICA et al., 2020b). In China, when YU et al. (2020) detected the genetic diversity of *B. cereus* isolates by MLST, 192 different sequence types were obtained, and 93 of them were new. In this respect, we can say that molecular typing methods can help us better understand the molecular epidemiology of *B. cereus* strains. Conversely, PFGE has been accepted as the gold standard for the discrimination and molecular characterisation of bacterial strains. In addition, using more than one restriction enzyme in PFGE analysis could increase the discriminatory power of the method, but we used only one restriction enzyme (*SmaI*) in the present study.

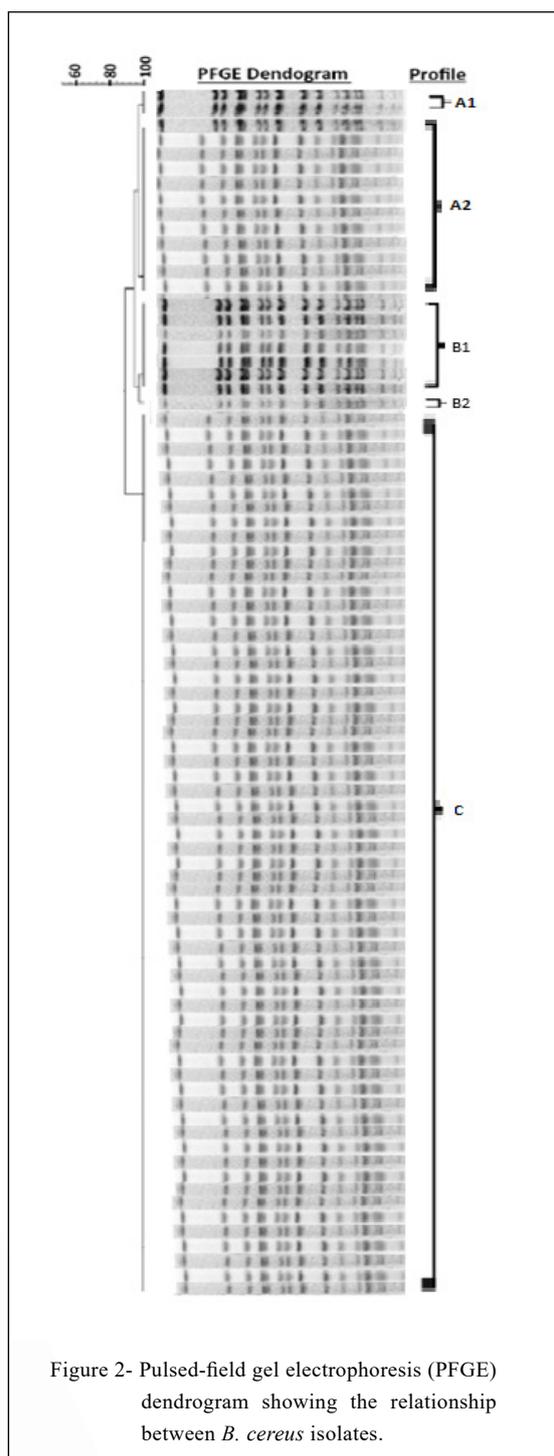


Figure 2- Pulsed-field gel electrophoresis (PFGE) dendrogram showing the relationship between *B. cereus* isolates.

## CONCLUSION

Our results indicated that the tested foods were frequently contaminated with *B. cereus* and that 31% of the *B. cereus* strains were both psychrotrophic

and enterotoxigenic, which may increase the potential risk for both food spoilage and food poisoning. In addition, most of the isolated strains show similar PFGE patterns indicating that there is a high genetic relationship between them. As a high genetic relationship was detected between our isolates from different samples, cross-contamination of samples with these bacteria may be considered. This study was conducted in a specific region where local people consume a great many of these products. The study reveals the potential sources of *B. cereus* and provides a scientific basis for further investigation. Of course, more studies should be carried out to better understand the virulence mechanism and molecular epidemiology of *B. cereus* strains.

## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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