Occurrence, growth, and virulence genes of *Bacillus cereus* in ready-to-cook plant-based meat analogues

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Abstract

The aim of this study was to monitor the growth of *Bacillus cereus* in 11 different types of naturally contaminated ready-to-cook plant-based meat analogues (PBMAs). The PBMAs were prepared according to the manufacturer's instructions and subsequently stored at room temperature for up to 24 h. *Bacillus* spp. represented the second most numerous group of microbiota detected in PBMAs after lactic acid bacteria. *Bacillus cereus sensu lato* (*s.l.*) was isolated from all 11 PBMA samples. Following heat treatment and 24-h storage at 22 °C, *B. cereus s.l.* was detected by plate counting in five samples, with one product reaching nearly 4 log CFU/g. The presence of *B. cereus sensu stricto* (*s.s.*) was detected in all 11 samples. *Bacillus thuringiensis* was present in 7 samples, and in 4 PBMA samples the species *B. weihenstephanensis*, *B. mycoides*, and *B. cytotoxicus* were found. A total of 95.7% of *B. cereus s.l.* strains contained genes for toxin production. The presence of *nhe* genes was demonstrated in 92.5%, *hbl* genes in 59.0% of the analysed *B. cereus s.l.* strains, the *cyt*K-1 gene in 5 strains (*B. cytotoxicus*). The presence of the *ces* gene was confirmed in 5% of *B. cereus s.l.* strains. In total, 47 different combinations of genes for toxin production were detected in *B. cereus s.l.* strains. The almost 100% presence of virulence genes associated with the possibility of *B. cereus* growth during inappropriate storage of PBMAs thus poses a risk for the development of foodborne diseases.

Total viable count, heat treatment, hbl genes, nhe genes, ces gene, Bacillus cytotoxicus

In 2023, *Bacillus cereus* caused 474 foodborne outbreaks in EU member states, making *B. cereus* the second most common known bacterial agent after *Salmonella* (EFSA 2024). In the group of bacterial toxins, *B. cereus* ranked first, same as in 2022 (EFSA 2023). The number of outbreaks caused by *B. cereus* toxins increased by 55% year-on-year. Globally, the officially reported share of *B. cereus* in foodborne outbreaks ranges between 1.4 and 12%, but this is considered to be a major underestimate (Grutsch et al. 2018).

Bacillus cereus is widespread in the environment, including dust, soil, plant surfaces, the rhizosphere and in the intestinal tract of insects and mammals, as well as in ingredients and final food products (Jovanovic et al. 2022). Mild outbreaks or individual cases have been associated with many types of food, including cereals/grain products, herbs, spices, dairy products, confectionery, canned goods, seafood, vegetables, egg products, beverages and dried food products (Jovanovic et al. 2022; Zhao et al. 2025). Li et al. (2025) analysed 266 studies selected from PubMed and Chinese National Knowledge Infrastructure databases. Rice was identified as the most frequently contaminated food, and other significant categories included multi-ingredient foods, desserts, meat, and dairy products (Li et al. 2025).

Bacillus cereus may be introduced into the food service environment via raw materials, with the proportion of positive swab samples from work surfaces or staff hands reported to

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range tens of percent (Bogdanovičová et al. 2019; Dorotíková et al. 2022). Dušková et al. (2024) isolated *B. cereus* following enrichment from 81% of ready-to-cook plant-based meat analogues (PBMAs).

Bacillus cereus is capable of growth within a temperature range of 6 °C to 48 °C (Webb et al. 2019), whereas *Bacillus cytotoxicus* exhibits thermotolerance, with growth observed at temperatures up to 55 °C (Rau et al. 2025). Storing food at temperatures outside the growth limit of *B. cereus* is considered the most effective preventive measure against disease caused by this pathogen. These diseases can occur in the form of an emetic syndrome caused by the cereulide toxin, or as a diarrhoeal syndrome associated with three enterotoxins, namely non-haemolytic enterotoxin (Nhe), haemolysin BL (Hbl) and cytotoxin K (CytK-1, CytK-2) (Fiedler et al. 2019; Yu et al. 2020). Cereulide production typically begins during the late exponential growth phase, with maximum accumulation levels gradually reached in the stationary phase (Rouzeau-Szynalski et al. 2020). A concentration of *Bacillus cereus* in the range of 5 to 8 log CFU/g of food is considered sufficient to produce cereulide levels capable of causing food poisoning (Tirloni et al. 2022). For diarrhoeal syndrome, the infectious dose is 5–7 log of total ingested *B. cereus* cells. In contrast to cereulide, *B. cereus* enterotoxins produced in food are unlikely to contribute to the onset of disease, as they are sensitive to heat, acids, and proteases (Dietrich et al. 2021). Ingestion of *B. cereus* spores or viable cells therefore leads to colonization of the small intestine, where toxin(s) are produced between the exponential and stationary phases, together with other virulence factors (Griffiths and Schraft 2017).

Due to its ability to form spores, *Bacillus cereus* can survive pasteurization temperatures commonly used in food processing and meal preparation. In mashed potatoes, the generation time of *B. cereus* is less than 1 h under favourable temperature conditions (Turner et al. 2006) and at 40 °C, the cell count can reach 4 log CFU/g after 4 h (Kameník et al. 2025). In cooked rice, Juneja et al. (2019) observed growth at temperatures between 13 and 46 °C. While the growth of *B. cereus* has been demonstrated in various types of food, studies investigating its growth in PBMAs are currently lacking in the literature.

The aim of this study was to analyse the growth of *B. cereus* in naturally contaminated ready-to-cook PBMA samples prepared according to the manufacturer's instructions and subsequently stored at room temperature for up to 24 h. The *B. cereus* isolates obtained from PBMAs were also analysed for genes encoding toxin production.

Materials and Methods

PBMA samples

In the months of April–May and August–October 2023, PBMAs of the 'ready-to-cook' category were purchased in retail stores. The selection of purchased PBMAs was focused on products in which *B. cereus* was detected in previous analyses focused on microbial contamination (Dušková et al. 2024). A total of 11 products were purchased in each of the mentioned periods (i.e. a total of 22 samples). The products were divided into 4 categories according to Dušková et al. (2024). Five products were purchased in the 'burgers' category, 2 products in the 'steaks' category, 1 product in the 'balls' category and 3 products in the 'others' category. Immediately after purchase, the samples were placed in a thermobox and transported to the laboratory within 60 min of purchase. Until the moment of processing, the PBMAs were stored at a temperature of 4.0 ± 1.0 °C.

Preparation of PBMA samples

The samples were subjected to the cooking treatment recommended by the manufacturer and indicated on the product packaging (Table 1). When PBMAs were prepared in a pan, the internal temperature after heat treatment ranged between 73–82 °C. When a convection oven was used as recommended by the manufacturer, the internal temperature reached 58–68 °C. A Testo 104-IR thermometer (Testo, Prague, Czech Republic) was used for testing. After heat treatment, part of the samples was kept for 24 h at a temperature of 22.0 ± 2.0 °C on sterile plates without foil covering, while the other part of the samples on sterile plates was covered with high-density polyethylene microthin foil (HDPE foil) to prevent excessive drying. Immediately afterwards, the microbiological analysis of the samples was performed again. This combination of time and temperature was chosen to simulate inappropriate consumer behaviour in the home, where for various reasons, a portion of prepared food is kept until the next day without being stored in the refrigerator.

Table 1. Instructions for preparing PBMAs according to the information on the product packaging.

Type of PBMA	Preparation instructions
Plant Chef burger patties	Heat a small amount of oil in a heavy-bottomed pan over medium heat for 2–3 minutes. Fry the product for 5–6 min, turning occasionally.
Burger with broccoli	Heat a little oil in a frying pan, fry the burgers lightly coated with oil over medium heat for 2–4 min on each side.
Vegetarian southern-style chicken fillet burgers	Preheat fan oven to 180 °C. Place vegetarian burger onto a preheated baking tray and cook for 28–30 min, turning halfway through cooking.
Chickpea falafel, frozen	Fry in a pan with oil for 10 min, turning during frying.
Vegan burger	Heat a little oil in a frying pan and fry over medium heat for 3 min on each side until golden brown.
Vegan mince	Heat a little oil in a frying pan and fry over medium heat for 7 min until golden brown. Stir carefully while heating.
Vegetable pea noodles	Heat a little oil in a pan, fry over medium heat for 3 min.
Vegetarian southern-style chicken, frozen	Pour 1 tablespoon of oil into a heated pan. Add the product and fry for 7–8 min over low heat, stirring constantly.
Vegan schnitzel	Fry for 7–9 min on medium heat in a small amount of heated oil, turning halfway through frying.
Vegetarian chicken roast	Preheat the fan oven to 170 °C, place the product in the baking dish in the middle of the oven. Bake for 45 min.
Vegetarische Klösschen	Fry for 4–7 min on medium heat with a little oil in a pan, turn after 2 min.

PBMA – plant-based meat analogue

Microbiological analysis of PBMA samples

The total viable mesophilic count (TVC), the number of *Enterobacteriaceae*, yeasts and moulds, and the number of lactic acid bacteria (LAB), *B. cereus sensu lato* (*s.l.*), mannitol-positive bacilli and *Clostridium* spp. were monitored in the samples. A total of 225 ml of buffered peptone water (Oxoid, Hampshire, UK) was added to 25 g of sample, and further decimal dilutions were prepared as necessary after homogenisation (Star-Blender™ LB 400 Stomacher; VWR, Leuven, Belgium). The samples were analysed in duplicates.

The TVC was determined in accordance with ISO 4833-1:2013. Samples were cultured at 30 °C for 72 h under aerobic conditions on plate count agar (Oxoid) with tryptone, glucose, and yeast extract (TGYE agar).

Agar with crystal violet, neutral red, bile salts and glucose (Oxoid) was used to monitor the number of bacteria of the family *Enterobacteriaceae* in accordance with ISO 21528-2:2017. Incubation took place at 37 °C for 24 h. Up to five colonies were selected at random from each plate for confirmation using oxidase test (JK Trading, Prague, Czech Republic).

Yeast and mould counts were determined on chloramphenicol agar with dichlorane and Bengal red (Oxoid) in accordance with ISO 21527-1:2008 and incubated at 25 °C for 5 days.

Determination of the number of LAB was performed on de Man, Rogosa and Sharpe agar (Oxoid) under anaerobic conditions during incubation at 30 °C for 72 h in accordance with ISO 15214:1998. All colonies showing different morphological characteristics were selected from each counted plate and post-propagation plate and tested for the presence of catalase and oxidase.

Mannitol yolk polymyxin B agar (MYP agar, Oxoid) was used for the determination of bacteria of the genus *Bacillus* in accordance with ISO 7932:2004 at 30 °C for 24 h. Qualitative determination of *Bacillus* spp. was performed with inoculation from the homogenate after 24 h of cultivation at 37 °C on MYP agar (30 °C for 24 h). Suspected *B. cereus* colonies were identified by PCR on the basis of detection of the *gyr*B gene encoding DNA gyrase subunit B (Yamada et al. 1999). Colonies of *B. cereus* and colonies showing a mannitol-positive reaction on MYP agar were both identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS).

Determination of *Clostridium* spp. was performed in accordance with ISO 7937:2004 on tryptone sulphite cycloserine agar (Oxoid) under anaerobic conditions at 37 °C for 48 h.

Identification of bacterial isolates by MALDI-TOF mass spectrometry

Individual colonies of suspected *B. cereus* isolates were treated by the ethanol-formic acid extraction protocol

(Freiwald and Sauer 2009). The samples were subjected to MALDI-TOF MS analysis (UltrafleXtreme instrument, Bruker Daltonics, Bremen, Germany; FlexControl software version 3.4; BioTyper software version 3.1; Bruker Daltonics; BioTyper database version 10.0, 9607 entries). Only identification outputs with a BioTyper log(score) above 2.0, which means highly reliable identification at the species level, were taken into consideration.

PCR species-specific identification

Polymerase chain reaction (PCR) was used for *B. cereus sensu stricto* (*B. cereus s.s.*) confirmation. Colonies grown on TGYE agar under cultivation conditions of 30 °C/24 h were used for the isolation of DNA by heat. Briefly, one bacterial colony was suspended in 100 µl of sterile saline and heated at 100 °C/10 min, followed by centrifugation at 2,082 × *g* for one min. The supernatant was then transferred to a new microcentrifuge tube and an aliquot of 2 µl was used as template DNA in the PCRs. The species identification of *B. cereus s.s.* was performed on the basis of detection of the *gyr*B gene encoding DNA gyrase subunit B with used primers BC1 [5′-ATTGGTGACACCGATCAAACA-3′] and BC2 [5′-TCATACGTATGGATGTTATTC-3′] (Yamada et al. 1999). The amplified products were separated by electrophoresis on a 2% agarose gel in 0.5× Tris-borate-EDTA buffer. The gels were stained with Midori Green (Nippon Genetics, Düren, Germany) and visualised using a UV transilluminator (VWR, Radnor, USA).

Virulence gene distributions

All *B. cereus s.l.* isolates (n = 246) were screened for the presence of toxin genes *nhe*ABC, *hbl*ACD, *cyt*K and *ces* encoding for non-haemolytic enterotoxin (Nhe), haemolysin BL (Hbl), cytotoxin K (CytK), and cereulide (Ces) production, respectively. Five sets of multiplex PCRs were employed to amplify the genes under study. The detection of genes encoding the production of binding component B (*hbl*A) and lytic component L2 (*hbl*C) of Hbl was based on the procedure of Rowan et al. (2003). In the second and third reaction, *nhe*A along with *nhe*C and *nhe*B with *hbl*D genes were targeted using specific primers (Ghelardi et al. 2002; Guinebretière et al. 2002; Rowan et al. 2003). The primers used for *ces* and *cyt*K-1 and *cyt*K-2 gene detection were described by Horwood et al. (2004) and Guinebretière et al. (2006).

Determination of pH values and water activity

The pH value was measured in an aqueous solution of the sample (1:10) using a combined 211 electrode and pH meter (Hanna Instruments, USA) at a temperature of 25 ± 1 °C. The water activity (a_w) was determined in a well-homogenised sample using a Lab-Master aw-meter (Novasina AG, Switzerland) at a temperature of 25.0 ± 0.1 °C.

Statistical analysis

All data were entered into spreadsheets (Microsoft Office Excel 2019). The obtained experimental data (CFU/g) were $\log_{10}$ transformed, and the mean values and standard deviations were calculated. The differences were compared using paired-samples *t*-test because Shapiro–Wilks tests were not able to reject the normality of the data. Statistical significance was accepted at $P < 0.05$.

Results

Changes in the microbiota of PBMAs after heat treatment and storage at room temperature

The TVC in PBMA samples before treatment ranged from 1.8 to 5.3 log CFU/g, the average value was 3.6 log CFU/g (Table 2). *Enterobacteriaceae* could not be detected in eight samples, in the remaining samples their count ranged between 1.0–2.0 log CFU/g. Lactic acid bacteria contributed significantly to the present microbiota, their average level ranged between < 1.7 to 5.2 log CFU/g. Yeasts represented a negligible proportion of the present microbiota, in most samples their number was below the detection limit. *Clostridium* spp. and moulds were not detected in the analysed samples (Table 2).

Heat treatment according to the manufacturer's instructions reduced the TVC level in the samples studied ($P < 0.001$), although not in all samples with the same intensity. A significant reduction was found after heat treatment for LAB; in 6 samples LAB could no longer be detected by plate counting. *Enterobacteriaceae* were also not detected in any of the analysed samples after heat treatment.

In the simulation of inappropriate storage (time storage/temperature abuse), an increase in TVC occurred within 24 h after heat treatment. The differences were significant ($P < 0.001$) compared to TVC in samples immediately after heat treatment. In some samples, the number of TVC after 22 °C/24 h exceeded the level of TVC before heat

Table 2. Results of microbiological analyses of selected PBMAs (n = 11) before and after treatment (in accordance with the manufacturer's recommendations) and after 24 h of storage. The results are given in log CFU/g and as means ± standard deviation.

Analysed microorganisms	Before treatment	After treatment	Storage 22 °C/24 h	Storage 22 °C/24 h under foil
Total viable count	3.6 ± 1.2	2.2 ± 0.9	3.2 ± 1.2	3.9 ± 1.7
Enterobacteriaceae	1.0 ± 0.6	< 1.0	< 1.0	<1.0
Lactic acid bacteria	3.0 ± 1.6	1.4 ± 0.2	1.6 ± 0.7	1.8 ± 1.2
Yeasts	1.8 ± 0.3	< 2.0	< 2.0	<2.0
Moulds	< 2.0	< 2.0	< 2.0	<2.0
Clostridium spp.	< 1.7	< 1.7	< 1.7	<1.7
Mannitol positive *Bacillus* spp.	2.1 ± 0.7	1.8 ± 0.6	2.5 ± 1.2	3.0 ± 1.5
Bacillus cereus s.l.	1.6 ± 0.4	1.4 ± 0.2	1.9 ± 0.8	2.0 ± 1.0
Bacillus cereus s.l. species	*B. cereus s. s.* *B. mycoides* *B. weihenstephanensis* *B. thuringiensis* *B. cytotoxicus*	*B. cereus s. s.* *B. mycoides* *B. weihenstephanensis* *B. thuringiensis*	*B. cereus s. s.* *B. mycoides* *B. weihenstephanensis* *B. thuringiensis* *B. cytotoxicus*	*B. cereus s. s.* *B. mycoides* *B. weihenstephanensis* *B. thuringiensis* *B. cytotoxicus*
Water activity	0.966 ± 0.005	NA	0.933 ± 0.047	0.959 ± 0.012

PBMA – plant-based meat analogue; NA – not analysed

treatment. When the samples were covered with HDPE foil after heat treatment and left at 22 °C until the next day, the number of TVC increased even more and the changes compared to uncovered samples were significant ($P = 0.02$). The reason was the higher water activity value in the samples wrapped in foils compared to the samples without foil covering ($P = 0.031$). After heat treatment, bacteria of the *Enterobacteriaceae* family could not be detected in any of the samples. In two samples out of a total of 11 analysed, LAB were detected even after heat treatment. Upon further storage at 22 °C, further LAB growth occurred.

Growth of *Bacillus cereus* in PBMAs

Bacillus spp. represented the second most numerous group of microbiota detected in PBMAs after LAB (Table 2). *Bacillus cereus* group bacteria (*B. cereus s.l.)* were isolated from all 11 PBMA samples, but only in 4 samples by plate counting. In two of the samples, the number of *B. cereus s.l.* was at the level of 2 log CFU/g. In seven samples, *B. cereus s.l.* was detected only after enrichment in liquid nutrient medium. The presence of *B. cereus s.s.* was detected in all 11 samples. *Bacillus thuringiensis* was present in seven samples, and in four PBMA samples the species *B. weihenstephanensis, B. mycoides*, and *B. cytotoxicus* were found.

When PBMA samples were subjected to culinary heat treatment according to the manufacturer's instructions (Table 1), immediately afterward, *B. cereus* was detected by plate counting in only one sample. After heat treatment, the possibility of detecting *B. cereus* from partial PBMA samples after enrichment also decreased. While before heat treatment, *B. cereus* was isolated from several PBMA species in 100% (i.e. from all four samples of one PBMA species), this was no longer the case after heat treatment.

After heat treatment and subsequent storage for 24 h at 22 °C, *B. cereus s.l.* was detected by plate counting in five samples, in two samples even from those PBMAs where plate counting was negative before heat treatment. In one PBMA product, the average number of *B. cereus* approached 4 log CFU/g. When the samples were stored with HDPE foil

overwrapping, there was no significant difference in the numbers of *B. cereus* compared to the samples without overwrapping ($P = 0.123$). In two PBMA samples with overwrapping, the numbers of *B. cereus* reached the level of 3 log CFU/g.

Virulence genes in *B. cereus s.l.* isolates

A total of 246 isolates of *B. cereus s.l.* were obtained from PBMA samples. By analysing virulence genes taking into account individual PBMA products and preparation phases (before heat treatment, immediately after heat treatment, after storage for 24 h/22 °C with or without HDPE foil), 140 strains of *B. cereus s.l.* were obtained from the PBMA samples. The *gyr*B gene was detected positively in 102 strains (*B. cereus s.s.*). MALDI-TOF MS identified 15 strains of *B. thuringiensis*, 10 strains of *B. mycoides*, eight strains of *B. weihenstephanensis* and five strains of *B. cytotoxicus*.

Only in six strains of *B. cereus s.l.* (4.3%) out of a total of 140 strains, no genes encoding toxin production were detected by PCR. The *nhe* genes were detected in 124 strains, and the *hbl* genes in 79 strains. The *cyt*K-2 gene was detected in 76 strains, the *cyt*K-1 gene in five (*B. cytotoxicus*). The *ces* gene was detected in seven strains of *B. cereus s.l.*, always in combination with genes for enterotoxin production. In total, 47 different combinations of genes for toxin production were detected in *B. cereus s.l.* strains. Figure 1 contains 24 variants of gene combinations that were detected in two or more strains. There were 23 variants that were always obtained in only one strain from PBMAs, i.e. 23 strains had a different gene profile than the 111 strains included in Fig. 1.

The *hbl*ACD gene cluster was found in 42.5% of the strains, with *hbl*A being detected in 45.5%, *hbl*C in 52.2% and *hbl*D in 58.2% of the strains. The *nhe*A gene was found in 74.6%, *nhe*B in 63.4% and *nhe*C in 50.7% of the strains. The frequently detected gene combinations were six (*cyt*K-2, *hbl*ACD, *nhe*AB) or seven virulence genes (*cyt*K-2, *hbl*ACD, *nhe*ABC).

Discussion

Bacillus cereus spreads easily from its natural environment to many types of food, especially of plant origin, due to the resistance of endospores to various stress factors and their ability to survive for a long time (Tewari et al. 2015). In a study by Rahnama et al. (2023), the highest prevalence of *B. cereus* was recorded in cereals and beans, with 41.5% and 44.9% positive samples, respectively, of the eight food groups examined. Fiedler et al. (2019) isolated 147 isolates of *B. cereus* from 137 samples of fresh vegetables including ready-to-eat mixed salads obtained from a retail chain in Germany. In China, ready-to-eat foods from retail stores were examined for the presence of *B. cereus* by Yu et al. (2020). *Bacillus cereus* was detected in 302 samples out of 860 analysed. Jovanovic et al. (2022) examined 250 samples of food products intended as ingredients of 'mixed food products' obtained from retail in Belgium, the Netherlands or Serbia. 90.4% of the samples were positive for *B. cereus*. Of the 51 ice cream samples in Italy, 65% of the samples were positive for *B. cereus s.l.* (Fraccalvieri et al. 2022). Dušková et al. (2024) demonstrated *B. cereus s.l.* by plate counting in only three samples out of a total of 43 analysed PBMAs. However, after enrichment, *B. cereus s.l.* was isolated from 35 samples. The results of this study are consistent with these findings.

In the spore form, *B. cereus* can remain dormant without nutrient uptake for an indefinite period of time. Once conditions become favourable, spores are able to transform back into vegetative cells within minutes (Cho and Chung 2020; Vidic et al. 2020). Some forms of food processing, such as exposure to sublethal temperatures during heat treatment (65–85 °C), can act as triggers for germination (Luu et al. 2015). Spore germination can also be induced by the presence of germinants such as amino acids (especially L-alanine), glucose, or lysozyme (Soni et al. 2016).

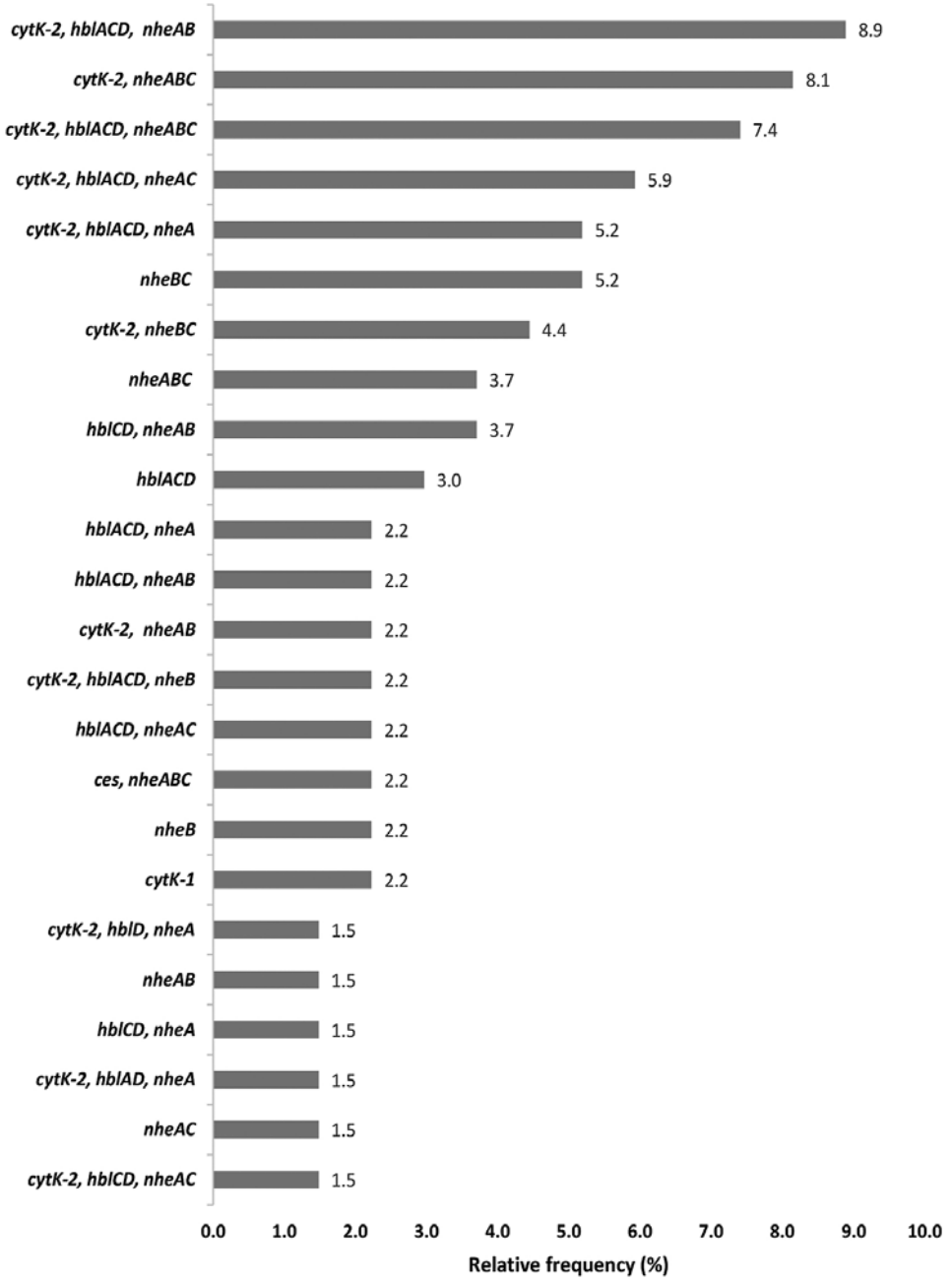

Fig. 1. Relative frequency (%) of gene combinations for the production of non-haemolytic enterotoxin (Nhe), haemolysin BL (Hbl), cytotoxin K (CytK) and cereulide (Ces) in *Bacillus cereus sensu lato* strains (n = 134) carrying at least one gene isolated from 11 samples ready-to-cook plant-based meat analogues. The graph shows combinations with at least two strains.

It is evident that the heat treatment used in this study, according to the instructions of the PBMA manufacturers, acted as a trigger for spore germination and subsequent growth of *B. cereus* at a temperature of 22 °C. This temperature was chosen as a simulation of possible (but inappropriate) storage conditions by consumers in the case of unconsumed heated PBMAs for later consumption. In 4 products, after 24 h of storage, the numbers of *B. cereus* increased to levels of 3 log CFU/g. According to Yu et al. (2020), more than 3 log *B. cereus* per gram of food is considered risky for consumption. For comparison, Bursová et al. (2024) reported that the critical value of 5 log CFU/g for *B. cereus* in cooked rice stored at 24 °C was reached in less than 10 h. The results of the presented study thus emphasize the need for proper handling of PBMA products in consumers' kitchens.

In general, sterilization temperatures (121 °C) are required to inactivate bacterial spores (Soni et al. 2016). Le Marc et al. (2022) found that *B. cereus s.l.* isolates were variably resistant to heat, with D_{90} values ranging from 4.5 to 164 min with a median of 16.8 min (tested in different bacterial culture media). It is evident that short-term heat treatment of ready-to-cook PBMAs was not capable of inactivating *B. cereus* spores, but rather served as a trigger for spore germination and subsequent bacterial cell growth.

Bacillus cereus toxins cause two forms of gastrointestinal disease: diarrhoeal and emetic (Ehling-Schulz et al. 2006; Jovanovic et al. 2022). The toxins haemolysin BL, nonhaemolytic enterotoxin, and cytotoxin K are associated with diarrhoeal foodborne illness (Fiedler et al. 2019). The Hbl and Nhe enterotoxins are tripartite toxins consisting of subunits HblC, HblD, and HblA for the Hbl toxin and subunits NheA, NheB, and NheC for the Nhe toxin. The emetic toxin is encoded by a gene cluster (*ces*ABCDPTH) located on a 270 kb megaplasmid pCER270 for nonribosomal peptide synthesis (Jacobs et al. 2025).

It is alarming that 95.7% of *B. cereus s.l.* strains isolated from PBMAs in this study contained genes for toxin production. Dorotíková et al. (2022) demonstrated the presence of genes encoding at least 1 toxin in 100% of *B. cereus s.l.* isolates obtained from the hands of food service personnel. Our results are consistent with the study by Kowalska et al. (2024), in which toxin production genes were detected in 95.8% of a total of 550 isolates obtained from ready-to-eat foods on the Polish market.

In this study, the presence of *nhe* genes was demonstrated in 92.5% of the obtained strains and these virulence genes were detected most frequently. This was a similar proportion found in the study by Kowalska et al. (2024) with 91.8%. Jacobs et al. (2025) reported a proportion of 98% of *nhe* genes detected in 169 isolates of *B. cereus s.l.*, obtained from 12 types of food and soil over two decades in Belgium. Fiedler et al. (2019) demonstrated the presence of *nhe* genes by multiplex PCR in 73.5% of *B. cereus s.l.* isolates from fresh vegetable samples from retail in Germany. Among *B. cereus s.l.* isolates from Italian ice cream samples, Fraccalvieri et al. (2022) found 100% presence of *nhe* genes. Moravek et al. (2006) analysed 100 isolates of *B. cereus* isolated from different sources (food, patient isolates) in different countries between 1972 and 2002. They demonstrated the presence of *nhe* genes in 99 strains by PCR.

The presence of *hbl* genes was confirmed in this study in 59.0% of the analysed *B. cereus s.l.* strains. Kowalska et al. (2024) reported a proportion of 43.8%, Fraccalvieri et al. (2022) 44% and Jacobs et al. (2025) 52%. Moravek et al. (2006) demonstrated *hbl* genes in 42% of *B. cereus* strains. On the contrary, Fiedler et al. (2019) found that 91.2% of *B. cereus* isolates from vegetable samples from the German retail had the presence of *hbl* genes. The presence of the *cyt*K-1 gene is clearly associated with the thermotolerant representative of *B. cereus s.l.,* which is *B. cytotoxicus* (Jacobs et al. 2025). The *cyt*K-1 gene was detected in five *Bacillus cytotoxicus* strains isolated from PBMA and confirmed by MALDI-TOF MS. The accuracy of *B. cytotoxicus* identification using MALDI-TOF MS

has recently been successfully improved and according to Rau et al. (2025) the method is suitable for routine use in an accredited laboratory setting.

The *ces* gene is rarely found in *B. cereus s.l.* isolates. Fidler et al. (2019) did not detect the presence of *ces* genes in any of the isolates from vegetable and herb samples from German retail. Kowalska et al. (2024) described the occurrence in 4.2% of isolates from ready-to-eat foods in Poland, Yu et al. (2020) isolated *B. cereus* strains from ready-to-eat foods in China with the presence of the *ces* gene in 7%. In Italian ice cream samples, the *ces* gene was found in 8% of strains (Fraccalvieri et al. 2022). From PBMAs, the presence of the *ces* gene was found in 5% of *B. cereus s.l.* strains. In three strains, the *ces* gene was demonstrated in combination with the *nhe*ABC genes, and in one strain in combination with *nhe*BC; or *nhe*AC; or *hbl*ACD, *nhe*AB; or *nhe*ABC, *cyt*K-2. The combination of *ces* and *nhe* genes is not uncommon, Thery et al. (2022) described a case of severe multi-organ failure in an 11-year-old girl after eating improperly stored lasagna (72 h after cooking). Cereulid and NHE toxins were detected in her stool. *Bacillus cereus* has been isolated from stool samples and vomit. The *cyt*K-2 gene is widely distributed in *B. cereus s.l.* isolates, but its contribution to the development of the disease remains a subject of debate (Jacobs et al. 2025). In PBMAs, *cyt*K-2 genes were detected in 75 strains (53.2%).

In conclusion, in ready-to-cook PBMA samples from retail, *B. cereus s.l.* with the potential to produce toxins can be detected. Culinary heat treatment according to the manufacturer's instructions does not lead to devitalization of the *B. cereus* spores. On the contrary, it acts as a trigger for the germination of bacterial spores. Inappropriate storage at room temperatures can allow the growth of *B. cereus* to levels exceeding 3 log CFU/g within 24 h. Of the 140 isolated *B. cereus s.l.* strains, only six strains did not show any genes for toxin production. The common combination was the presence of six (*cyt*K-2, *hbl*ACD, *nhe*AB) or seven virulence genes (*cyt*K-2, *hbl*ACD, *nhe*ABC) in PBMAs.

Thus, strains with the potential to cause foodborne disease also occur in PBMAs. Given the low level of contamination at a maximum of 2 log CFU/g in products from the retail, the risk of disease is practically zero if good hygiene practices are followed in households. On the other hand, the almost 100% presence of virulence genes associated with the possibility of *B. cereus* growth during inappropriate storage of PBMA thus poses a risk for the development of foodborne diseases.

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