JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EVALUATION OF *BACILLUS CEREUS* GROWTH IN COOKED RICE

Šárka Bursová[1], Danka Haruštiaková[2,3], Lenka Necidová[1], Eliška Krobotová[1], Zuzana Mlejnková[1], Matej Ťkáč[1], Kateřina Stojanová[1], Jozef Golian[4]*

Address(es): Bc. Ing. Kateřina Stojanová,
[1] University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Palackého tř. 1946/1, 612 42 Brno, Czech Republic.
[2] Masaryk University, Faculty of Science, RECETOX, Kamenice 753/5, 625 00 Brno, Czech Republic.
[3] Masaryk University, Faculty of Medicine, Institute of Biostatistics and Analyses, Kamenice 126/3, 625 00 Brno, Czech Republic.
[4] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Food Hygiene and Safety, Akademická 1755, 949 01 Nitra, Slovak Republic.

*Corresponding author: stojanovak@vfu.cz

https://doi.org/10.55251/jmbfs.10985

ARTICLE INFO

Received 2. 2. 2024
Revised 23. 5. 2024
Accepted 1. 7. 2024
Published 1. 8. 2024

Regular article

OPEN ACCESS

ABSTRACT

The present study aimed to evaluate the dependence of growth dynamics of toxigenic *B. cereus* on rice type, inoculum type, storage time, and temperature using appropriate mathematical models. Samples of cooked white (milled) and brown (husked) rice inoculated with vegetative cell/spore suspension of *B. cereus* were stored for 48 h at 4 °C (correct storage) and 24 °C (inappropriate storage method). No growth of *B. cereus* was observed during 48 hours of storage at 4 °C and was characterised by a linear model. To assess the growth of *B. cereus* in the cooked rice stored at 24 °C, the Baranyi-Roberts, Gompertz and Buchanan mathematical models were used. The Gompertz model characterized the growth of *B. cereus* best. The type of rice and the type of inoculum did not have a significant effect on the bacterial growth. After 48 h of storage at 24°C, the *B. cereus* counts were in the range of 8-9 log cfu.g^{-1}, critical value of 5 log cfu.g^{-1} was reached in less than 10 hours of storage. The study results indicate that cooked rice stored at 24 °C quickly becomes a health risk for consumers due to the rapid multiplication of *B. cereus* within a few hours. In conclusion, it is crucial to cool cooked rice immediately to 4 °C if it is not to be consumed right after cooking.

Keywords: predictive microbiology, Gompertz, Baranyi-Roberts, Buchanan model, storage temperature

INTRODUCTION

Bacillus cereus, a subdivision of the genus *Bacillus*, is a group of bacteria also known as *Bacillus cereus sensu lato* (*s.l.*). This group includes a variety of sporulating, closely related Gram-positive bacteria. The most important are: *B. cereus sensu stricto (s.s.), B. anthracis, B. mycoides, B. pseudomycoides B. cytotoxicus, B. thuringiensis, B. toyonensis* and *B. weihenstephanensis* **(Messelhäusser & Ehling-Schulz, 2018)**. Members of the genus *Bacillus cereus* are ubiquitous throughout the world. They are found in soil, water, plants and in the digestive tracts of mammals and insects **(Marrollo, 2016)**. They are able to grow in a wide range of temperatures and conditions (aerobic and anaerobic). They form highly resistant endospores which are very difficult to inactivate and remove from their environment. Because of these properties, members of the genus *Bacillus cereus* are well-known as important foodborne pathogens **(Messelhäusser & Ehling-Schulz, 2018; Glasset *et al.*, 2021)**.

Bacillus cereus spreads easily from the environment to food and causes 2 types of foodborne illnesses, namely diarrhoeal and emetic. The diarrhoeal form is caused by vegetative cells which produce enterotoxins of a protein nature in the small intestine. Ingestion of viable spores and their germination in the small intestine is a prerequisite of this condition **(Berthold-Pluta *et al.*, 2015)**. The diarrhoeal disease is caused by enterotoxins, which are produced by vegetative *Bacillus cereus* cells in the human digestive tract. *B. cereus* expresses 3 main types of these cytotoxins, namely: cytotoxin K, haemolysin BL (Hbl) and non-haemolytic enterotoxin (Nhe) **(Marrollo, 2016)**. The infective dose in this case is considered to be about 10^6 viable spores per 1 g of food. In the case of diarrhoeal disease, the incubation period is 8-16 hours. The length of time between the consumption of contaminated food and the onset of symptoms is influenced by the length of passage of the contaminated food through the beginning of the digestive tract into the intestine, the time required for *B. cereus* to accumulate in the intestine to a dangerous level and the time required for spore germination. Symptoms of diarrhoeal disease include mainly cramps, abdominal pain and watery diarrhoea. Nausea may also appear, and vomiting appears quite sporadically. The disease usually persists for 12-24 hours, rarely for several days, and is similar in symptoms to that caused by *Clostridium perfringens* **(Pexara & Govaris, 2010)**. The emetic form of the disease caused by *Bacillus cereus* is more acute and severe. The emetic form is caused by ingestion of food that is already contaminated with the emetic toxin (cereulide) - a cyclic, heat-stable peptide **(Rouzeau-Szynalski *et al.*, 2020; Meng *et al.*, 2022)**. It takes about 10^5-10^8 *Bacillus cereus* cells per 1 g of food to produce a toxic dose of cereulide. Symptoms of intoxication, which include abdominal cramps, nausea and vomiting, appear about 1 to 6 hours after consumption of the contaminated food and usually disappear within 24 hours. The length of the incubation period and the symptoms are similar to the case of food poisoning caused by *Staphylococcus aureus* **(Tewari & Abdullah, 2015)**.

Bacillus cereus is considered one of the main pathogens in mass catering **(Schmid *et al.*, 2016; Osimani *et al.*, 2018)**. It survives at refrigerator temperatures while being resistant to heat as well, produces toxins and cannot be eliminated by conventional sanitation or pasteurisation procedures. In fact, it can easily contaminate a wide range of foods as it is capable of proteolysis, lipolysis or saccharolysis. It can, therefore, threaten, for example, the microbiological quality of eggs or meat dishes; still, rice and milk are among the most commonly contaminated foods **(Tewari & Abdullah, 2015)**. The potential relationship between toxin production and biofilm formation in *B. cereus* was evaluated **Huang *et al.* (2020, 2021)**. A review of causative agents of the "fried rice syndrome" emphasizes the prevalence of *B. cereus* in starchy food contamination and the possibility of contamination in protein-rich food **(Leong *et al.*, 2023)**. Rice and pasta meals are the most important sources of *B. cereus* spores causing intoxication **(Rodrigo *et al.*, 2021)**. Rice is consumed by almost half of the human population and is classified as a staple food. Rice can be classified into several types – raw, brown and white according to the degree of processing. Raw rice is unhusked and contains husks, hulls and bran. Brown rice (husked) is produced when the husk is removed. White rice (milled) is dehulled and dehusked **(Choi *et al.*, 2014)**. Rice can be contaminated with micro-organisms during the growing, harvesting, milling, storage or other processing operations. The heat-resistant spores of *Bacillus spp*. then germinate, reproduce and contaminate the rice, which can lead to foodborne illness **(Kim *et al.*, 2014)**. As a prevention of diseases caused by *Bacillus cereus*, it is recommended to cool cooked food as quickly as possible and to avoid so-called danger zone temperatures, i.e. 5-60 °C **(Juneja *et al.*, 2018)**. According to the requirements of current legislation, the finished food must be kept at a temperature of at least 60 °C after cooking; foods that are not consumed shortly must be cooled quickly to a temperature not exceeding 4 °C **(Act No. 121/2023)**. To ensure food safety, it is important to prevent the growth of undesirable micro-organisms or to destroy them. Methods of predictive microbiology can help this prevention **(Stavropoulou & Bezirtzoglou, 2019)** through modelling of the behaviour of micro-organisms depending on various factors such as temperature,

humidity, pH, water activity or different storage conditions. For example, a stochastic model that predicts the maximum specific growth rate (μmax) of *Bacillus cereus sensu lato* as a function of temperature was developed by **Le Marc *et al.* (2021)**. The Baranyi model was used for describing the growth rate of mesophilic *Bacillus cereus* in reconstituted infant formula by **Buss da Silva *et al.* (2017)** and **Bursová *et al.* (2018)**. The thermal inactivation of *B. cereus* spores was modelled by **Le Marc *et al.* (2022)**. Using mathematical modelling, it is also possible to predict the growth of micro-organisms in food and to evaluate the whole process of food production. Finally, it can be decided whether or not a given food will be safe from a microbiological point of view under the paticular conditions **(Stavropoulou & Bezirtzoglou, 2019)**.

This study aimed to evaluate the effect of storage temperature (4 °C, 24 °C), inoculum type (vegetative cells, spores) and product composition (brown rice, white rice) on growth of toxigenic *Bacillus cereus* strains in cooked rice. The growth dynamics were evaluated using three primary models, namely, the Baranyi-Roberts, the Gompertz and the three-phase Buchanan models, in order to determine which model is the most appropriate to describe the growth of *B. cereus* in this commodity.

MATERIAL AND METHODS

Bacterial strains and their toxigenic profile

To evaluate the growth dynamics of *Bacillus cereus* in cooked rice, toxigenic strains of *B. cereus* available from collections of microorganisms were used. Tables 1 and 2 give an overview of the strains used, their selected characteristics and their origin.

Table 1 Used *Bacillus cereus* strains and their origin

Strain	Origin (sample)	Collection
Bacillus cereus 055	milk powder	VETUNI
Bacillus cereus 120	fresh goat's cheese	VETUNI
Bacillus cereus 149	fresh sheep's cheese	VETUNI
Bacillus cereus 207	bun dumpling	VETUNI
Bacillus cereus 255	bun dumpling	VETUNI
Bacillus cereus 284	bun dumpling	VETUNI
Bacillus cereus CCM 869	control strain	CCM
Bacillus cereus CCM 2010	control strain	CCM
Bacillus cereus DSM 4312	food poisoning incident	DSZM

Legend: CCM – Czech Collection of Microorganisms (Brno, Czech Republic); DSM – German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany); VETUNI – Collection of the Department of Animal Origin Food & Gastronomic Sciences, University of Veterinary Sciences Brno (Brno, Czech Republic)

Table 2 Toxigenic profile of *Bacillus cereus* strains

Strain	Haemolysin BL (Hbl)		Non-haemolytic enterotoxin (Nhe)		Emetic toxin (cereulide)
	RPLA	Duopath®	ELISA	Duopath®	PCR
B. cereus 055	-	-	+	+	-
B. cereus 120	+	+	+	+	-
B. cereus 149	-	-	+	+	+
B. cereus 207	-	-	+	+	-
B. cereus 255	-	-	+	+	+
B. cereus 284	+	+	+	+	-
B. cereus CCM 869	-	-	+	+	-
B. cereus CCM 2010	+	+	+	+	-
B. cereus DSM 4312	-	-	+	+	+

Legend: Duopath® – GLISA Duopath® Cereus Enterotoxins (Merck); ELISA – Bacillus Diarrhoeal Enterotoxin Visual Immunoassay (BDE VIA™, 3M Tecra); PCR – Polymerase Chain Reaction; RPLA – *Bacillus cereus* Enterotoxin Reversed Passive Latex Agglutination (BCET-RPLA, Oxoid). The Duopath®, ELISA and RPLA tests were performed according to the manufacturer's instructions.

The polymerase chain reaction (PCR) was used for the detection of the gyrase B gene (*B. cereus* species confirmation), a gene encoding the non-ribosomal peptide synthetase (which plays a role in the production of emetic toxin) and the highly conserved regions of the bacterial 16S rRNA (internal control). For DNA isolation, colonies grown on blood agar plates (Bio-Rad, Marnes-la-Coquette, France) were used (after incubation at 30 °C for 24 h). The DNA isolation was carried out by heating the bacterial cells in 200 μl of 20% Chelex 100® Resin (Bio-Rad Laboratories, Hercules, CA, USA) at 95.5 °C for 10 min with consecutive centrifugations at 10,660 × g for 3 min. The supernatant was used as a template. The sequences of the used primers are given in Table 3, the composition of the reaction mixture was adapted to the PPP Master Mix use (Top-Bio, Ltd., Prague, Czech Republic). A total reaction volume of 25 μl was made up of 23 μl of the master mix and 2 μl of the template DNA. Amplification was performed on a PTC-200 thermocycler (MJ Research Watertown, Massachusetts, USA). The PCR amplification involved: the initial denaturation step of 10 min at 95 °C, followed by 30 cycles, each including 1 min of denaturation at 94 °C, 1 min of annealing at 54 °C, 1 min of elongation at 72 °C, and the completion with a final elongation at 72 °C for 5 min. The amplified products were separated by electrophoresis on a 2% agarose gel in 0.5× TBE buffer, followed by consecutive staining with ethidium bromide and visualization on a UV transilluminator.

Table 3 Primers used

Target gene	Primer	Primer sequence (5´ – 3´)	Amplicon size	Reference
gyrB	BC1	ATT GGT GAC ACC GAT CAA ACA	365 bp	**Yamada *et al.* (1999)**
	BC2	TCA TAC GTA TGG ATG TTA TTC		
NRPS*	CER1	ATC ATA AAG GTG CGA ACA AGA	188 bp	**Horwood *et al.* (2004)**
	CER2	AAG ATC AAC CGA ATG CAA CTG		
16S rRNA	InKo1	GGA AGG TGG GGA TGA CG	241 bp	**Martineau *et al.* (1996)**
	InKo2	ATG GTG TGA CGG GCG GTG TG		

* NRPS – non-ribosomal peptide synthetase

Preparation of stock *Bacillus cereus* spore suspensions

A spore suspension was prepared separately for each *B. cereus* strain used. Nutrient agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India; pH 7.4) enriched with 0.5% glucose and 0.003% manganese sulfate was used as the sporulation medium. Prior to inoculation onto sporulation medium, vegetative *Bacillus cereus* cells were activated by growth in a tube on Tryptone soy agar (HiMedia; pH 7) for 18-20 h at 30°C. The bacterial culture thus grown was injected in a Roux bottle onto the surface of the sporulation medium after resuspension in 2 ml of sterile saline. Incubation was carried out at 30 °C for 6 days, aerobically.

Using sterile glass beads and 20 ml of sterile saline solution, the culture mass was aseptically removed from the surface of the sporulation medium. The total suspension volume was aseptically transferred to a sterile test tube and centrifuged at 1700 × g for 10 min. The supernatant was discarded, and the pellet was resuspended in a sterile saline solution and centrifuged again. This procedure was repeated twice. After washing, the pellet was resuspended in 50 ml of sterile saline solution (approx. 8 log spores ml^{-1}), treated by ultrasound (15 min at 38 kHz), and stored at 4 °C. Spore formation was examined microscopically (by hot staining with 5% malachite green and carbolfuchsin staining).

Rice samples, inoculation mix preparation, inoculation and storage

The test media included two types of rice, namely long-grain milled ("white") and husked ("brown") rice (*Oryza sativa* L.; Podravka - Lagris, a.s., Dolní Lhota). Both types of rice were purchased in the retail market and then stored at laboratory temperature. Information on the composition of both rice samples (see Table 4) was obtained from the packaging. Prior to their use in model studies, the number of *Bacillus cereus* was determined in both rice samples according to EN ISO 7932 (2005). Neither of the rice samples contained *Bacillus cereus* (determined at < 1 log $cfu.g^{-1}$, i.e. below the detection limit of the plate method used).

Table 4 Composition of rice used

Type of rice	Selected nutrients
long-grain white rice	fat 1.0 g/100 g, carbohydrate 77.0 g/100 g, fibre 1.1 g/100 g, protein 7.5 g/100 g
long-grain brown rice	fat 2.6 g/100 g, carbohydrate 70.0 g/100 g, fibre 7.2 g/100 g, protein 7.7 g/100 g

Rice samples were always prepared according to the manufacturer's instructions immediately before the experiment. 250 g of white rice was mixed with 750 ml of drinking water and 5 g of NaCl and kept at a gentle boil for 15 min. 250 g of brown rice was mixed with 750 ml of drinking water and 5 g of NaCl, and kept at a gentle boil for 30 min. Cooked rice was cooled immediately after the boiling was completed by rinsing with sterile distilled water at 5-6 °C.

Rice samples were inoculated with spore suspension or vegetative cell suspension. For spore suspension inoculation, the stock spore suspension of each of the test strains was first heated to 85 °C for 10 min and subsequently, after cooling, a mixed suspension containing the same proportion of each of the six stock suspensions was prepared. The resulting suspension mixtures were diluted as required after thorough mixing.

For inoculation with a suspensions of vegetative cells, individual strains of *B. cereus* were aerobically cultured for 24 h at 30 °C. Subsequently, a suspension in sterile saline solution with a density of about 8 log cfu.ml^{-1} was prepared for each strain and the individual suspensions were mixed in the same proportion.

Brown and white cooked rice was aseptically distributed into sterile homogenization bags of 10 g each. A total of 9 samples were prepared in 4 sets for both temperatures evaluated. One set of samples was without the addition of *B. cereus* and served as a control. The other sets of samples (3 parallels) were inoculated with a mixed suspension of *B. cereus* strain (cells or spores) to give a starting concentration of approx. 2-3 log cfu.g^{-1}. These cooked rice samples were then stored at 4 °C and 24 °C for 48 h. Temperatures were chosen to simulate both the correct procedure for storing rice (up to 4 °C) and the incorrect method of leaving rice at room temperature for long periods after cooking.

Quantitative detection of *B. cereus*

Individual samples were examined immediately after inoculation (0 h) and at the following time intervals: 3, 6, 8, 10, 12, 24, 31, and 48 h. For both types of rice (white and brown), storage temperature (4 °C and 24 °C) and inoculum type (spores/vegetative cells), the experiment was carried out in 3 parallels and 1× for control. The count was determined on selective Mannitol Yolk Polymyxine B agar (MYP agar, Oxoid Ltd.). The Petri dishes were incubated aerobically at 30 °C for 24 h.

Determination of the pH value and water activity

Along with the bacterial count, the pH value and water activity were measured at each time point. The water activity of the cooked rice was measured at 25 °C with a LabMaster aw-meter (Novasina AG, Lachen, Switzerland). The pH value was determined using a 211 microprocessor pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA). The pH was measured at 25 °C from the aqueous leachate (10 g of rice sample was mixed with 100 ml of deionized water for 15 min at laboratory temperature).

Statistical analysis

All the data (cfu.g^{-1}) were logarithmically transformed to the log 10 scale, and the mean values and standard deviations were calculated in IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY) or Statistica, version 13 (StatSoft, Tulsa, OK, USA). *B. cereus* dynamics was examined using a primary mathematical model for microbial growth (the change in bacterial number over time). Data were fitted to the Baranyi-Roberts model **(Baranyi & Roberts, 1994)**, the Gompertz model **(Gibson *et al.*, 1988; Zwietering *et al.*, 1990)**, and the three-phase Buchanan model **(Buchanan *et al.*, 1997)**. The following parameters were chosen to interpret each model: growth rate μ_{max}, lag phase duration λ and maximum number of microorganisms. The individual models were then compared with each other using the residual standard error (RSE) value and the squared correlation between the observed values and the values predicted by the model (R^2). The R software, version 3.4.3 and the nlsMicrobio library **(Baty & Delignette-Muller, 2013)** were used to model the relationships. The generated growth models were also used to calculate the time needed for *B. cereus* to achieve a critical concentration causing poisoning in humans.

RESULTS AND DISCUSSION

The growth dynamics of *Bacillus cereus* in cooked long-grain brown and white rice were evaluated using three primary models – Baranyi-Roberts, Gompertz and three-phase Buchanan models (R software, version 3.4.3 and nlsMicrobio library). The effect of storage temperature and inoculum type was also investigated. Mean *Bacillus cereus* counts are listed in Table 5. After 48 h of storage at 24 °C, the maximum *B. cereus* counts in cooked rice were in the order of 8-9 log cfu.g^{-1}, with minimal differences between the different types of rice and used inocula. In all control samples (cooked rice without added *B. cereus*) the number of *B. cereus* was < 1.70 log cfu.ml^{-1}, i.e. below the limit of detection of the plate method used (no increase in typical colonies occurred at 0,2 ml of primary sample dilution).

Table 5 Mean ± standard deviation of *Bacillus cereus* counts (log cfu.g^{-1}) in cooked long-grain white and brown rice stored for 48 hours at 4 °C and 24 °C (n = 3).

Time (hours)	Long-grain white rice				Long-grain brown rice			
	Inoculum – cells		Inoculum – spores		Inoculum – cells		Inoculum – spores	
	4 °C	24 °C	4 °C	24 °C	4 °C	24 °C	4 °C	24 °C
0	2.21 ± 0.08	2.05 ± 0.30	3.37 ± 0.03	3.34 ± 0.08	2.23 ± 0.05	2.12 ± 0.13	3.25 ± 0.02	3.28 ± 0.00
3	2.18 ± 0.07	2.38 ± 0.07	3.38 ± 0.07	3.74 ± 0.05	2.49 ± 0.29	2.39 ± 0.02	3.39 ± 0.08	3.78 ± 0.09
6	1.96 ± 0.07	3.31 ± 0.12	3.26 ± 0.10	5.09 ± 0.08	2.18 ± 0.11	3.40 ± 0.13	3.21 ± 0.09	5.11 ± 0.11
8	2.10 ± 0.09	4.36 ± 0.07	3.22 ± 0.07	6.08 ± 0.06	2.21 ± 0.19	4.39 ± 0.19	3.28 ± 0.05	6.31 ± 0.09
10	2.00 ± 0.12	5.32 ± 0.05	3.24 ± 0.09	6.97 ± 0.19	2.06 ± 0.22	5.28 ± 0.06	3.31 ± 0.03	7.14 ± 0.12
12	2.08 ± 0.07	6.15 ± 0.11	3.10 ± 0.11	7.31 ± 0.09	2.10 ± 0.21	5.86 ± 0.10	3.24 ± 0.01	7.70 ± 0.05
24	1.96 ± 0.07	8.19 ± 0.16	3.20 ± 0.05	8.39 ± 0.13	2.14 ± 0.23	7.98 ± 0.11	3.40 ± 0.03	8.63 ± 0.08
31	1.76 ± 0.10	8.54 ± 0.09	3.09 ± 0.05	8.69 ± 0.07	1.76 ± 0.10	8.45 ± 0.00	3.33 ± 0.06	8.92 ± 0.02
48	1.82 ± 0.10	8.83 ± 0.08	3.20 ± 0.10	9.01 ± 0.03	1.80 ± 0.17	8.63 ± 0.61	3.38 ± 0.07	9.04 ± 0.06

In a study by **Finlay *et al.* (2002)** assessing the growth of emetic strains *B. cereus* in cooked rice, with an initial count of approximately 4 log spores per gram of rice, mean maximum *B. cereus* counts of 7–8 log cfu.g^{-1} at 15 °C and 8 log cfu.g^{-1} at 20 °C as well as 30 °C, respectively, were found over 72 to 96 h, depending on storage temperature. The authors individually tested three mesophilic emetic strains of *B. cereus*; in our study, a mixture of six strains producing different types of toxins, not only the emetic toxin, was used to inoculate rice, which may explain the higher maximum counts and shorter time required to achieve them. However, **Finlay *et al.* (2002)** confirmed the high growth rate of *B. cereus* during the first 24 h at temperatures of 20 °C and 30 °C, when the presence of the emetic toxin was also detected in the tested food. In another study, the bacterial counts ranging from 3.51 to 5.95 log cfu.g^{-1} were identified in 34/100 samples of ready-to-eat (RTE) cooked rice randomly collected from food outlets in Malaysia. A high percentage (82.4 %) of the isolates carried at least 1 toxin gene, with higher detection rates of diarrheal (58.8 - 76.5%) than emetic toxin (14.7 %) genes **(Navaneethan & Effarizah, 2021)**.

The generated growth curves of *B. cereus* for each storage temperature and inoculum type are shown in Figure 1. At 4 °C, *B. cereus* growth was not observed during 48 h of storage and was characterised by a linear model (Figure 1, Table 6). Changes in the number of *B. cereus* were minimal, the bacterial population remained in the lag phase of growth throughout and no spore germination occurred. At 24 °C, the resulting growth curves were complete. Of the models used, the Gompertz model was selected as the most valid based on the lowest value of the residual standard error RSE and the highest correlation between the observed values and the values predicted by the R2 model (Table 7).

Table 6 Parameters of the linear growth model of *B. cereus* in cooked rice at 4 °C. Rice inoculated with vegetative cells and spores at a starting concentration of 2-3 log cfu.g^{-1}.

Rice	Inoculum	Intercept	Regression coefficient of time	RSE	R^2	F	df	P value
White	cells	2.136 ± 0.031	-0.008 ± 0.001	0.109	0.573	33.58	1; 25	<0.001
White	spores	3.287 ± 0.029	-0.004 ± 0.001	0.103	0.234	7.62	1; 25	0.011
Brown	cells	2.291 ± 0.057	-0.012 ± 0.003	0.203	0.439	19.54	1; 25	<0.001
Brown	spores	3.274 ± 0.021	0.002 ± 0.001	0.075	0.190	5.88	1; 25	0.023

Legend: RSE - residual standard error, R^2- coefficient of determination; df - degrees of freedom

Table 7 Parameters of growth patterns of *B. cereus* in cooked rice stored at 24 °C. Rice inoculated with vegetative cells and spores at a starting concentration of 2-3 log cfu.g^{-1}. Estimate ± standard error of parameter.

Inoculum	Model	lag phase duration (hours)	growth rate (ln cfu.g^{-1}.h^{-1})	initial count (log cfu.g^{-1})	maximum count (log cfu.g^{-1})	RSE	R^2
Long-grain white rice							
Cells	Baranyi	3.224 ± 0.457	1.079 ± 0.058	2.079 ± 0.110	8.521 ± 0.070	0.210	0.994
	Gompertz	3.104 ± 0.384	1.106 ± 0.045	1.974 ± 0.099	8.662 ± 0.061	0.156	0.997
	Buchanan	2.561 ± 0.404	0.991 ± 0.043	2.049 ± 0.131	8.521 ± 0.076	0.227	0.993
Spores	Baranyi	1.827 ± 0.541	0.975 ± 0.054	3.303 ± 0.133	8.692 ± 0.078	0.234	0.988
	Gompertz	2.072 ± 0.478	1.096 ± 0.054	3.220 ± 0.131	8.733 ± 0.065	0.183	0.993
	Buchanan	1.769 ± 0.449	0.951 ± 0.044	3.344 ± 0.133	8.698 ± 0.077	0.230	0.989
Long-grain brown rice							
Cells	Baranyi	2.796 ± 0.667	0.975 ± 0.071	2.099 ± 0.151	8.355 ± 0.092	0.276	0.989
	Gompertz	2.481 ± 0.694	0.989 ± 0.060	1.944 ± 0.172	8.533 ± 0.095	0.228	0.992
	Buchanan	2.454 ± 0.531	0.923 ± 0.053	2.123 ± 0.159	8.355 ± 0.092	0.276	0.989
Spores	Baranyi	1.873 ± 0.374	1.070 ± 0.042	3.254 ± 0.103	8.856 ± 0.060	0.180	0.994
	Gompertz	2.412 ± 0.270	1.246 ± 0.041	3.233 ± 0.081	8.886 ± 0.044	0.126	0.997
	Buchanan	1.742 ± 0.324	1.037 ± 0.034	3.279 ± 0.104	8.863 ± 0.060	0.181	0.993

Legend: RSE - residual standard error, R^2 - the square of the correlation between the observed values and the values predicted by the model

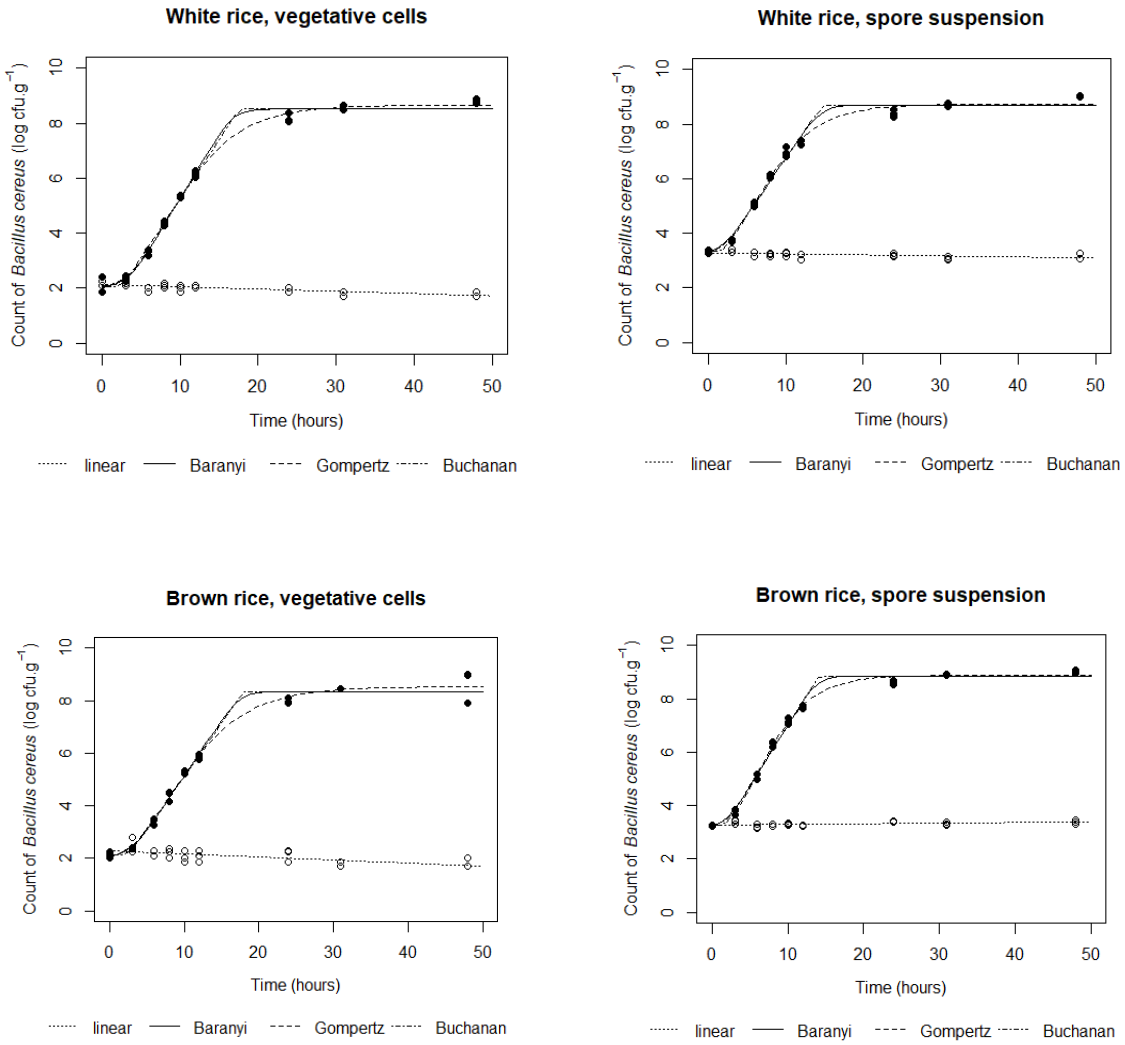

Figure 1 Observed (symbol) and predicted (lines) values of *B. cereus* counts in cooked rice stored at 4 °C (○) and 24 °C (●) for 48 h. Rice inoculated with vegetative cell suspension and spore suspension at a starting concentration of 2-3 log cfu.g^{-1}.

The main factors influencing the growth of microorganisms in food include pH and water activity in food **(Rodrigo *et al.*, 2021)**. Vegetative cells of *B. cereus* are able to grow and proliferate in the pH range of 4.5-9.5 and the minimum water activity is reported to be 0.93 **(Fernandes, 2009; Rodrigo *et al.*, 2021)**. The pH values of cooked long-grain white and brown rice immediately after cooking ranged from 5.92-6.41 and did not change significantly after 48 h of storage at 4 °C and 24 °C. There was no significant difference between the two types of rice. Similarly, the water activity of cooked rice (white, brown) varied only slightly and did not fall below 0.982, which is in agreement with the data by **Finlay *et al.* (2002)** who evaluated the water activity in cooked rice inoculated with *B. cereus* (aw 0.97-0.99 immediately after cooking, 0.97-0.98 after reaching the stationary growth phase, i.e. in 48-72 h at 20-30 °C). The results of a study by **Park & Yoon (2019)** showed that a combination of acidification and low-temperature pasteurization could improve the safety of packaged rice cakes and significantly inhibit the growth of *B. cereus* (< −2.43 log cfu/g at 95% percentile). The initial pH of the rice cakes was 5.92 ± 0.06. The target pH of the product was set using 0.1M of lactic acid to 5.0 to inhibit microbial growth and maintain the quality of the rice cake. **Cetin-Karaca & Newman (2018)** evaluated the *B. cereus* and its spores growth in infant rice cereals reconstituted with infant formula (pH 6.8, aw 0.92) and stored at 7, 23, or 37 °C. The *B. cereus* population significantly increased with the increase in storage temperatures (23 and 37 °C) during 24 h storage.

As far as the content of essential nutrients is concerned, the main difference between white (milled) and brown (husked) rice is in the fibre content, which is 7× higher in brown rice than in white rice; the protein content is comparable, and the fat content is slightly higher in brown rice. As can be seen from these results, the growth dynamics of *B. cereus* in cooked white and brown rice did not differ, and no statistically significant difference was found for the lag phase length or growth rate when comparing the two types of rice. In both cases, the length of the lag phase was around 2-3 hours and the determined growth rate was approximately 1 ln cfu.g^{-1} per hour. Since there was no difference between pH and water activity of the two types of cooked rice, it can be concluded that the differences in the nutritional composition of the tested rice did not have a significant effect on the growth of *B. cereus*.

Similarly, the type of inoculum (vegetative cells, spores) had no significant effect on the lag phase length or growth rate. Based on a visual comparison of the observed values, it can be concluded that a faster onset of proliferation (shorter lag phase) was observed in most cases in samples inoculated with spore suspension, which may be related to the higher initial count of spores in the solution (see Table 7) or the heat treatment and thus activation of spores immediately before inoculation of the samples. However, these differences were not statistically significant. It is necessary to emphasize that the type of inoculum is crucial for pathogen inactivation. Inducing spore germination before subsequent heat treatment (or generally, any eradication treatment) is one of the most effective ways to control *B. cereus* spores **(Choi & Kim, 2020)**.

McElroy *et al.* (2000) assessed the growth of a psychrotrophic emetic strain *B. cereus* 404 in cooked long grain rice at 15 °C, 20 °C and 30 °C. The Gompertz model was used to generate a growth curve and assess growth dynamics. The lag phase duration was 6.7 h at 20 °C and 2.1 h at 30 °C. In our study, the lag phase duration was 2.07 ± 0.48 h in the case of white rice inoculated with spore suspension and stored at 24 °C using the Gompertz model, with an initial spore concentration of 2-3 log cfu.g^{-1}. The growth rate was another evaluated kinetic parameter. The authors of the cited study reported values of 0.13 log cfu.g^{-1}.h^{-1} at 20 °C and 0.37 log cfu.g^{-1}.h^{-1} at 30 °C. In our study, the growth rate of *B. cereus* in cooked white rice determined by the Gompertz model (24 °C, spore suspension) was 1.042 to 1.150 ln cfu.g^{-1}.h^{-1} (i.e. 0.453-0.499 log cfu.g^{-1}.h^{-1}). These differences may be due to the fact that in our study: a) a mixture of 6 toxigenic strains was used, where not only emetic strains but also strains producing only diarrheagenic enterotoxins were represented; b) mesophilic, not psychrotrophic, strains were used; c) heat treatment of spores (85 °C, 10 min) was performed just before inoculation, which may have activated dormant spores.

McElroy *et al.* (2000) also compared the observed kinetic parameters (exponential growth rate, generation time, and lag phase duration) with those of a model predicted by the Pathogen Modeling Program (PMP; R. Whiting, U.S. Deparment of Agriculture - Agriculture Research Service, Philadelphia, PA, USA). They, however, modelled the growth of vegetative cells of mesophilic *B. cereus* in a nutrient broth (specific model not shown). The reported duration of the lag phase in their study was 4.4 h at 20 °C and 1.2 h at 30 °C. This is essentially consistent with the values found in our study where, at 24 °C, the duration of the lag phase was determined to be 3.224 ± 0.457 h (Baranyi-Roberts model), 3.104 ± 0.384 (Gompertz model) and 2.561 ± 0.404 (Buchanan model) for white rice inoculated with vegetative cells. The growth rates predicted by PMP were 0.26 log cfu.g^{-1}.h^{-1}at 20 °C and 0.82 log cfu.g^{-1}.h^{-1}at 30 °C. In our study, the rates for cooked white rice (vegetative cell inoculum) ranged from 1.021 to 1.137 ln cfu.g^{-1}.h^{-1} (i.e. 0.443-0.494 log cfu.g^{-1}.h^{-1}; Baranyi-Roberts model), 1.061 to 1.151 ln cfu.g^{-1}.h^{-1} (i.e. 0.461-0.500 log cfu.g^{-1}.h^{-1}; Gompertz model), and 0.948-1.034 ln cfu.g^{-1}.h^{-1} (i.e. 0.412-0.449 log cfu.g^{-1}.h^{-1}; Buchanan model), respectively.

Three values of bacterial counts were considered critical from the perspective of causing alimentary disease in healthy and immunocompromised individuals – 3, 5 and 8 log cfu.g^{-1}. **Hwang & Park (2014)** have indicated that consumption of food contaminated with more than 5 log *B. cereus* cells g^{-1} may cause food poisoning. **Granum & Lindbäck (2013)** have concluded that food containing more than 3 log *B. cereus* spores g^{-1} cannot be considered completely safe for consumption. Based on the published data, the real infectious dose of viable vegetative cells or spores may vary from about 5 to 8 log *B. cereus* per gram of food **(Bae *et al.*, 2012; Berthold-Pluta *et al.*, 2015; Granum & Lindbäck, 2013)**. Finally, outbreaks in neonates and infants have been attributed to consumption of even lower numbers of *B. cereus* cells in the order of 3–5 log cells ml^{-1} **(Hwang & Park, 2015)**. The concentration of 3 log cfu.g^{-1} is considered a health hazard to consumers according to the US Food Safety Inspection Service (FSIS) **(Juneja *et al.*, 2018)**. The Gompertz model was used for the calculation (Table 8). No growth of *B. cereus* was observed in rice stored at 4 °C; therefore, none of the considered critical concentrations were reached during the duration of any of the sub-experiments. The only exception was rice inoculated with spore suspension, where the amount of inoculum already corresponded to the lowest considered critical concentration of 3 log cfu.g^{-1}. In the case of storage of cooked rice at 24 °C, the time required to reach the critical concentration of *B. cereus* was different for the different types of inoculum, reflecting the different initial concentrations of cells and spores in the inoculum (approx. 2 log cfu.g^{-1} for vegetative cells, 3 log cfu.g^{-1} for spores). When inoculated with spores, the critical concentration was reached in a shorter time. On the other hand, the differences between the different types of rice were minimal (see Table 8).

Table 8 Storage time required to multiply *Bacillus cereus* in cooked rice to critical levels. Rice inoculated with vegetative cell suspension and spore suspension at a starting concentration of 2-3 log cfu.g^{-1}. Storage time (hours) predicted by the Gompertz model.

Temperature, inoculum	**Time (hours) required to reach the critical concentration of *B. cereus***		
	3 log cfu.g^{-1}	5 log cfu.g^{-1}	8 log cfu.g^{-1}
Long-grain white rice			
4 °C, vegetative cells	> 48[a]	> 48[a]	> 48[a]
4 °C, spores	0[b]	> 48[a]	> 48[a]
24 °C, vegetative cells	5.0	9.4	19.8
24 °C, spores	0[b]	5.8	14.7
Long-grain brown rice			
4 °C, vegetative cells	> 48[a]	> 48[a]	> 48[a]
4 °C, spores	0[b]	> 48[a]	> 48[a]
24 °C, vegetative cells	4.7	9.6	22.1
24 °C, spores	0[b]	5.7	13.1

[a] the critical concentration has not been reached within 48 hours of the experiment
[b] a concentration of 3 log cfu.g^{-1} was achieved immediately after sample inoculation

As suggested by the results of the presented study and the conclusions by **Rouzeau-Szynalski *et al.* (2020)** and **Rodrigo *et al.* (2021)**, effective cooling of cooked rice, if not consumed quickly, is one of the essential steps of controlling the reproduction of *B. cereus* and, thus, the possible production of toxins. This statement is supported by the results of **Juneja *et al.* (2018)**, who focused their work on assessing the effect of cooling rate on the ability of *B. cereus* spores to germinate and grow. Among others, cooked long-grain rice was used as a test medium in their study, either alone or in combination with chicken or beef and vegetables. Foods were inoculated with a mixture of spores of four emetic and diarhogenic strains of *B. cereus* at a starting concentration of the magnitude of approx. 2 log cfu.g^{-1}. Cooling of rice was carried out exponentially from 54.5 °C to 7.2 °C during 6, 9, 12, 15, 18, and 21 h. The authors conclude that if the cooling period of the cooked rice to 7.2 °C is longer than 12 h, spore germination and subsequent reproduction of vegetative cells to values exceeding 3 log cfu.g^{-1} (which is considered a health hazard to consumers according to the FSIS) can occur **(Juneja *et al.*, 2018)**. Also, **Heo *et al.* (2009)** reported that long and slow cooling of cooked rice contaminated with *B. cereus* spores that survived the cooking process to a room temperature leads to the germination of spores and subsequent reproduction of vegetative cells. The multiplication of *B. cereus* can be reduced by refrigeration, storage time or by adjusting the pH value (e.g. by addition of wine vinegar in sushi rice).

In summary, it can be concluded that improper handling of cooked rice (insufficient cooling and storage at room temperature, contamination with spores or vegetative cells of *B. cereus*) can lead to the multiplication of the bacterium to levels posing a health risk to consumers within a few hours.

CONCLUSION

Ensuring food safety is one of the top priorities. The behaviour of microorganisms in food is influenced by a variety of factors, including the composition and physico-chemical parameters of the product, the presence of natural or contaminating microflora or storage conditions. The selection of an appropriate primary model that accurately describes the growth or devitalisation kinetics of the microorganisms in question is an important part of the development, evaluation and validation of predictive models. The use of appropriate mathematical models and their application to specific foods allows the behaviour of foodborne pathogens to be analysed in a more comprehensive way, including the assessment of potential health risks to the consumer.

According to the requirements of the current legislation, the finished dishes must be kept at a temperature of at least 60 °C after cooking. If they are not consumed quickly, they must be cooled to a temperature not exceeding 4 °C. Cooked rice is a suitable medium for the growth and multiplication of toxigenic *B. cereus* strains. *B. cereus* spores can survive cooking and can be even activated by heating. If the cooling of cooked rice is slow and/or insufficient and/or stored at room temperature, the bacteria can multiply to levels posing a health risk to consumers within a few hours. The growth dynamics of toxigenic *B. cereus* in cooked rice are best characterised by the Gompertz model, and neither the type of inoculum nor the composition of the product significantly affect the behaviour of the bacterium.

Acknowledgments: This study was supported by the Internal Grant Agency of the University of Veterinary Sciences Brno (grant No. IGA 211/2019/FVHE).

REFERENCES

Act No. 121/2023. Decree on food requirements of the Ministry of Agriculture of the Czech Republic. Collection of Laws. 63:1763–1768.

Bae, Y. M., Kim, B. R., Lee, S. Y., Cha, M. H., Park, K. H., Chung, M. S. & Ryu, K. (2012). Growth and predictive model of *Bacillus cereus* on blanched spinach with or without seasoning at various temperatures. *Food Science and Technology*, 21 (2), 503–508. https://doi.org/10.1007/s10068-012-0064-7

Baranyi, J. & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*, 23 (1994), 277–294. https://doi.org/10.1016/0168-1605(94)90157-0

Baty, F. & Delignette-Muller, M. L. (2014). nlsMicrobio: data sets and nonlinear regression models dedicated to predictive microbiology. R package version 0.0-1.

Berthold-Pluta, A., Pluta, A. & Garbowska, M. (2015). The effect of selected factors on the survival of *Bacillus cereus* in the human gastrointestinal tract. *Microbial Pathogenesis*, 82 (2015), 7–14. https://doi.org/10.1016/j.micpath.2015.03.015

Buchanan, R. L., Whiting, R. C. & Damert, W. C. (1997). When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiology*, 14 (4), 313–326. https://doi.org/10.1006/fmic.1997.0125

Bursová, Š., Necidová, L. & Haruštiaková, D. (2018). Growth and toxin production of *Bacillus cereus* strains in reconstituted initial infant milk formula. *Food Control*, 93 (2018), 334–343. https://doi.org/10.1016/j.foodcont.2017.05.006

Buss da Silva, N., Baranyi, J., Carciofi, B. A. M. & Ellouze, M. (2017). From Culture-Medium-Based Models to Applications to Food: Predicting the Growth of *B. cereus* in Reconstituted Infant Formulae. *Frontiers in Microbiology*, 8 (2017), 01799. https://doi.org/10.3389/fmicb.2017.01799

Cetin-Karaca, H. & Newman, M. C. (2018). Antimicrobial efficacy of phytochemicals against *Bacillus cereus* in reconstituted infant rice cereal. Food Microbiology, 69 (2018), 189–195. https://doi.org/10.1016/j.fm.2017.08.011

Choi, S., Kim, H., Kim, Y., Kim, B. S., Beuchat, L. R. & Ryu, J. H. (2014). Fate of *Bacillus cereus* and naturally occurring microbiota on milled rice as affected by temperature and relative humidity. *Food Microbiology*, 38 (2014), 122–127. https://doi.org/10.1016/j.fm.2013.08.016

Choi, W. & Kim, S. S. (2020). Outbreaks, Germination, and Inactivation of *Bacillus cereus* in Food Products: A Review. *Journal of Food Protection*, 83 (9), 1480–1487. https://doi.org/10.4315/0362-028X.JFP-19-429

Fernandes, R. (2009). *Microbiology handbook: Dairy products* (Vol. 3). UK: Leatherhead Publishing, and Royal Society of Chemistry. ISBN: 978-1-905224-62-3.

Finlay, W. J. J., Logan, N. A. & Sutherland, A. D. (2002). *Bacillus cereus* emetic toxin production in cooked rice. *Food Microbiology*, 19 (5), 431–439. https://doi.org/10.1006/fmic.2002.0505

Gibson, A. M., Bratchell, N. & Roberts, T. A. (1988). Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *International Journal of Food Microbiology*, 6 (1988), 155–178. https://doi.org/10.1016/0168-1605(88)90051-7

Glasset, B., Sperry, M., Dervyn, R., Herbin, S., Brisabois, A. & Ramarao, N. (2021). The cytotoxic potential of *Bacillus cereus* strains of various origins. *Food Microbiology*, 98 (2021), 103759. https://doi.org/10.1016/j.fm.2021.103759

Granum, E. P. & Lindbäck, T. (2013). *Bacillus cereus*. In M. P. Doyle, & R. L. Buchanan (Eds.), Food Microbiology: Fundamentals and Frontiers, Fourth edition (pp. 491–502). Washington, DC: ASM Press.

Heo, S. K., Lee, J. Y., Baek, S. B. & Ha, S. D. (2009) A Response Surface Model To Describe the Effect of Temperature and pH on the Growth of *Bacillus cereus* in Cooked Rice. *Journal of Food Protection*, 72 (6), 1296–1300. https://doi.org/10.4315/0362-028X-72.6.1296

Horwood, P. F., Burgess, G. W. & Oakey, H. J. (2004). Evidence for non-ribosomal peptide synthetase production of cereulide (the emetic toxin) in *Bacillus cereus*. FEMS Microbiology Letters, 2004, 236 (2), 319–324. https://doi.org/10.1111/j.1574-6968.2004.tb09664.x

Huang, Y., Flint, S. H. & Palmer, J. S. (2020). *Bacillus cereus* spores and toxins – The potential role of biofilms. *Food Microbiology*, 90 (2020), 103493. https://doi.org/10.1016/j.fm.2020.103493

Huang, Y., Flint, S. H., Yu, S., Ding, Y. & Palmer, J. S. (2021). Phenotypic properties and genotyping analysis of *Bacillus cereus* group isolates from dairy and potato products. *LWT – Food Science and Technology*, 140 (2021), 110853. https://doi.org/10.1016/j.lwt.2021.110853

Hwang, J. Y. & Park, J. H. (2014). Characteristics of enterotoxin distribution, hemolysis, lecithinase, and starch hydrolysis of *Bacillus cereus* isolated from infant formulas and ready-to-eat foods. *Journal of Dairy Science*, 98 (3), 1652–1660. https://doi.org/10.3168/jds.2014-9042

Hwang, J. Y. & Park, J. H. (2015). Distribution of six enterotoxin genes and production of L2-HBL and NheA proteins in six Bacillus cereus isolates from infant formula and produce. *Food Science and Biotechnology*, 24 (1), 379–382. https://doi.org/10.1007/s10068-015-0050-y

International Standard ISO 7932 (2005). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony-count technique at 30 °C (p. 20). Czech office for standards, metrology and testing.

Juneja, V. K., Mohr, T. B., Silverman, M. & Snyder, Jr., O. P. (2018). Influence of Cooling Rate on Growth of *Bacillus cereus* from Spore Inocula in Cooked Rice, Beans, Pasta, and Combination Products Containing Meat or Poultry. *Journal of Food Protection*, 81 (3), 430–436. https://doi.org/10.4315/0362-028X.JFP-17-397

Kim, B., Bang, J., Kim, H., Kim, Y., Kim, B. S., Beuchat, L. R. & Ryu, J. H. (2014). *Bacillus cereus* and *Bacillus thuringiensis* spores in Korean rice: Prevalence and toxin production as affected by production area and degree of milling. *Food Microbiology*, 42 (2014), 89–94. https://doi.org/10.1016/j.fm.2014.02.021

Le Marc, Y., Buss da Silva, N., Postollec, F., Huchet, V., Baranyi, J. & Ellouze, M. (2021). A stochastic approach for modelling the effects of temperature on the growth rate of *Bacillus cereus sensu lato*. *International Journal of Food Microbiology*, 349 (2021), 109241. https://doi.org/10.1016/j.ijfoodmicro.2021.109241

Le Marc, Y., Postollec, F., Huchet, V. & Ellouze, M. (2022). Modelling the thermal inactivation of spores from different phylogenetic groups of *Bacillus cereus*. *International Journal of Food Microbiology*, 368 (2022), 109607. https://doi.org/10.1016/j.ijfoodmicro.2022.109607

Leong, S. S., Korel, F. & King, J. H. (2023). *Bacillus cereus*: A review of "fried rice syndrome" causative agents. *Microbial Pathogenesis*, 185 (2023), 106418. https://doi.org/10.1016/j.micpath.2023.106418

Marrollo, R. (2016). *Bacillus cereus* Food-Borne Disease. In: V. SAVINI, ed. The diverse faces of *Bacillus cereus* London: Elsevier. https://doi.org/10.1016/B978-0-12-801474-5.00005-0

Martineau, F., Picard, F. J., Roy, P. H., Ouellette, M. & Bergeron, M. G. (1996). Species-specific and ubiquitous DNA-based assays for rapid identification of *Staphylococcus epidermidis*. *Journal of Clinical Microbiology*, 34 (12), 2888–2893. https://doi.org/10.1128/jcm.34.12.2888-2893.1996

McElroy, D. M., Jaykus, L. A. & Foegeding, P. M. (2000) Validation and Analysis of Modeled Predictions of Growth of *Bacillus cereus* Spores in Boiled Rice. *Journal of Food Protection*, 63 (2), 268–272. https://doi.org/10.4315/0362-028X-63.2.268

Meng, J. N., Liu, Y. J., Shen, X., Wang, J., Xu, Z. K., Ding, Y., Beier, R. C., Luo, L., Lei, H. T. & Xu, Z. L. (2022). Detection of emetic *Bacillus cereus* and the emetic toxin cereulide in food matrices: Progress and perspectives. *Trends in Food Science & Technology*, 123 (2022), 322–333. https://doi.org/10.1016/j.tifs.2022.03.023

Messelhäusser, U. & Ehling-Schulz, M. (2018). *Bacillus cereus* – a Multifaceted Opportunistic Pathogen. *Current Clinical Microbiology Reports*, 5 (2018), 120–125. https://doi.org/10.1007/s40588-018-0095-9

Navaneethan, Y. & Effarizah, M. E. (2021). Prevalence, toxigenic profiles, multidrug resistance, and biofilm formation of *Bacillus cereus* isolated from ready-to eat cooked rice in Penang, Malaysia. *Food Control*, 121 (2021), 107553. https://doi.org/10.1016/j.foodcont.2020.107553

Osimani, A., Aquilanti, L. & Clementi, F. (2018). *Bacillus cereus* foodborne outbreaks in mass catering. *International Journal of Hospitality Management*, 72 (2018), 145–153. https://doi.org/10.1016/j.ijhm.2018.01.013

Park, H. W. & Yoon, W. B. (2019). A quantitative microbiological exposure assessment model for *Bacillus cereus* in pasteurized rice cakes using computational fluid dynamics and Monte Carlo simulation. *Food Research International*, 125 (2019), 108562. https://doi.org/10.1016/j.foodres.2019.108562

Pexara, A. & Govaris, A. (2010). *Bacillus cereus*: an important foodborne pathogen. *Journal of the Hellenic Veterinary Medical Society*, 61 (2), 127–133. http://dx.doi.org/10.12681/jhvms.14881

Rodrigo, D., Rosell, C. M. & Martinez, A. (2021). Risk of *Bacillus cereus* in Relation to Rice and Derivatives. *Foods*, 10 (2), 302. https://doi.org/10.3390/foods10020302

Rouzeau-Szynalski, K., Stollewerk, K., Messelhäusser, U. & Ehling-Schulz, M. (2020). Why be serious about emetic *Bacillus cereus*: Cereulide production and industrial challenges. *Food Microbiology*, 85 (2020), 103279. https://doi.org/10.1016/j.fm.2019.103279

Schmid, D., Rademacher, C., Kanitz, E. E., Frenzel, E., Simons, E., Allerberger, F. & Ehling-Schulz, M.. (2016). Elucidation of enterotoxigenic *Bacillus cereus* outbreaks in Austria by complementary epidemiological and microbiological investigations, 2013. *International Journal of Food Microbiology*, 232 (2016), 80–86. https://doi.org/10.1016/j.ijfoodmicro.2016.05.011

Stavropoulou, E. & Bezirtzoglou, E. (2019). Predictive Modeling of Microbial Behavior in Food. *Foods*, 8 (12), 654. https://doi.org/10.3390/foods8120654

Tewari, A. & Abdullah, S. (2015).*Bacillus cereus* food poisoning: international and Indian perspective. *Journal of Food Science and Technology*, 52 (5), 2500–2511. https://doi.org/10.1007/s13197-014-1344-4

Yamada, S., Ohashi, E., Agata, N. & Venkateswaran, K. (1999). Cloning and nucleotide sequence analysis of gyrB of *Bacillus cereus*, *B. thuringiensis*, *B. mycoides*, and *B. anthracis* and their application to the detection of *B. cereus* in rice. *Applied and Environmental Microbiology*, 65 (4), 1483–1490. https://doi.org/10.1128/AEM.65.4.1483-1490.1999

Zwietering, M. H., Jongenburger, I., Rombouts, F. M. & Van´t Riet, K. (1990). Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology*, 56 (6), 1875–1881. https://doi.org/10.1128/aem.56.6.1875-1881.1990