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Paw Dalgaard:

Bacillus-Predict – Mejeri-prædiktionsværktøj

Bacillus-Predict – Predictive tool for dairy products

Mejeribrugets ForskningsFond

September 2026

Final report

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1. Title of the project

Danish: Mejeri-prædiktionsværktøj (Bacillus-Predict)

English: Predictive tool for dairy products (Bacillus-Predict)

2. Project manager

Paw Dalgaard, DTU National Food Institute, Technical University of Denmark, Kemitorvet, Building 204, 2800 Kgs. Lyngby, email: pada@food.dtu.dk.

3. Other project staff

- Maryam Maktabdar, PhD student, DTU National Food Institute (DTU Food), Technical University of Denmark, Kemitorvet, Building 204, 2800 Kgs. Lyngby, email: mmak@food.dtu.dk.
- Elissavet Gkogka, Research Microbiologist, Arla Innovation Centre, Arla Foods amba, Agro Food Park 19, 8200 Aarhus N, email: elgko@arlafoods.com.
- Ellen Wemmenhove, Senior Scientist Microbiology, Arla Foods Ingredients (AFI), Nr. Virum, Sønderupvej 19, 6920 Videbæk, Denmark, email: elwem@arlafoods.com.

4. Sources of funding

The Milk Levy Fund, DTU National Food Institute (DTU Food), Arla Innovation Center, and Arla Foods Ingredients.

5. Project period

Project period with DDRF funding:	January 2022 – December 2024
Total project period, if sub-project within a larger project:	Project started in October 2021

6. Project summary

Danish:

Bacillus cereus er fødevarebårne humanpatogene bakterier med høj forekomst I mange mejeriprodukter, men hidtil har der ikke været retningslinjer eller anbefalinger for produktsammensætninger, der hæmmer deres vækst. Derfor er validerede prædiktive fødevaremikrobiologiske modeller interessante til styring af vækst samt til at gøre det lettere at udvikle mejeriprodukter, der er stabiliseret mod vækst af *B. cereus.* Dette projekt udvikler og validere nye prædiktive modeller for vækst og vækst-grænse af både mesofile og kuldetolerante *B. cereus* således at de kan bidrage til produkt

-udvikling, risikovurdering samt dokumentation af fødevaresikkerhed for mejeriprodukter og ingredienser. De nye modeller er indbygget i det brugervenlige og frit tilgængelige Food Spoilage and Safety Predictor (FSSP) software således at korrekt anvendelse af modellerne gøres lettere og dermed kan gavne mejerisektoren. Forekomst af *B. cereus* i et bredt udsnit af mejeriprodukter, blev undersøgt og relevante isolater udvalgt til prædiktiv modellering. 49% af de 71 undersøgte mejeriprodukter var positive og 51 *B. cereus* isolater blev indsamlet, identificeret og karakteriseret. Højeste *B. cereus* forekomst blev observeret for mælkepulver (83%), ostevalle (43%) og oste (42%). Mejeri-ioslater af *B. cereus* indeholdt gener for produktion af enterotoksiner (97 %) og toksinet cereulide, der forårsager opkast (2.9%). Blandinger af isolater for, henholdsvis, mesofile og kuldetolerante *B. cereus* blev udvalgt til studier af vækst og sporedannelse. Kardinal-parameter vækstmodeler for mesofile og kuldetolerante *B. cereus* isolate-blandinger blev udviklet til at inkludere den væksthæmmende effekt af 11 miljø-faktorer og deres interaktion (temperatur, pH, NaCl/a_w, eddike-, benzo-, citron-, mælke og sorbin-syre, samt ortho-, di-, og tri-fosfater). Vækst data fra både et flydende laboratoriesubstrat samt fra UF vallepermeat blev anvendt til udvikling af de nye og omfattende modeller. Mesofile *B. cereus* var mere resistente overfor eddike-, benzo-, sorbin-syre end den kuldetolerante isolat-blanding. 133 nye belastningsundersøgelser og 248 publicerede vækst-responser i mejeriprodukter blev anvendt til validering af de to udviklede modeller. Forudsigelser fra de nye modeller passede bedre med disse data for mejeriprodukter sammenlignet med eksisterende modeller for vækst af mesofile og kuldetolerante *B. cereus*. Vækst og sporerdannelse af *B. cereus* blev undersøgt gennem i alt 29 forsøg med kinetik for de mesofile og kuldetolerante isolate-blandinger i UF vallepermeat, valleprotein koncentrat, mælk og havredrik. En model blev udviklet således at sporedannelse relateres til vækst (som forudsagt med de nye modeller fra dette projekt). Sporer blev dannet af høje *B. cereus* celle koncentrationer. Vækst skal styres således at sporedannelse i mejeriprodukter/ingredienser undgås og de nye modeller fra dette projekt kan bidrage til denne opgave.

English:

Bacillus cereus is foodborne human pathogenic bacteria with high prevalence in many dairy foods but so far there are no guidelines or recommendations on suitable formulations to prevent their growth. Therefore, validated predictive models are interesting to manage growth and facilitate the development of dairy products stabilized against growth of *B. cereus.* This project developed and validated new predictive models for growth and growth-boundary of both mesophilic and psychrotolerant *B. cereus* so that they can contribute to product development, risk assessments and documentation of food safety for dairy foods and ingredients. The new models are included in the user-friendly and freely available Food Spoilage and Safety Predictor (FSSP) software to make correct use of the new models easier and thereby benefit the dairy sector. Prevalence of *B. cereus* in a broad range of dairy products was studied to select relevant isolates for predictive modelling. 49% of 71 studied dairy products were positive and 51 *B. cereus* isolates were obtained, identified and characterized. Highest *B. cereus* prevalences were observed for dairy powders (83%), cheese whey (43%) and cheeses (42%). *B. cereus* dairy isolates included genes for production of enterotoxins (97 %) and the emetic toxin cereulide (2.9%). Strain cocktails of, respectively, mesophilic and psychrotolerant *B. cereus* were selected for studies of growth and sporulation. Cardinal parameter growth models for mesophilic and psychrotolerant *B. cereus* strain cocktails were developed to include the growth inhibiting effect of 11 environmental factors and their interactions (temperature, pH, NaCl/a_w, acetic, benzoic, citric, lactic, and sorbic acids, as well as ortho-, di-, and tri-phosphates). Growth data in both liquid laboratory broth and UF whey permeate was used to develop the new and extensive models. Mesophilic *B. cereus* was more resistant to acetic, benzoic, and sorbic acids than the psychrotolerant strain cocktail. 133 new challenge tests and 248 literature growth responses in dairy products were used to validate the developed models. The new models performed better than available growth models of mesophilic and psychrotolerant *B. cereus* in dairy foods. *B. cereus* spore formation was studied for a total of 29 kinetics of mesophilic and psychrotolerant strain cocktails in UF whey permeate, whey protein concentrate, milk and oat drink. A spore formation model was developed to link growth, as predicted by the new models from the present project, and sporulation. Spores are formed by high *B. cereus* cell concentrations. Growth should be managed to avoid spore formation in dairy foods and ingredients and new models from the present project can facilitate this task.

7. Project aim

Danish:

Formålet med det foreslåede projekt er at udvikle et prædiktivt fødevaremikrobiologiske redskab som tillader forudsigelse af vækst, vækstgrænse og sporedannelse for kuldetolerante samt mesofile/termotolerante varianter af *Bacillus cereus* i mejeriprodukter og ingredienser. Denne forskning er opdelt i fire arbejdspakker:

- Bestem forekomst af *B. cereus* og andre *Bacillus* arter i relevante mejeriprodukter og ingredienser.
- Udvid vækst- og vækstgrænse-modeller til at forudsige den hæmmende effekt af relevante tilsætningsstoffer og procesbetingelser for mejeriprodukter og ingredienser.
- Modeller og forudsig dannelse af resistente *B. cereus* sporer under forarbejdning af udvalgte mejeriprodukter og ingredienser.
- Inkluder de nye og validerede modeller i et prædiktivt fødevaremikrobiologiske redskab.

English:

The overall objective of the proposed project is to develop a predictive food microbiology tool that allows the growth boundaries, growth potential and spore formation to be predicted for psychrotolerant and mesophilic/thermo-tolerant variants of *Bacillus cereus* in dairy products and ingredients. The research is structured to meet four aims:

- Determine occurrence of *B. cereus* and other *Bacillus* species in relevant dairy products and ingredients.
- Expand growth and growth boundary models to predict the inhibiting effect of relevant additives and processing conditions for dairy products and ingredients.
- Model and predict the formation of resistant *B. cereus* spores during processing of selected dairy products and ingredients.
- Include the new and validated models in a predictive tool.

8. Background for the project

B. cereus is a human pathogen that forms spores and these are present in many environments including milk and dairy products/cheeses. Disease outbreaks associated with *B. cereus* have been reported for cheeses, milk and milk powder/infant formula. *B. cereus* cause two distinct forms of food poisoning due to enterotoxins (diarrhoeal illness) or the toxin cereulide produced in food (emetic illness). Cereulide producing *B. cereus* has been detected in various dairy products and the toxin can be produced by high concentrations of the pathogen in for example sweetened dairy-based desserts and soft cheeses. *B. cereus* spores survive heating (pasteurization; spray drying) and may occur in milk and whey powder. Growth of psychrotolerant and mesophilic/thermo-tolerant variants of *B. cereus* can be observed from 5 to > 50°C and they are important to control in dairy products including reconstituted milk and whey powders. So far, there are no recommendations on suitable formulations to prevent their growth and predictive models will facilitate the development of dairy products stabilized against growth of *B. cereus.* Regulations for *B. cereus* are currently limited to specific foods such as infant formula and dairy powders. Traditionally, assessing food safety relies on challenge tests and storage trials. However, these methods are often time-consuming and expensive, requiring extensive experimentation across numerous product formulations and storage conditions. This makes it impractical to explore every potential scenario. Predictive models provide an alternative, cost-effective, and efficient approach to assess and improve food safety. These models must account for general product factors (temperature, NaCl, pH) and dairy-specific ingredients like phosphate melting salts and organic acids. While extensive models exist for other foodborne pathogens like *Listeria* *monocytogenes* and *Clostridium botulinum*, comparable extensive models for *B. cereus* subgroups were lacking, hindering effective risk management. Several growth and growth boundary models are available for *B. cereus*. However, available models are either not validated for dairy products or they do not contain the effect of relevant factors including organic acids and ranges of pH and temperature needed to predict growth in different dairy products and ingredients. New validated dairy models are needed to help manage growth of the pathogen. However, *B. cereus* spores can also be formed during processing of dairy products depending on time, storage conditions and product characteristics. Therefore, models for *B. cereus* spore formation is relevant to identify conditions that prevent this formation. Mechanistic models for sporulation kinetics have been proposed but these are complex and include numerous parameters that cannot be estimated for growth and spore formation in food. More recently, a kinetic approach was suggested to model and predict growth and spore formation by *Bacillus subtilis*. This approach previously used for liquid laboratory media, will be used in the present study for *B. cereus* in dairy products and ingredients.

9. Sub-activities in the entire project period

To meet objectives and aims the project has been divided into four work-packages (WP). Description of these WPs are included below as taken from the original Expression of Interest for the present project.

Activity	Start date (yyyy-mm-dd)	End date (yyyy-mm-dd)	Half year period 1	2	3	4	5	6	7	8
Literature review- data collection, model evaluation, identification methods	2021-10-02	2021-11-30								
WP1 - Collection and identification of Bacillus isolates	2021-12-01	2022-07-30								
WP2 - Experimental work for growth models development	2022-08-01	2024-03-30								
Article writing - WP 1	2023-03-01	2023-04-30								
WP2 - Experimental work for growth models validation	2023-01-01	2024-06-30								
WP3 - Quantification of spores, Spore formation model development	2023-01-01	2024-01-01								
Programing growth models in Excel	2024-03-01	2024-05-30								
External stay	2024-04-02	2024-04-12								
Article writing - WP 2	2024-05-01	2024-06-30								
Article writing - WP 2	2024-07-01	2024-08-30								
Article writing - WP 3	2024-09-01	2024-10-30								
Thesis writing	2024-11-01	2024-12-31								

WP1: Selection of psychrotolerant and mesophilic/thermo-tolerant *B. cereus* isolates from dairy products and ingredients

Collections of *B. cereus* isolates from the dairy environment are already available at both DTU Food and Arla Foods. Isolates from these collections will be used in the present study together with selected isolates from other/international culture collections. Cocktails of 3-5 psychrotolerant and 3-5 mesophilic/thermo-tolerant *B. cereus* isolates will be used for studies of growth and sporulation. Isolates will be selected on the basis of their growth rates at 10 °C and 45 °C, presence of selected genes (*cspA*, *panC*) as determined by PCR (Carroll et al. 2019; Guinebretière et al. 2008). Furthermore, isolates will be analysed by MALDI-TOF for identification and evaluation of cereulide formation (Ducrest et al., 2019). <u>Deliverable:</u> Selection of strain cocktails for growth and sporulation studies. <u>Milestone:</u> Internal project report (6 months).

WP 2: Growth and growth boundary models for psychrotolerant and mesophilic/thermo-tolerant *B. cereus*

DTU Food quantifies growth responses for cocktails of respectively psychrotolerant and mesophilic/ thermo-tolerant *B. cereus* isolates and develops extensive growth and growth-boundary models including the effect of relevant dairy ingredients, additives and processing conditions. The growth inhibiting effect of individual factors is quantified using automated growth analyzers (Bioscreen C). Subsequently, mathematical terms for their effect are developed and included in cardinal parameter growth and growth-boundary models (Carlin et al. 2013). These models are little data demanding, can be expanded with terms for various growth inhibiting factors and allow the effect of interaction between factors to be taken into account. Arla Foods and AFI contribute with information on dairy products, ingredients and processing conditions. The expanded models are product calibrated and evaluated by using data from both the scientific literature, accessible databases, colleagues and companies. Further, DTU Food quantify growth of psychrotolerant and mesophilic/ thermo-tolerant *B. cereus* in selected and well characterized dairy products and ingredients provided by Arla Foods and AFI. Conditions where model evaluation is successful are used to determine the range of applicability for the final models. Deliverable: Fitted and validated growth and growth-boundary models for psychrotolerant and mesophilic/thermo-tolerant *B. cereus*. Milestone: Scientific papers on model development and validation (Months 24)

WP 3: Models to predict spore formation by *B. cereus*.

During storage of selected dairy products and ingredients growth kinetics and the kinetics of spore formation will be quantified. Spores will be quantified by enumeration of *B. cereus* before and after heat treatment of samples. The Gauvry-approach is studied to model and predict growth and spore formation by psychrotolerant and mesophilic/thermo-tolerant *B. cereus* (Gauvry et al 2019, 2021). This modelling approach relates growth and maximum probability for sporulation (P_{max}) with the time when P_{max} is observed (t_{max}) and variability of t_{max} (σ). Experiment with different and well characterized dairy products and ingredients are used for fitting and evaluation/validation of this quantitative spore formation models. Deliverable: Fitted and validated spore formation models for psychrotolerant and mesophilic/thermo-tolerant *B. cereus*. Milestone: Scientific paper on sporulation models for dairy products and ingredients (Months 32).

WP 4: Tool to predict *B. cereus* growth and sporulation

Application software is developed at two different levels. Firstly, the successfully validated models will be programmed in MS Excel or R allowing project participants to predict *B. cereus* growth and sporulation for relevant products and processes. Secondly, models will be included in the Food Spoilage and Safety Predictor (FSSP) software (http://fssp.food.dtu.dk) which is more user-friendly and available for free. FSSP will include a multiple growth model to provide simultaneous predictions for psychrotolerant and mesophilic/thermo-tolerant *B. cereus*, *L. monocytogenes* and *C. botulinum*. The FSSP software will be developed by DTU Food in collaboration with Anchor Lab K/S (sub-contractor). Arla Foods and AFI evaluate and provide feedback on a beta-version of the software. Deliverable: FSSP software including new growth and sporulation models for psychrotolerant and mesophilic/thermo-tolerant *B. cereus*. Milestone: Updated and released new version of the FSSP software (Months 36).

10. Deviations

WP1: In addition to the initially planned criteria for characterization and selection of *B. cereus* isolates, tolerance to pH, NaCl, and ability to ferment lactose were performed. Also, *B. cereus* were isolated from a range of dairy products from Danish supermarkets but other Bacillus species were not studied. Thermotolerant *B. cereus* isolate was not detected or identified among the dairy isolates. Therefore, the mesophilic stain-cocktail exclusively contained *panC* group III isolates. This workpackage was significantly expanded compared to the original plans and instead of a internal project report after 6 months a scientific paper was produced including results from WP 1.

WP2: Mesophilic and psychrotolerant *B. cereus* subgroups showed higher tolerance to environmental conditions in dairy products compared to standard laboratory broth (BHI). Therefore, growth rates were generated in both BHI and a specific dairy product (UF permeate solution). The model parameters were selected to reflect the widest growth range observed in these two media. Therefore, model development, evaluation/validation and manuscript writing took longer than expected (36 month instead of originally planned 24 month).

WP3: A selection of sporulation models, including those by Gauvry et al. (2019, 2021), were assessed to determine the most suitable for *B. cereus* sporulation in dairy products. The Baril et al. (2012) model was chosen and subsequently modified to fit the specific needs of this project for modelling the sporulation of mesophilic and psychrotolerant *B. cereus* subgroups. Results showed spores to be formed by high cell concentrations and these should not be reached in dairy foods or ingredients e.g. due to the risk of cereulide formation (heat resistant emetic toxin). Therefore, it has been decided not to develop a specific predictive tool for spore formation within the Bacillus-Predict project.

WP4: The developed and validated growth and growth boundary models for mesophilic and psychrotolerant *B. cereus* subgroups have been programmed into the FSSP software. However, a multiple growth model to provide simultaneous predictions for psychrotolerant and mesophilic/thermo-tolerant *B. cereus*, *L. monocytogenes* and *C. botulinum* has not been developed. The reasons being that ranges of applicability for these model ended-up being markedly different and because a model for mesophilic *C. botulinum* in dairy foods were delayed and not completed until spring of 2026. Thus, the FSSP software include models to predict growth/growth boundary for psychrotolerant and mesophilic *B. cereus*, *L. monocytogenes* and psychrotolerant and mesophilic *C. botulinum*. These models, however, cannot be used as a multiple growth model to provide simultaneous predictions.

11. Project results

WP1: Selection of psychrotolerant and mesophilic *B. cereus* isolates from dairy products and ingredients

Prevalence of *B. cereus* sensu lato in dairy products and ingredients were studied to select isolates for predictive modelling. 71 products were examined, yielding 51 isolates from 35 positive samples (49% prevalence). These isolates, along with 18 additional dairy and 12 reference strains, were characterized using MALDI-TOF, *panC* gene sequencing, toxin gene analysis, lactose fermentation, and growth rate (μ_{max}) determination under diverse conditions including 10°C, 45°C, 0.5% NaCl, 6% NaCl, pH 5.1 and pH 6.7. Dairy powders (83% prevalence), cheese whey (43% prevalence), and cheeses (42% prevalence) showed the highest *B. cereus* prevalences. The *panC* groups II, III, IV, VI, and VIII were identified and of the 69 dairy isolates, 97% carried genes for enterotoxin production and 42% fermented lactose. Exclusively, two dairy isolates included the *ces-gene* for production of the emetic toxin cereulide and these two isolates belonged to *panC* groups III (Table 1).

Table 1: Presence of toxin genes and the ability to ferment lactose for *B. cereus* isoalates from panC groups.

***panC* groups**	**No. of isolates**	**Emetic**	**Enterotoxins (%)**			**Cold shock**	**Lactose fermenter (%)**
		ces	*hbl*	*nhe*	*cytK*	*cspA*	
II	9	0	44	100	0	0	11
III	34	5.9	8.8	94	21	0	53
IV	20	0	80	100	100	0	45
VI	4	0	50	100	0	100	0
VIII	2	0	0	100	0	0	50
Total[a]	69	2.9	36.2	97.1	39.1	5.8	42.0
DSM strains[b]	12	16.7	41.7	100	50.0	16.7	16.7

a: Total dairy isolates.
b: DSM strains from *panC groups* II, III, IV, and VI

Significant variation in growth rate of isolates was observed both within and among *panC* groups. As expected, mesophilic isolates of *panC* group III and IV grew slower at 10°C, than psychrotolerant isolates (*panC* group II and VI) and the latter did not grow at 45°C (Maktabdar et al., 2024). Resistance to high salt (6% NaCl) and low pH (pH 5.1) also varied within and among *panC* groups but differences between mesophilic and psychrotolerant isolates was less pronounced than for temperature (Fig. 1). One mesophilic and one psychrotolerant cocktail of isolates were selected based on growth rates at the studied (extreme) conditions, *panC* group, toxin profiles, and lactose fermentation (Fig. 1; Maktabdar et al., 2024). These cocktails (Mix-Bc_{mes} and Mix-Bc_{psy}) were used for challenge tests with dairy foods and for development and validation of predictive growth and sporulation models in WP2 and 3.

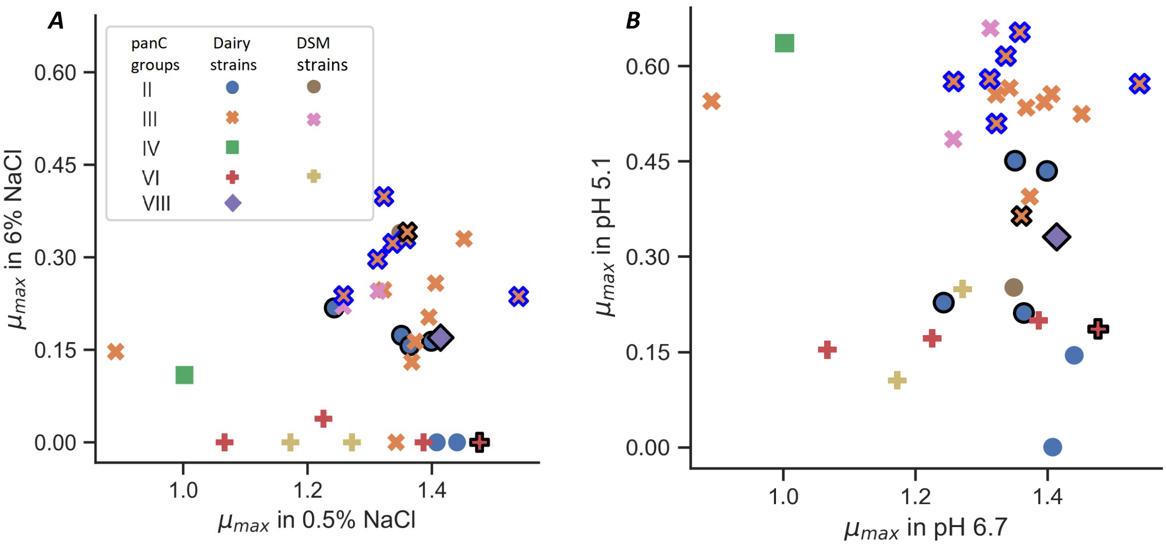

Figure 1. Maximum specific growth rates (μ_{max}, h^{-1}) at 25°C for 27 dairy and 5 DSM *B. cereus s.l.* isolates as divided into *panC* groups. A) pH 6.7 and with 0.5 % NaCl vs 6 % NaCl and B) with 0.5 % NaCl and pH 6.7 vs pH 5.1. The μ_{max}-values were determined over a period of up to 7 days. Strains selected for the mesophilic or psychrotolerant cocktails are shown with symbols having blue or black edges, respectively.

Table 2: Fitted cardinal parameter values and their standard errors estimated for a cocktail of mesophilic *B. cereus* (Mix-Bc$_{mes}$) using both BHI broth and UF permeate from whey (Maktabdar et al. 2025a).

Model parameters	**BHI data**		**UF permeate data**	
	Value	Optimum	Values	Optimum
$\mu_{ref,T}$ (h^{-1})	**2.99 ± 0.03**[a]	-	ND[b]	-
T_{min} (°C)	**7.06 ± 0.26**	-	ND	-
T_{opt} (°C)	**39.8 ± 0.18**	-	ND	-
T_{max} (°C)	**45.4 ± 0.04**	-	ND	-
$a_{w,\ min}$	0.960 ± 0.00	0.997 (fixed)	**0.955 ± 0.00**	**0.997 (fixed)**
pH_{min}	4.98 ± 0.02	6.96 ± 0.10	**4.75 ± 0.01**	**6.32 ± 0.11**
pH_{max}	9.5 (fixed)	-	**9.5 (fixed)**	-
Minimum inhibitory concentrations (*MIC*s) of undissociated acids (mM)				
Acetic acid (mM)	**15.6 ± 0.53**	-	6.80 ± 0.43	-
Benzoic acid (mM)	**0.36 ± 0.01**	-	0.19 ± 0.02	-
Lactic acid (mM)	2.34 ± 0.06[c]	≤0.61 ± 0.14[c]	**2.98 ± 0.058**[c]	**≤0.62 ± 0.15**[c]
Sorbic acid (mM)	**1.49 ± 0.04**	-	0.64 ± 0.01	-
Minimum inhibitory concentration (*MIC*) of total citric acid (mM)				
Citric acid (mM)	82.5 ± 1.0[c]	≤45 ± 2.2[c]	**169.1 ± 11.5**[c]	**0 (fixed)**[c]
Minimum inhibitory concentrations (*MIC*s) of phosphate ions (%)				
Orthophosphate	**4.96 ± 0.22**	-	ND	-
Diphosphate	**2.36 ± 0.07**	-	ND	-
Triphosphate	**2.32 ± 0.13**	-	ND	-

[a] Values indicated by bold were selected for the developed model.
[b] Not determined (ND).
[c] n1 = 1 and n2 =1 in Eq. (6) and Eq. (7) in Maktabdar et al. 2025a.

WP 2: Growth and growth boundary models for psychrotolerant and mesophilic *B. cereus*

Extensive growth models were developed to facilitate management of *B. cereus* in dairy foods and exclusively the two strain cocktails selected in WP1 were used to develop and validate models. Two cardinal parameter models, one for mesophilic (Mix-Bc_{mes}) and one for psychrotolerant (Mix-Bc_{psy}) strains, were developed to incorporated the effect of 11 environmental factors (temperature, pH, NaCl/a_w, acetic, benzoic, citric, lactic, and sorbic acids, as well as ortho-, di-, and tri-phosphates). Furthermore, the effect of interaction between these factors was included in the two models (Maktabdar et al 2025a, b). Growth rate data (μ_{max}-values) was generated using a standard liquid laboratory broth (BHI broth). Furthermore, these growth responses were evaluated in UF permeate to capture responses in a specific dairy solution. Most interestingly, both strain cocktails showed increased acid tolerance in UF permeate, with lower pH_{min} and higher lactic and citric acid minimum inhibitory concentration values (MICs) (Table 2). Figure 2 shows differences in growth responses between BHI broth and UF permeate for both of the studied mesophilic (Mix-Bc_{mes}) and psychrotolerant (Mix-Bc_{psy}) strains cocktails. To mathematically model these growth responses, a conservative approach was used for cardinal parameters values. This approach is illustrated in Table 2 for the mesophilic (Mix-Bc_{mes}) *B. cereus* strain cocktail. To include the widest growth range cardinal parameters values for NaCl/a_w, pH, lactic acid and citric acid were estimated from data obtained using the UF permeate solution whereas the remaining cardinal parameters values (temperature, acetic, benzoic, and sorbic acids, as well as ortho-, di-, and tri-phosphates) were estimated from data obtained using BHI broth. Furthermore, the model term for citric acid relied on the total concentration of this acid rather than concentrations of undissociated acids as typically used and as included in terms for other organic acids studied (Table 2; Maktabdar et al. 2025a, b).

The developed extensive growth and growth boundary models were both evaluated for their ability to predict growth responses in various dairy products for mesophilic and psychrotolerant *B. cereus*. These validation studies included 133 new challenge tests and 248 literature growth responses in dairy products. The mesophilic model had a good performance, showing accurate and correct predictions for new challenge tests and literature data. The psychrotolerant model also showed accurate and correct predictions for new challenge tests. However, to obtain an acceptable performance for literature growth responses in dairy products at low temperatures (≤ 10°C) the model temperature term had to be expanded, which markedly improved the performance of this model (Maktabdar et al. 2025b). Compared to existing models, the two new models developed and validated within the present study offer broader applicability by incorporating more environmental factors as well as the effect of interaction between factors, resulting in unbiased predictions. These validated models can be valuable for risk assessment, development or re-formulation of product recipes to manage growth of *B. cereus* in dairy foods.

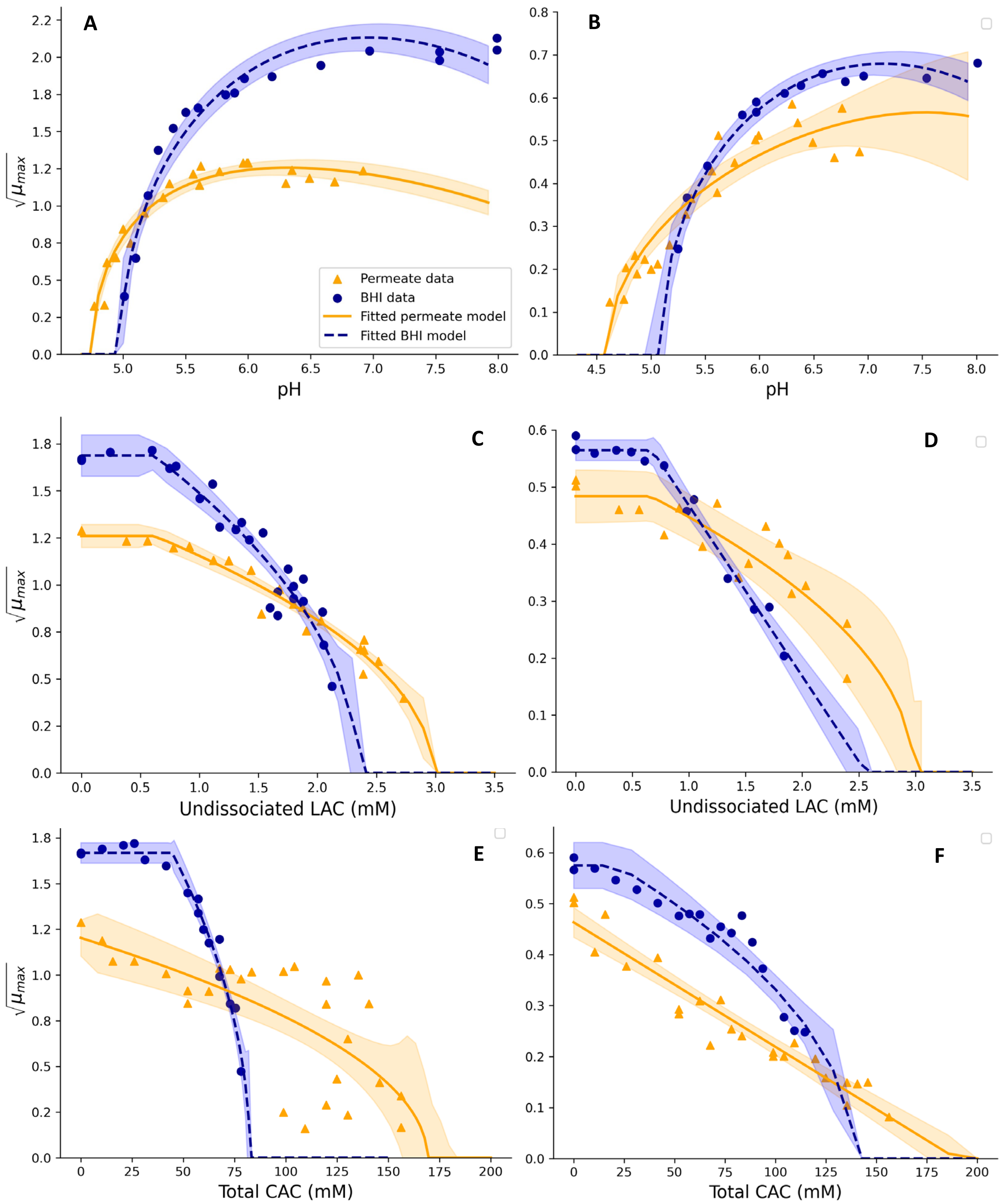

Figure 2. Effect of pH (A, B), undissociated lactic acid (C, D) and total citric acid (E, F) on square root transformed maximum specific growth rates (μ_{max}, $h^{-0.5}$) of mesophilic (A, C, and E) and psychrotolerant (B, D, and F) *B. cereus* in BHI broth and UF permeate solution. Fitted model terms for BHI (dashed blue lines) and UF permeate (solid yellow lines) were obtained by fitting equations from Maktabdar et al. (2025a) to the observed μ_{max} values in BHI (blue dots) and UF permeate (yellow triangles). 95% confidence intervals of the fits are shown in blue and yellow shades.

WP 3: Models to predict spore formation by *Bacillus cereus*.

B. cereus spore formation was studied for a total of 29 kinetics of mesophilic (Mix-Bc$_{mes}$) and psychrotolerant (Mix-Bc$_{psy}$) strains cocktails in UF whey permeate, whey protein concentrate, milk and oat drink (Maktabdar et al., 2026). An available primary spore formation model was expanded to link growth, as predicted by the growth and growth boundary models developed in WP 2, and sporulation. In this way, time to first spore formed (t_{1S}), t_{delay} and spore formation rate (μ_S) was predicted from the growth models. In contrast, the maximum spore concentration (S_{max}) was matric dependent and S_{max} had to be estimated for each matrix studied (Fig. 3). Prediction of *B. cereus* spore formation is possible; however, spores are formed by high cell concentrations and these should not be reached in dairy foods or ingredients e.g. due to the risk of cereulide formation (heat resistant emetic toxin). Therefore, it has been decided not to develop a specific predictive tool for spore formation within the Bacillus-Predict project.

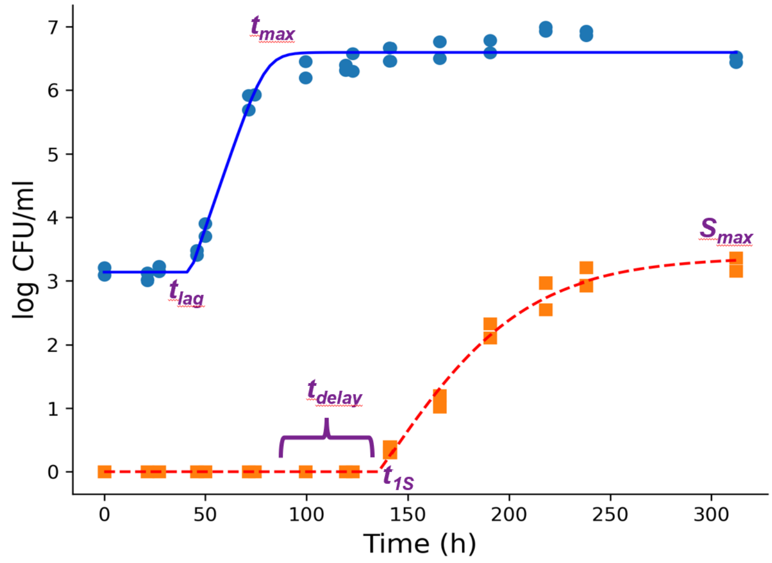

Figure 3. Growth and sporulation of mesophilic *B. cereus* at 15 °C and pH 5.5. Blue dots represent the total concentration of mesophilic *B. cereus*, orange squares indicate concentration of mesophilic *B. cereus* spores, blue line and orange dashed line indicate the fitted growth and sporulation model.

WP 4: Tool to predict *B. cereus* growth

The developed and validated extensive growth and growth boundary models for mesophilic and psychrotolerant *B. cereus* were programmed in MS Excel and distributed to the industrial project participants for use and commenting. These models were then included in the Food Spoilage and Safety Predictor (FSSP) software (fssp.food.dtu.dk). The FSSP software includes guidance for users of the new models e.g. boundary conditions that prevent the models being used outside their range-of-applicability (within which the models have been successfully validated) with respect to food matrix and ranges of environmental factors. The FSSP software has been developed to predict growth and growth boundary of both mesophilic and psychrotolerant *B. cereus* from a specific set of environmental conditions. It is recommended to use these models with relative lag times (RLT) of respectively 4.2 for the psychrotolerant subgroup and 6.1 for mesophilic sub-group but models can also be used without lag time.

As example, the new models included in the FSSP software predict that mesophilic, but not psychrotolerant, *B. cereus* can grow in processed cheese at 25°C, pH 5.7, 2.50% ortho-phosphate melting salt in water phase and water phase concentrations of acetic, citric and lactic acids of 1500 ppm, 5500 ppm and 14700 ppm (Fig. 4). More specifically it is predicted that mesophilic *B. cereus* grows from 10 cfu/g to 1000 cfu/g in 39 days and from 10 cfu/g to 100.000 cfu/g in 59 days (Fig. 4). If, for this product, the water phase citric acid concentration is increased from 5500 ppm to 7500 ppm and pH is reduced from 5.7 to 5.6 - the models then predict no growth of both mesophilic (psi-value of 2.0) and psychrotolerant (psi-value > 10) *B. cereus* (Fig. 4).

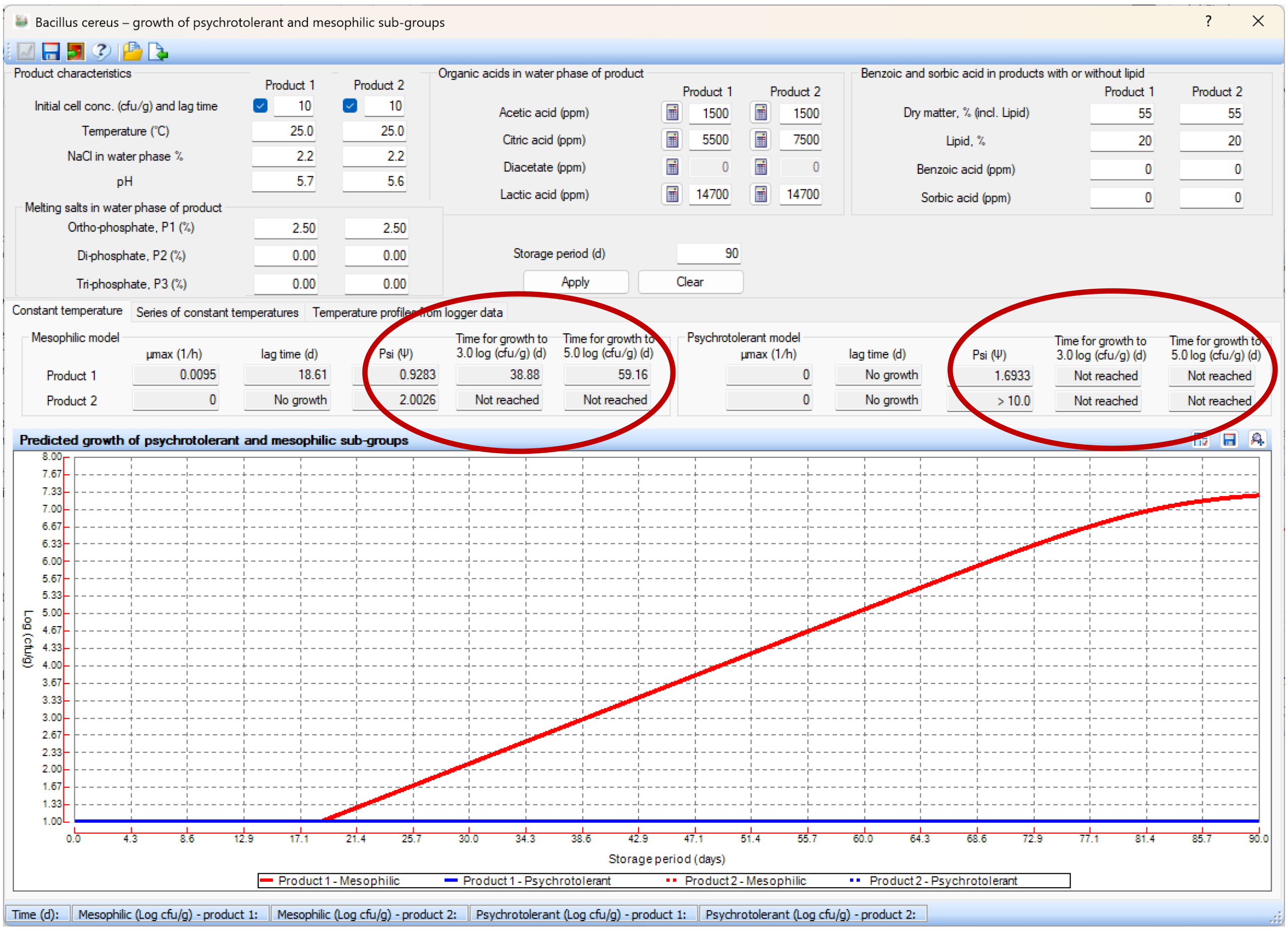

Figure 4. Example showing how the new growth and growth boundary models for mesophilic and psychrotolerant *B. cereus* can be used within the FSSP software to evaluate growth and identify conditions that prevent growth in dairy foods.

The FSSP software is available for free and the new growth and growth boundary models for mesophilic and psychrotolerant *B. cereus* can be used by the entire dairy sector to evaluate growth and to identify conditions that prevent growth for a wide range of dairy foods.

12. The relevance of the results, including relevance for the dairy industry

Scientific Relevance:

The new predictive growth and growth boundary models go beyond existing tools by incorporating a greater number of environmental factors relevant to dairy products. This allows for more accurate predictions of *B. cereus* growth behaviors. Moreover, the new sporulation models contribute new insights into how environmental factors influence spore formation in dairy products and dairy ingredients.

Societal Relevance:

The developed models and predictive tool serve as valuable educational resources for food safety training as offered e.g. by DTU Food. Moreover, these tools can assist food safety authorities in establishing guidelines for product formulation and storage temperatures, improving food safety.

Industrial Relevance:

The new models and predictive tool provide a practical resource for the dairy industry, enabling optimized product formulations and processing conditions that effectively control *B. cereus* growth and prevent reaching critical *B. cereus* concentrations. This directly supports product safety and innovation.

13. Communication and knowledge sharing about the project

Papers in international journals:

Maktabdar, M., Hansen, L-T., Wemmenhove, E., Gkogka, E., Dalgaard, P. (2024). Prevalence, characteristics, and selection of *Bacillus cereus* subgroups from dairy products for challenge testing and predictive model development. J. Food. Prot. 87, 100367. https://doi.org/10.1016/j.jfp.2024.100367

Maktabdar, M., Wemmenhove, E., Gkogka, E., Dalgaard, P. (2025a). Development of extensive growth and growth boundary models for mesophilic and psychrotolerant *Bacillus cereus* in dairy products (Part 1). Frontiers in Microbiology, Section on Food Microbiology, Volume 16: 1553885. https://doi.org/10.3389/fmicb.2025.1553885

Maktabdar, M., Houmann, R.H., Scheel, N.H., Skytthe, K.B., Wemmenhove, E., Gkogka, E., Dalgaard, P. (2025b). Evaluation and validation of extensive growth and growth boundary models for mesophilic and psychrotolerant *Bacillus cereus* in dairy products (Part 2). Frontiers in Microbiology, Section on Food Microbiology, Volume 16: 1553903. https://doi.org/10.3389/fmicb.2025.1553903

Maktabdar, M., Houmann, R.H., Sina, M.R.H., Jensen, F.K., Gkogka, E., Wemmenhove, E., Dalgaard, P. (2026). Kinetics and modelling of spore formation by *Bacillus cereus* subgroups in dairy matrices. Manuscript in preparation to be submitted to Journal of Food Protection.

Easily read papers:

Dalgaard, P., Maktabdar, M. (2022). Mejeri-Prædiktionsværktøj (Bacillus-Predict). Mælkeritidende, No. 7, side 1-2. https://maelkeritidende.dk/sites/default/files/udgivelser/Forskningsartikler/4983557_mt_07_2022_forskning.pdf

Dalgaard, P., Maktabdar, M. (2026). Nyt mejeri-prædiktionsværktøj for *Bacillus cereus*. Manuscript in preparation to be published in Mælkeritidende.

Student theses:

B.Sc. thesis at DTU Food: Johansen, N., Gadekær, S. *Bacillus cereus* – vækst og overlevelse i udvalgte mejeriprodukter, September 2022-January 2023.

M.Sc. Thesis at DTU Food: Sina, M. R. H. Growth and spore formation of *Bacillus cereus* in dairy products, July 2023.

B.Sc. thesis at DTU Food: Jensen, F.K. Growth and spore formation by *Bacillus cereus* - Effect of selected environmental conditions, December 2023.

PhD thesis at DTU Food. Maktabdar, M. Predictive food microbiology tools for *Bacillus cereus* in dairy products, DTU National Food Institute, January 2025.

Oral presentations at scientific conferences, symposiums etc.:

Maktabdar (2023). Presentation at Dairy Research Day 2023. Prediction of *Bacillus cereus* growth in dairy products, 16 March 2023, Billund, Denmark.

Abstract and oral presentation at 12th International Conference on Predictive Modelling in Food, June 13-16, 2023 Sapporo, Japan. Maktabdar, M., Hansen, L.T., Wemmenhove, E., Gkogka, E., Dalgaard, P. *Bacillus cereus* in dairy products: Prevalence and development of extensive growth and growth boundary models for mesophilic and psychrotolerant sub-groups. Prize obtained for this oral presentation by M. Maktabdar.

Dalgaard., P. (2023). Predictive food microbiology models and software – tools to facilitate safety evaluation of new products. Invited presentation at "New products from idea to supply chain". Danmarks Mejeritekniske Selskab, 28 September 2023, Billund, Denmark.

Abstract and oral presentation for European Symposium of International Association for Food Protection, 30 April – 2 May 2024, Geneva, Switzerland. Maktabdar, M., Hansen, L.T., Wemmenhove, E., Gkogka, E., Dalgaard, P. Extensive Models Predicting Growth and Growth Boundaries of Bacillus cereus in Dairy Products. Prize obtained for this oral presentation by M. Maktabdar.

Abstract and oral presentation for European Symposium of International Association for Food Protection, 6-8 May 2025, Madrid, Spain. Dalgaard, P., Maktabdar, M., Koukou, I., Martnez-Rios, V. Food Spoilage and Safety Predictor Software (FSSP v. 5 from 2025) – Approach and New Developments.

Oral presentations at meetings:

Maktabdar, M. and Dalgaard P., 2022. Predictive tool for dairy products (Bacillus-Predict). Presentation at DDRF Steering group meeting on 12 May 2022.

Maktabdar, M. and Dalgaard P., 2022. Predictive tool for dairy products (Bacillus-Predict). Presentation at DDRF Steering group meeting on 23 October 2023.

Dalgaard P., 2025. Predictive tool for dairy products (Bacillus-Predict) – Final Reporting. Presentation at DDRF Steering group meeting on 23 April 2025.

Other:

Dalgaard., P. (2025). Hvor er vi i forhold til at kunne forudsige mikrobiel vækst?. Inviteret foredrag inden for sessionen ”Forudsigelse af mikrobiel vækst” ved Mejeribrugets dag 2025 ”Fra forskning til praktisk anvendelse”, 12. marts 2025, Herning, Danmark. https://mejerimedier.dk/nyhed/mejeribrugets-dag-samler-branchen-om-den-nyeste-viden/

14. Contribution to master and PhD education

One PhD student, Maryam Maktabdar (see section 13, student theses).

One 35 ECTS MSc-project and three 20 ECTS BSc-project (see section 13, student theses).

15. New contacts/projects

Results from the Bacillus-Predict project have contributed to development of the HotBacillus project supported by DDRF. “HotBacillus. Models to predict growth and toxin production of Bacillus in dairy processes running at temperatures above 40 °C”. HotBacillus is a research project to be conducted during 2027-2029 by DTU Food, Arla Food Ingredient and Arla Foods Amba.

16. References

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Carroll, L.M., Wiedmann, M., Mukherjee, M., Nicholas, D.C., Mingle, L.A., Dumas, N.B., Cole, J.A., Kovac, J., 2019. Characterization of emetic and diarrheal *Bacillus cereus* strains from a 2016 foodborne outbreak using whole-genome sequencing: addressing the microbiological, epidemiological and bioinformatics challenges. Frontiers in Microbiology 10, 144.

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Gauvry, E., Mathot, A.-G., Couvert, O., Leguérinel, I., Coroller, L., 2021. Effects of temperatura, pH and water activity on the growth and the sporulation abilities of *Bacillus subtilis* BSB1. International Journal of Food Microbiology 337, 108915.

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