# Quantifying the growth variability of pathogenic and spoilage sporeforming microorganisms of interest for chilled dairy foods using meta-analysis techniques

*Elissavet Gkogka[1*]; Yifan Zhang[1,2]; Tjakko Abee[2], Yinghua Xiao[1]*
*[1]Arla Strategic Innovation Centre, Aarhus, Denmark; [2]Wageningen University, Wageningen, The Netherlands*
*Corresponding author: elgko@arlafoods.com

## Introduction

To ensure the safety and quality of heat-treated, chilled products with a long shelf life, it is important to control pathogenic and spoilage sporeforming microorganisms that may grow at refrigeration temperatures, among which *Bacillus cereus* and spoilage *Bacillus* spp. are of particular concern for the industry [1]. Meta-analysis techniques can be a strong tool for evaluating the growth potential of these microorganisms under chilled conditions and for identifying control strategies.

## Aim

The aim of this study was to perform a meta-analysis of growth rates of spoilage and pathogenic psychrotrophic *Bacillus* spp. found in a publicly available predictive modelling database and to use this information for predicting their growth in chilled dairy products.

## Materials and Methods

ComBase [2] was screened for information on growth of *B. cereus*, *Bacillus subtilis*, *Bacillus licheniformis* and other spoilage *Bacillus* spp. in dairy matrices (cheese, milk, other dairy) and culture media at temperatures relevant for refrigerated storage including product abuse by the consumer (0-15 ℃).
The simple square root model of Ratkowsky [3] was fitted to filtered datasets when including a sufficient number of points spread over the selected temperature range, using the Solver add-in of Microsoft® Excel 2010 after a $\log_{10}$-transformation of the data to improve the stabilisation of the variance over the entire temperature range [4]. This allowed for the estimation of the $b$ and $T_{min}$ parameters describing the mean exponential growth rate (EGR, $\log_{10}$/ h) as a function of temperature.

## Results

Only *B. cereus* had a sufficient number of datasets over the selected temperature range to allow a meta-analysis of collected growth rates (Figure 1). The estimated $b$ and $T_{min}$ parameters of the model describing the mean exponential growth rate of all data as a function of temperature were 0.024 and 1.4°C respectively.
In practice, based on our mean estimations, an increase from the recommended chilled storage threshold of 5 ℃ [5] to 8 ℃ which is a common fridge temperature for many consumer households worldwide [6] results in a more than 200% increase of the EGR for *B. cereus* which in turn means a shelf-life shortened by about two thirds for the same product assuming an initial contamination of 1 CFU/ g and a qualitative limit of 1000 CFU/ g [7] (Figure 2).

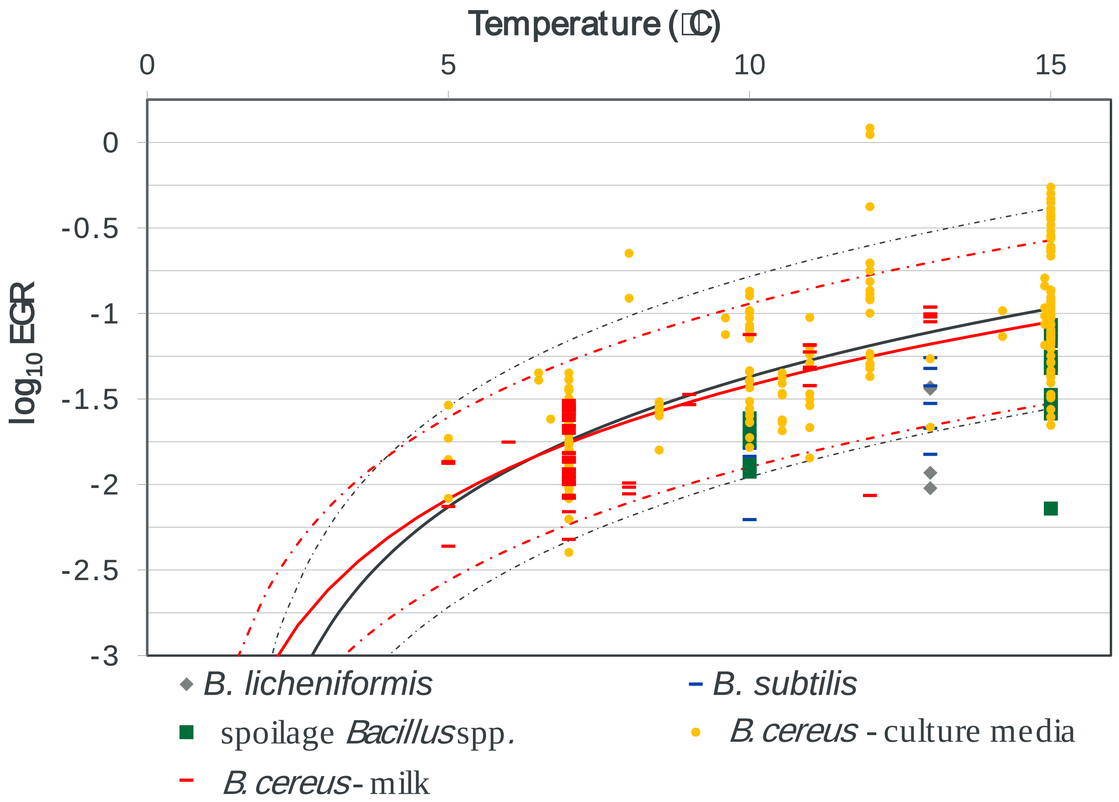


**Figure 1.** Growth kinetics of different *Bacillus* spp. in laboratory media and dairy matrices. Solid lines represent the fit of *B. cereus* growth rate models and dotted lines the 95% prediction levels for milk (red) and all matrices (black).

For a more abusive fridge temperature like 10 ℃ which is also not likely to be uncommon in some countries [6], shelf life would have been reduced to less than one fifth of that at 5 ℃.
Shelf life predictions with mean estimates were found to be in between predictions made with other models for milk [8] and predictive modelling software [9]. Shelf life predictions based on the lower 95% confidence interval are fail-safe (Figure 2).

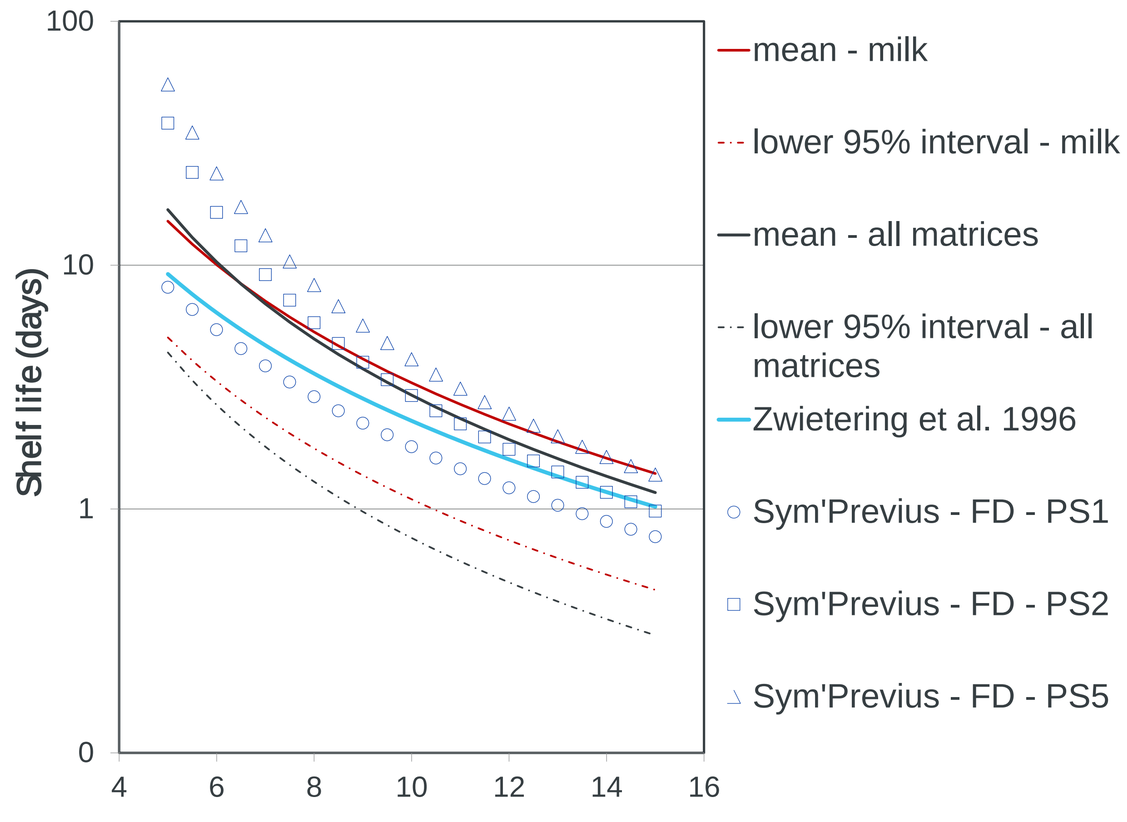


**Figure 2.** Shelf life at different refrigeration temperatures relevant for storage at consumer households. Results on the y-axis are presented on a logarithmic scale. Solid lines represent the mean *B. cereus* growth predictions and dotted lines the 95% prediction levels for milk (red) and all matrices (black) based on meta-analysis. Results are compared with other models for milk [8] and different psychrotrophic strains (PS) in fresh dairy (FD) with predictive modelling software [9].

## Conclusions

- *Bacillus* spp. were found to grow over a wide range of pH, water activity and temperatures relevant for the storage of chilled dairy products.
- The quality of extracted data varied depending on the species of microorganism and matrix with the best dataset obtained for *B. cereus* in milk.
- The availability and quality of data for spoilage sporeformers was relatively limited. This agrees with observations of other authors regarding the limited information on growth characteristics of sporeformers other than the *B. cereus* group [1].
- Quantifying the variability of growth rates as a function of temperature can allow for fail-safe shelf life estimations for chilled products.
- The meta-analysis also helped to identify important areas where limited information is available in ComBase for controlling psychrotrophic spore-formers in this specific product category. More specifically, we recommend addition of information on:
  - dairy matrices other than milk
  - dairy matrices and laboratory media containing antimicrobials
  - growth at recommended refrigeration temperatures (<5 ℃).

**References**
1. Samapundo et al. (2014). Food Microbiology, **44**: p. 288-295.
2. ComBase (2015). Available from: http://www.combase.cc/
3. Ratkowsky et al. (1982). Journal of Bacteriology, 1982. **149**(1): p. 1-5.
4. den Besten and Zwietering (2012). Trends in Food Science and Technology, **25**(1): p. 34-39.
5. WHO, *Five keys to safer food manual*, 2006, World Health Organization: Geneva.
6. James et al. (2008). Journal of Food Engineering, 2008. **87**: p. 2-10.
7. Bolton et al. (2009). *Guidelines for assessing the microbiological safety of ready-to-eat foods*, Health Protection Agency: London.
8. Zwietering et al. (1996). International Journal of Food Microbiology, **30** (1-2),p.55-70
9. Sym'Previus (2015). Available from: http://www.symprevius.net/