Growth Potential of *Bacillus cereus* Group Strains from Different Phylogenetic Groups in a Dairy Food Model

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Background and Objectives

The *Bacillus cereus* group are Gram-Positive, closely related spore-forming bacteria. Some members of this group are cytotoxic and cause foodborne illness, while others are used as bioinsecticides or probiotics in the agricultural industry[1].

The range of growth temperatures of the *B. cereus* group varies between *panC* phylogenetic clades[2].

Understanding the differences in the growth capabilities of cytotoxic *B. cereus* group strains belonging to different phylogenetic clades is needed to inform the development of exposure and risk assessment models in milk[3].

Objectives:
(1) **Evaluate the growth in skim milk broth (SMB) of spores** from 17 cytotoxic ***B. cereus* group isolates** with unique virulence gene profiles and *panC* phylogenetic clades.
(2) **Compare growth data from room temperature** (22°C), **exceeded refrigeration temperature** (10°C), and **refrigeration temperature** (4°C) to evaluate differences between clades in their **maximum population size**, **doubling time**, and the **maximum growth rate.**

Materials and Methods

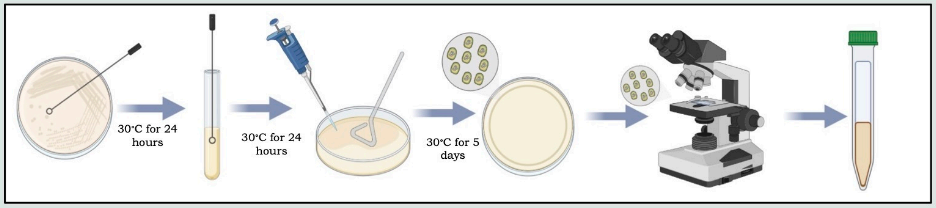

Figure 1: Isolates were streaked onto brain heart infusion agar (BHIA), incubated, and inoculated into BHI broth. Sporulation was induced using AK #2 agar for 5 days. Phase contrast microscopy was used to confirm the presence of spores. Spores were harvested and suspended in sterile deionized water. Suspensions were quantified in CFU/ml.

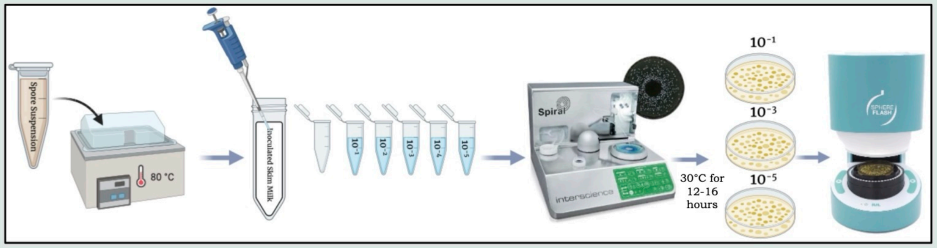

Figure 2: Spore suspensions were heat-treated at 80°C to promote germination and kill remaining vegetative cells. Spore suspensions were inoculated into sterile SMB at **~10^3 spores/ml.** The skim milk broth was sampled at the set time points based on the temperature. Dilutions were spiral plated onto BHIA and counted using the Neutec SphereFlash colony scanner.

Conclusions

(1) At both 22°C and 10°C, the growth of *B. cereus* species exhibited significant differences among phylogenetic groups.

(2) Tested *B. cereus* isolates did not grow at 4°C.

(3) Most isolates in this study grew **above 10^5 CFU/ml** at exceeded refrigeration temperature (10°C), which has previously been **associated with foodborne disease**[4].

Significance

Accurately predicting the growth of *B. cereus* species in milk is imperative to help mitigate the risk of foodborne illnesses and protect public health

Future Directions

(1) Use growth data to build an exposure assessment model for milk

(2) Test more isolates representing individual phylogenetic groups to improve the accuracy of the exposure assessment model

(3) Evaluate growth in another food model

References

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(3) Martin, N. H. et al. (2012). *J Dairy Sci* 2012, *95* (12), 7384–7390.
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Results

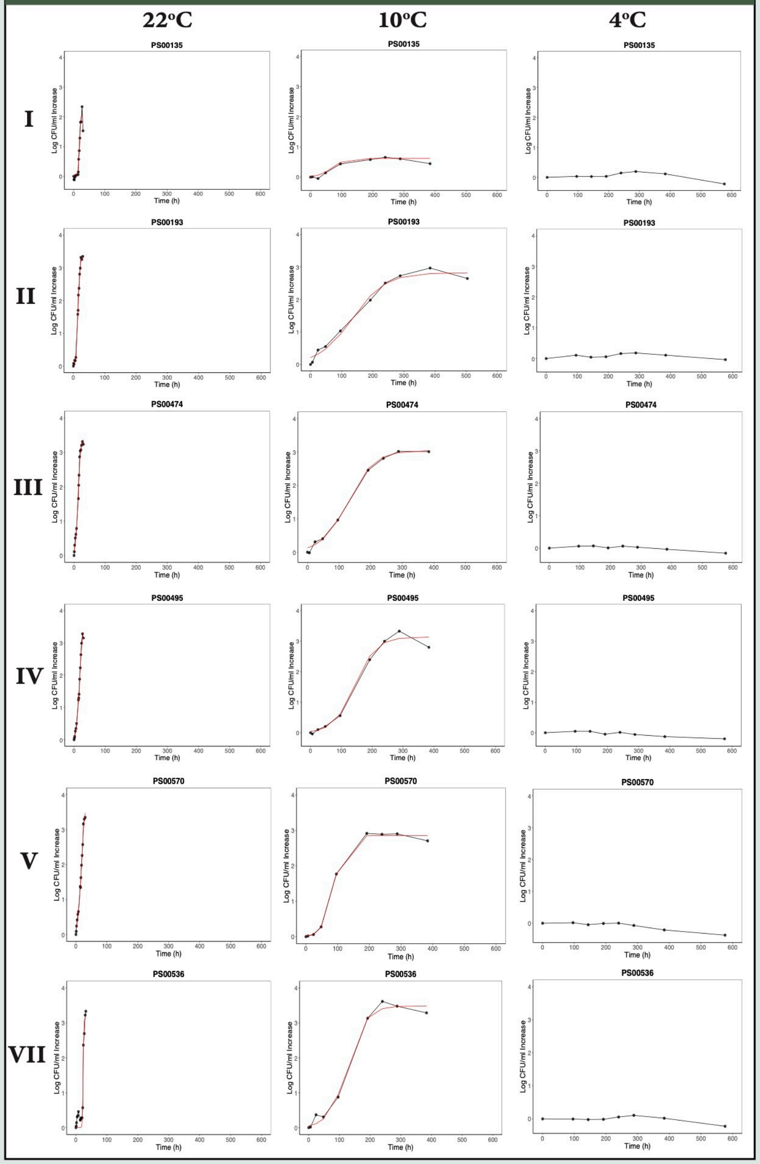

Figure 3: Growth of *B. cereus* species from different clades (I, II, III, IV, V, VII) in SMB at 22°C,10°C, and 4°C. The Growthcurver package in R was used to collect growth metrics. There was a **significant difference** (α=0.05) in the estimated **maximum population size** (p=0.0058), **doubling time** (p<0.001), and the **maximum growth rate** (p<0.001) between isolates grown at 10°C compared to 22°C. Isolates from *panC* clade I grew significantly less (α=0.05) at 22°C (p=0.00139) and 10°C (p=$7.76*10^{-7}$) compared to all other clades.

This work was supported by USDA NIFA Hatch Appropriations under project no. PEN04646 and accession no. 1015787, and the USDA NIFA GRANT 12686965.