Cytotoxicity Assessment of Psychrotolerant *Bacillus cereus* Isolates Across Varied Temperatures

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Background and Objectives

The *Bacillus cereus* group encompasses eight genomospecies such as *B. cereus sensu stricto (s.s.), B. anthracis,* and *B. thuringiensis*. It also includes members capable of secreting **cytotoxic enterotoxins at body temperature (37°C)**[1].

However, it is uncertain how growth **temperature affects the ability to express toxins and cause cytotoxicity** among strains that encode enterotoxin genes[2].

In some psychrotolerant strains of *B. cereus* belonging to **phylogenetic groups II and VI, high cytotoxicity** was detected at **15°C and 32 °C but not at 37°C**[2,3,4].

Objective 1

Assess differences in cytotoxicity among 69 **psychrotolerant** *B. cereus* isolates from groups II and VI when **grown at 32°C and 37°C** through ***in vitro* cytotoxicity assays**.

Objective 2

Evaluate changes in **enterotoxin gene transcription** among a subset of **psychrotolerant** *B. cereus* isolates from groups II and VI when grown at **32°C and 37°C using RT-qPCR**.

Materials and Methods

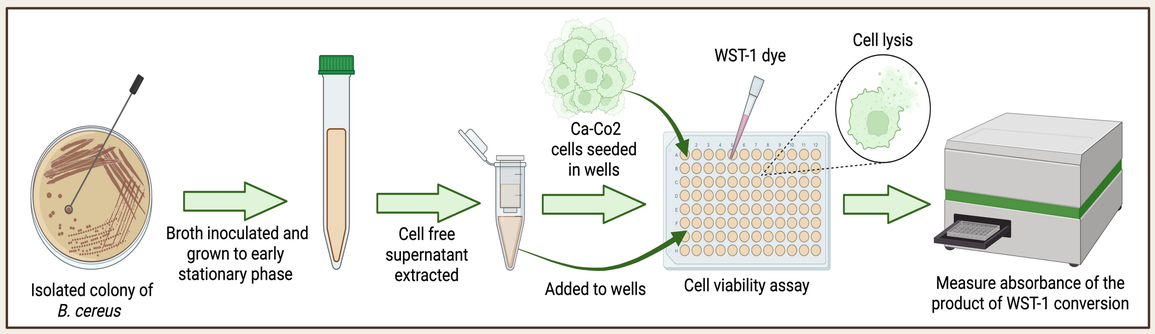

Figure 1: Isolates were grown in brain heart infusion (BHI) broth to early stationary phase **at 32°C and 37°C** to collect cell-free supernatants. Cytotoxicity was assessed using the **WST-1 assay using Caco-2 cells.** Caco-2 cells were exposed to 15% v/v of supernatants for 15 minutes before adding the WST-1 dye. **The absorbance data was min-max normalized to BHI and *B. cereus* ATCC 14579 to determine cytotoxicity.**

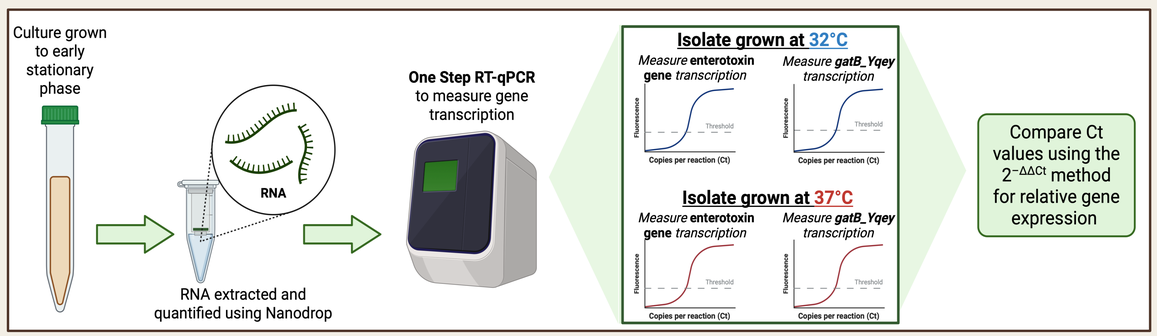

Figure 2: 13 isolates that had either a **5x change in cytotoxicity, or were cytotoxic at 32°C, but not 37°C** were selected and grown until early stationary phase in brain heart infusion broth (BHIB) at 32°C and 37°C. RNA was extracted and transcripts of ***hblC, nheA, cytK, sph, plc,* and *gatB_Yqey*** were detected using a **One-Step RT-qPCR** Kit. The **$2^{-\Delta\Delta Ct}$ method** was used to compare cycle threshold (Ct) values.

Results

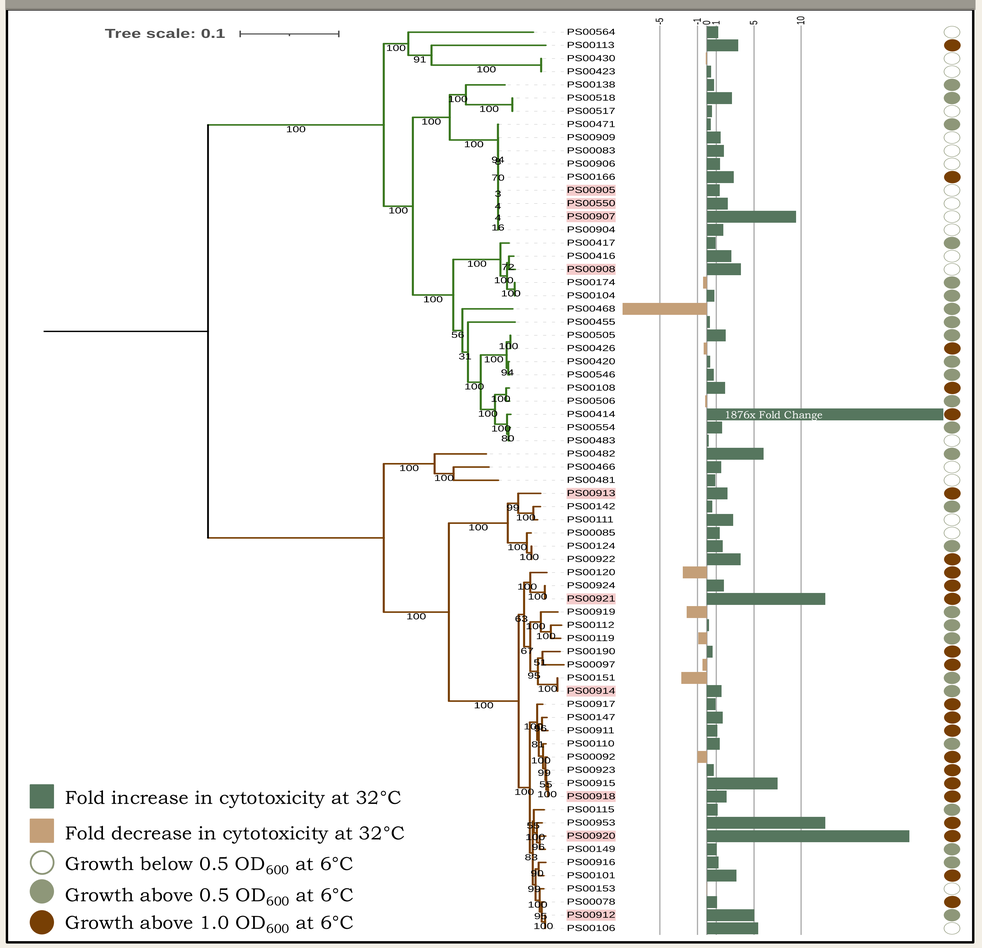

Figure 3: Phylogenetic tree of isolates from group II (green branches) and VI (brown branches), plotted with the fold change in cytotoxicity at 32°C compared to 37°C. **Isolates highlighted in red were cytotoxic at 32°C only. Growth at 6°C was evaluated after 30 days.** The CFSAN SNP pipeline and RAxML in GalaxyTrakr were used to build the phylogenetic tree and annotated using the Interactive Tree of Life (iTOL).

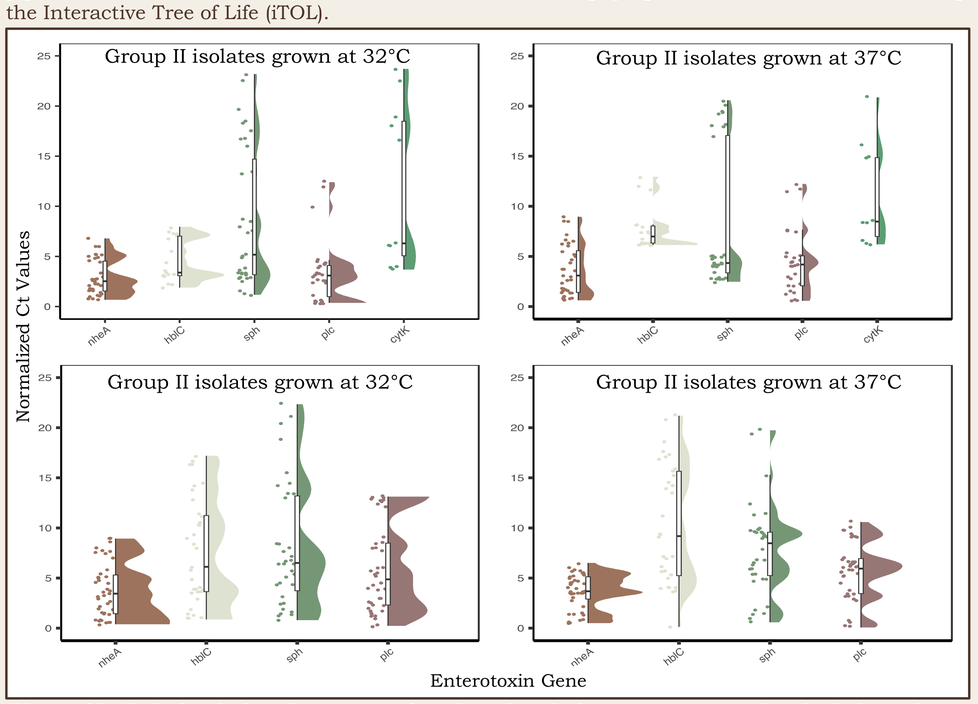

Figure 3B: Raincloud plots for average Ct values for virulence gene transcripts in isolates from groups II and VI grown at 32°C and 37°C, **normalized** to the housekeeping gene ***gatB_Yqey***. On average, there was a **significant difference ($p<0.001$)** between the normalized Ct values just **for *hblC* transcripts** for isolates from group II grown at 32°C compared to 37°C.

Conclusions

1. No isolates from groups II and VI were cytotoxic at 37°C, but 10/69 isolates were cytotoxic at 32°C.
2. On average, isolates from group II had a 7.82-fold increase in *hblC* transcripts when grown at 32°C compared to 37°C.
3. These findings illustrate a need to investigate if psychrotolerant isolates can secrete cytotoxic enterotoxins at refrigeration temperatures.

Significance

Evaluating how temperature affects transcription of virulence genes and cytotoxicity in *B. cereus* isolates can improve strain-specific predictions of virulence potential

Future Directions

(1) Evaluate the cytotoxic potential of psychrotolerant *B. cereus* isolates at 6°C.

(2) Investigate how exposure to pH, thermal treatment, and proteases affects the cytotoxic activity of secreted virulence factors.

References

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