Effects of Protein Stress Factors on the Cytotoxicity of Psychrotolerant *Bacillus cereus* isolates

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Background and Objectives

The *Bacillus cereus* group is a diverse group of spore-forming bacteria responsible for an **estimated 63,000 annual cases of foodborne illness** in the U.S. with illness cases commonly linked to foods such as cooked rice, pasta, and milk[1].

B. cereus diarrheal illness is caused by **protein enterotoxins** secreted in the intestinal tract. Some **psychrotolerant** strains of *B. cereus* from phylogenetic groups II (*B. mosaicus*) and VI (*B. mycoides*) **can produce cytotoxic enterotoxins in food**, potentially leading to intoxication.

Thermal treatment[2], exposure to **low pH[3]**, and presence of **proteolytic enzymes[4]** have been shown to **reduce the immunological activity** of toxins, though some monomers may retain their activity. However, it remains unclear whether these alterations in immunological activity correspond to **changes in the biological activity** of cytotoxic enterotoxins.

Objective 1

Measure the impact of protein stress factors on the cytotoxicity of 7 psychrotolerant *B. cereus* isolates' supernatants through ***in vitro* cytotoxicity assays.**

Objective 2

Assess the impact of serial dilution on the cytotoxicity of 7 psychrotolerant *B. cereus* isolates' supernatants to examine concentration-dependent cytotoxicity through ***in vitro* cytotoxicity assays.**

Materials and Methods

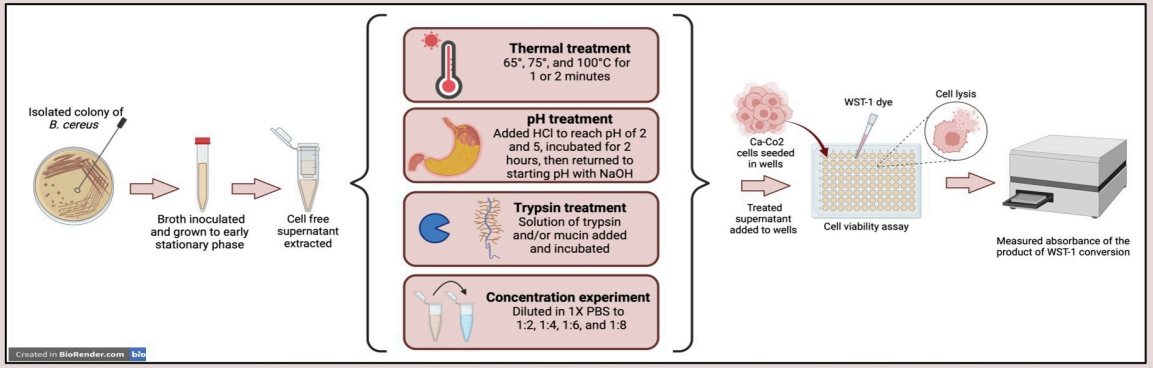

Figure 1: Isolates were grown in brain heart infusion (BHI) broth at 32°C until reaching early stationary phase, before the collection of supernatants. Supernatants were either thermally treated, exposed to low pH, or exposed to trypsin (10 µg/µl) and mucin (0.25%) solutions. Dilutions of supernatant with 1X PBS were also performed. 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.

Results

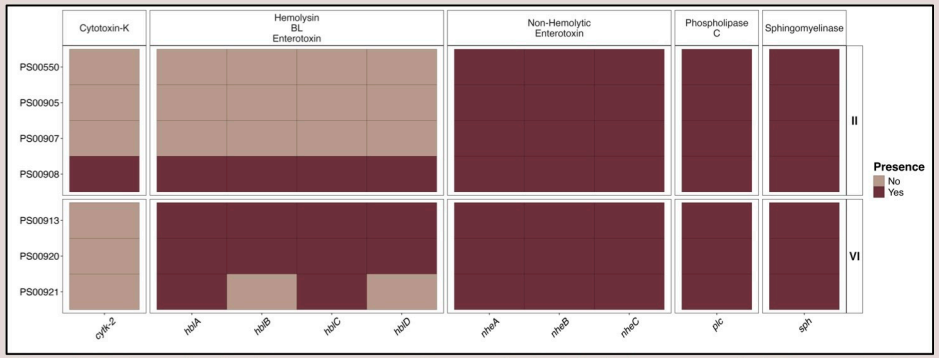

Figure 2: Heatmap showing the presence and absence of selected virulence genes in 7 *B. cereus* isolates. Virulence gene identification and *panC* phylogenetic group assignment were performed using BTyper3 (v3.4.0) using previously sequenced genomes. All isolates, regardless of clade, carried genes for the non-hemolytic enterotoxin (3/3), phospholipase C (1/1), and sphingomyelinase (1/1). Only one clade II isolate had all genes for hemolysin BL and cytotoxin-K, while clade VI isolates lacked *cytk-2*. Two clade VI isolates carried all hemolysin BL genes, but *hblB* and *hblD* were not detected in one isolate.

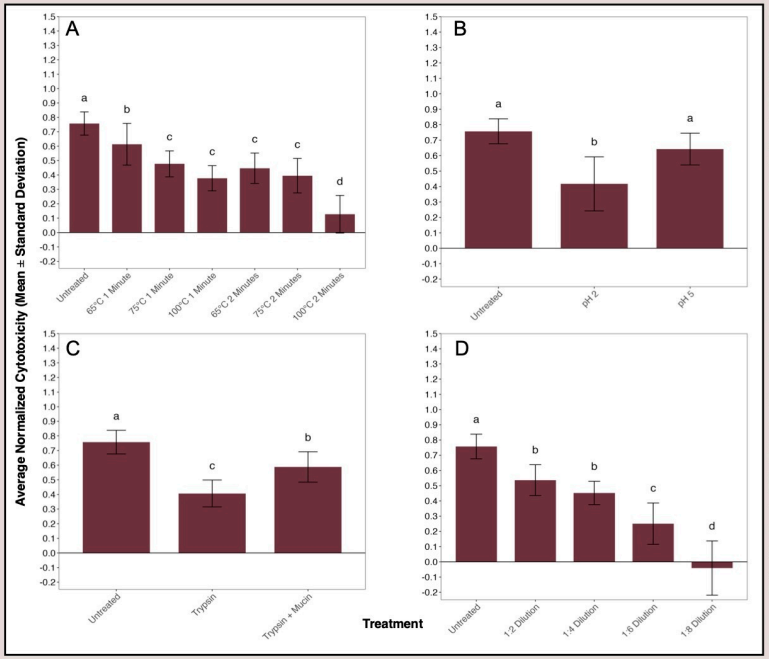

Figure 3: Bar charts showing the average ± the standard deviation of cytotoxicity values for all selected isolates with different letters indicating significant difference, as determined using ANOVA and Tukey tests. A: thermal treatment, B: pH treatment, C: Trypsin treatment, D: Dilutions.

On average, all thermally treated supernatants exhibited significantly reduced cytotoxicity compared to the untreated supernatant ($p<0.001$). The treatment at 65°C for 1 minute resulted in the lowest reduction and 100°C for 2 minutes resulted in the highest reduction in cytotoxicity. While pH 2 significantly impaired cytotoxicity, pH 5 did not ($p<0.001$). Furthermore, treatment with trypsin significantly reduced cytotoxicity and the addition of mucin with trypsin significantly increased cytotoxicity compared to just trypsin ($p<0.001$). Lastly, cytotoxicity of untreated supernatants was confirmed to be concentration- dependent.

Significance

Assessing the impact of protein stress factors on the cytotoxicity of secreted virulence factors in *B. cereus* isolates can improve predictions of food safety risks if enterotoxins are produced in food.

Conclusions

1. High temperature, low pH, and trypsin significantly reduced the cytotoxicity ($p<0.001$) of secreted virulence factors, potentially reducing food safety risks.
2. Cytotoxicity of untreated supernatants was concentration-dependent.
3. Further research is needed to investigate the stability of secreted enterotoxins in food matrixes and under simulated gastrointestinal conditions.

Future Directions

Assess the cytotoxic potential of secreted virulence factors in **food matrices** following treatments with protein stress factors.

Assess the cytotoxic potential of secreted virulence factors after exposure to **simulated gastrointestinal conditions.**

References

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