Effects of Protein Stress Factors on the Cytotoxicity of Supernatants from Psychrotolerant *Bacillus cereus* Isolates

PennState College of Agricultural Sciences

Tyler Chandross-Cohen[1], Brian Praul[1], Jasna Kovac[1]

[1]Department of Food Science, The Pennsylvania State University, University Park, PA 16802

kovac lab Precision Food Safety

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. **Psychrotolerant** strains of *B. cereus* from phylogenetic groups II (*B. mosaicus*) and VI (*B. mycoides*) **can produce cytotoxic enterotoxins in food**, potentially contributing to intoxication.

Thermal treatment[2], exposure to **low pH[3]**, and the presence of **proteolytic enzymes[4]** have **reduced the immunological activity** of toxins. However, it remains unclear whether these alterations in immunological activity correspond to **changes in the biological activity** of the toxins.

Objective

Evaluate the impact of protein stress factors and dilutions on the cytotoxicity of supernatants from seven psychrotolerant *Bacillus cereus* isolates using *in vitro* cytotoxicity assays.

Significance

Evaluating how protein stress factors impact the cytotoxicity of secreted virulence factors can enhance our understanding of the toxicity of enterotoxins ingested with food

Materials and Methods

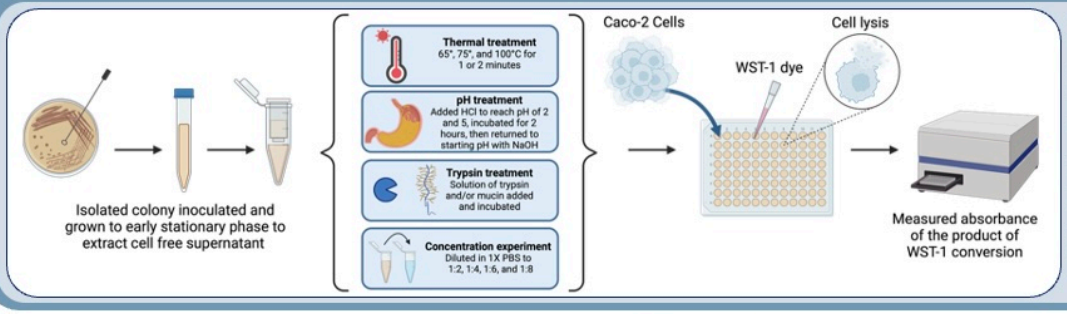

Figure 1: Isolates were grown in brain heart infusion (BHI) broth at 32°C until reaching early stationary growth phase, before the collection of supernatants. Supernatants were either thermally treated, exposed to low pH, or exposed to trypsin (10 ug/ul) and mucin (0.25%) solutions. Dilutions of supernatant 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, respectively, to determine cytotoxicity.

Results and Discussion

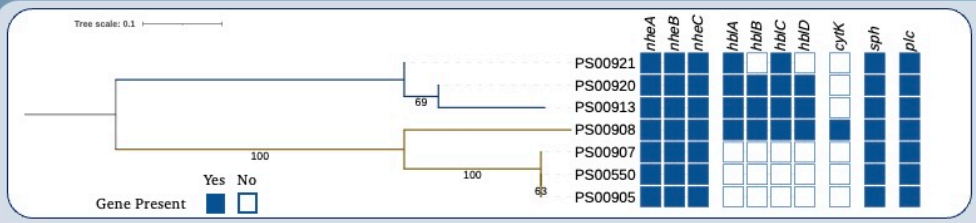

Figure 2: Phylogenetic tree and heatmap showing the presence and absence of selected virulence gene sequences in 7. *B. cereus* isolates. The group II branch is blue, and the group VI branch is brown. Virulence gene identification and *panC* phylogenetic group assignment were performed using BTyper3 (v3.4.0) and NCBI Blast using previously sequenced genomes. The CFSAN SNP pipeline and RAxML were used in GalaxyTrakr to build the phylogenetic tree. The tree was plotted using the Interactive Tree of Life (iTOL).

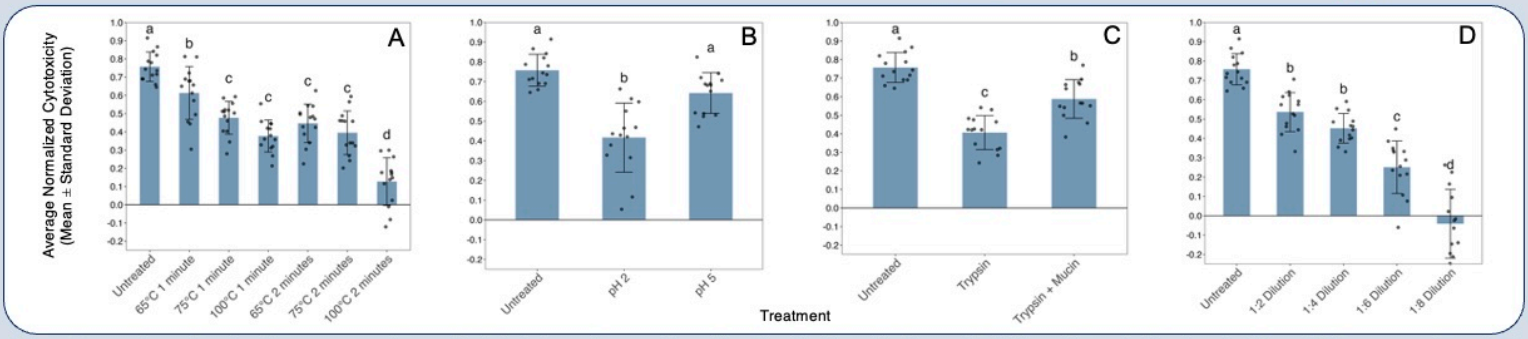

Figure 3: Bar charts showing the average ± the standard deviation of cytotoxicity values for all selected isolates with different letters indicating significant differences, as determined using ANOVA and Tukey tests. **A: Thermal treatment, B: pH treatment, C: Trypsin treatment, D: Dilutions.** On average, 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 and trypsin with mucin significantly reduced cytotoxicity compared to the untreated control, however, the reduction in the presence of mucin was significantly lower compared to just trypsin ($p<0.001$).

Conclusions

1. Stress factors reduced cytotoxicity, potentially mitigating food safety risks, but treated supernatants still exhibited residual cytotoxicity toward Caco-2 cells.
2. Cytotoxicity of untreated supernatants was concentration-dependent.

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 in food after exposure to simulated gastrointestinal conditions.

References

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