

Characterization of *Bacillus cereus* Group Isolates from Infant Formula and Cereal by Whole-Genome Sequencing and Cytotoxicity Analysis

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Background and Objective

The *Bacillus cereus* group is a diverse group of spore-forming bacteria classified into 8 phylogenetic groups, responsible for an estimated 63,000 cases of foodborne illness annually in the United States¹.

Although *B. cereus* is not routinely monitored in U.S. infant formula, a 2022 study detected *B. cereus* group species harboring toxin genes in 26.7% of powdered infant formula and 36.7% of milk-cereal-based infant formula².

A recent outbreak associated with *B. cereus* in powdered infant formula has raised concerns regarding the microbiological safety of these products and underscores the need to better characterize the diversity of *B. cereus* group species present in these products³.

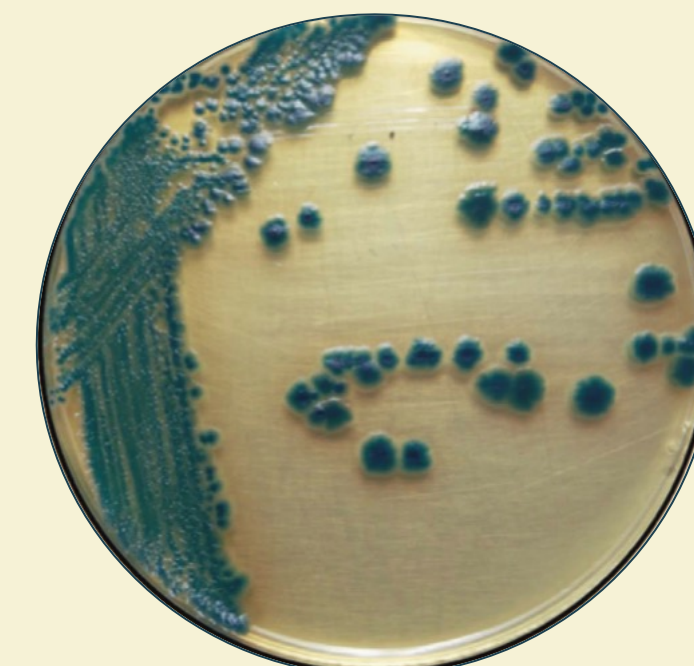


Figure 1. *B. cereus* growth on RF BCG media

Objective

Characterize *Bacillus cereus* from infant foods using whole-genome sequencing and cytotoxicity analysis to better understand their virulence potential

Significance

Infant foods can harbor low concentrations of cytotoxic *B. cereus* isolates missed by routine testing, posing a hidden food safety risk

Materials and Methods

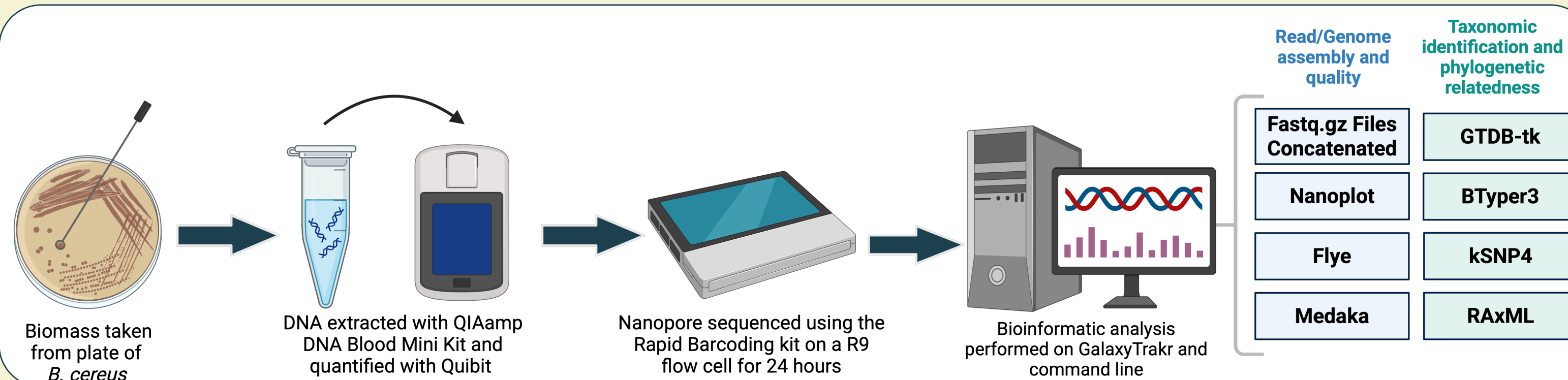


Figure 2. Genome assembly, polishing, and quality assessment were performed through GalaxyTrakr using Flye (v 2.9.5), Medaka (v 1.3.2), and QUAST (v 5.2.0), respectively. Taxonomic classification and virulence gene identification were performed with BType3 (v 3.4.0), and phylogenetic grouping with kSNP4 (v 4.1.0). RAxML (Galaxy v8.2.12) was used to build a maximum likelihood phylogenetic tree and visualized in the interactive tree of life (iTOL) (v6) software.

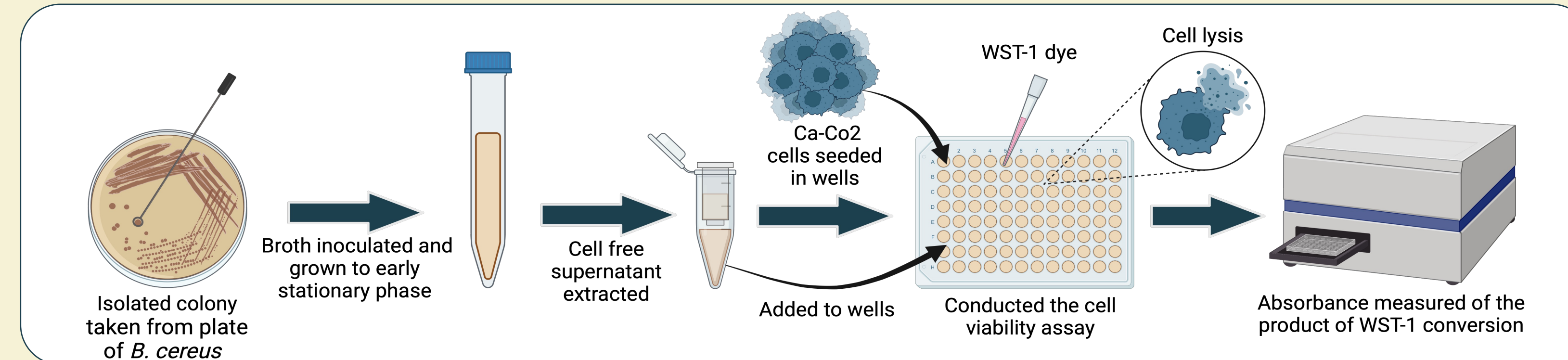


Figure 3. Isolates were grown in brain heart infusion (BHI) broth to early stationary phase at 37°C to collect cell-free supernatants. Cytotoxicity of supernatants was assessed using the WST-1 assay on Caco-2 cells. Caco-2 cells were exposed to 15% v/v of supernatants for 15 minutes before adding the WST-1 dye. After completing incubation, cytotoxicity values were min-max normalized to BHI and *B. cereus* ATCC 14579, respectively, to determine cytotoxicity.

Results and Discussion

What *B. cereus* group species are present in infant formula and cereal products, and do they carry virulence genes?

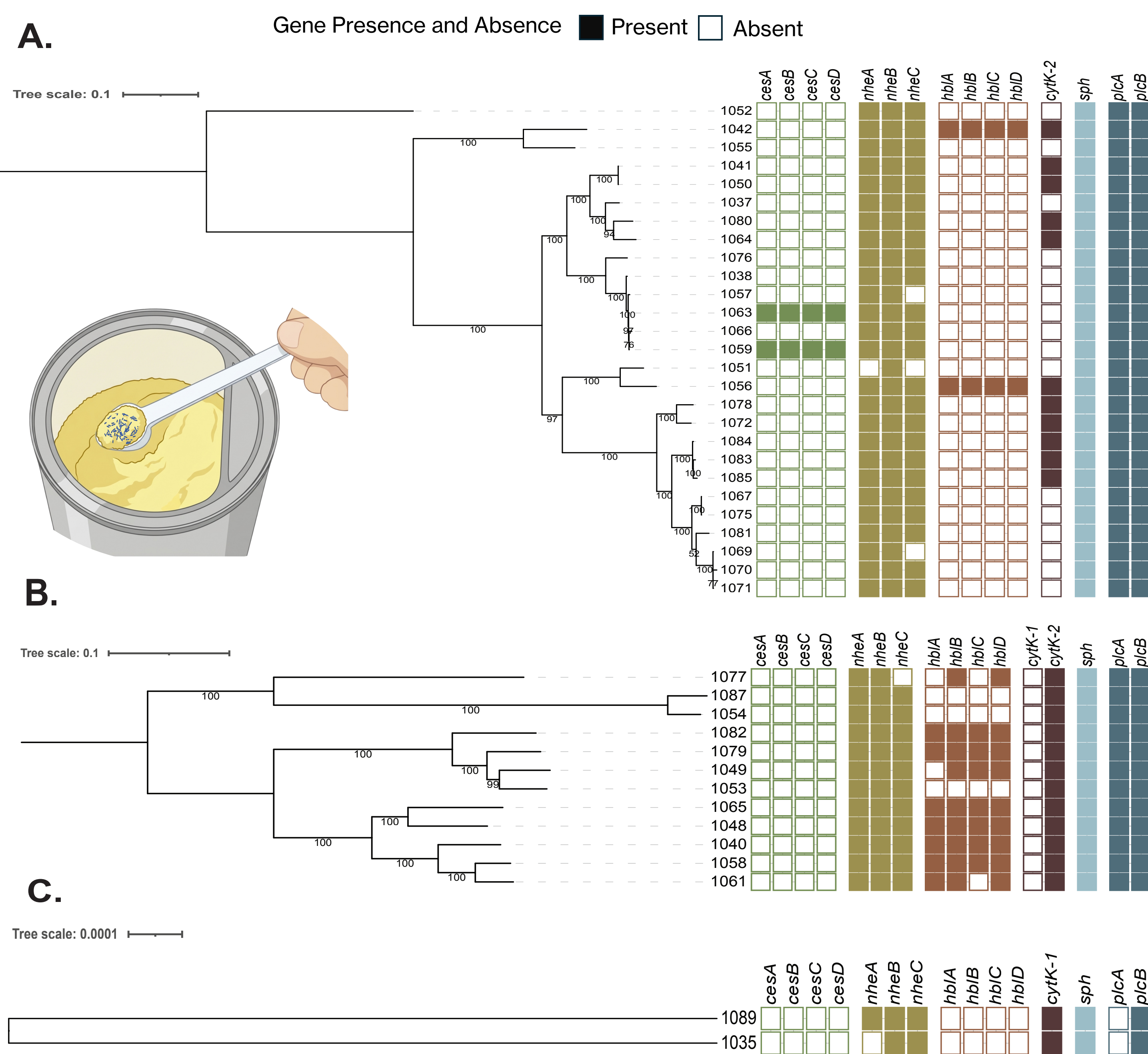


Figure 4. Maximum-likelihood phylogenetic tree of *B. cereus* group isolates recovered from various infant food products, showing the presence of virulence genes associated with emetic and diarrheal illness. Isolates were assigned to (A) phylogenetic group III, (B) group IV, and (C) group VII. *B. cereus* isolates were identified as *B. cytotoxicus* (n=2), *B. mosaicus* (n=27), and *B. cereus* s. s. (n=12), using the 92.5% average nucleotide identity (ANI) species threshold with BType3⁴.

Do *B. cereus* isolates recovered from infant foods exhibit toxicity toward human cells?

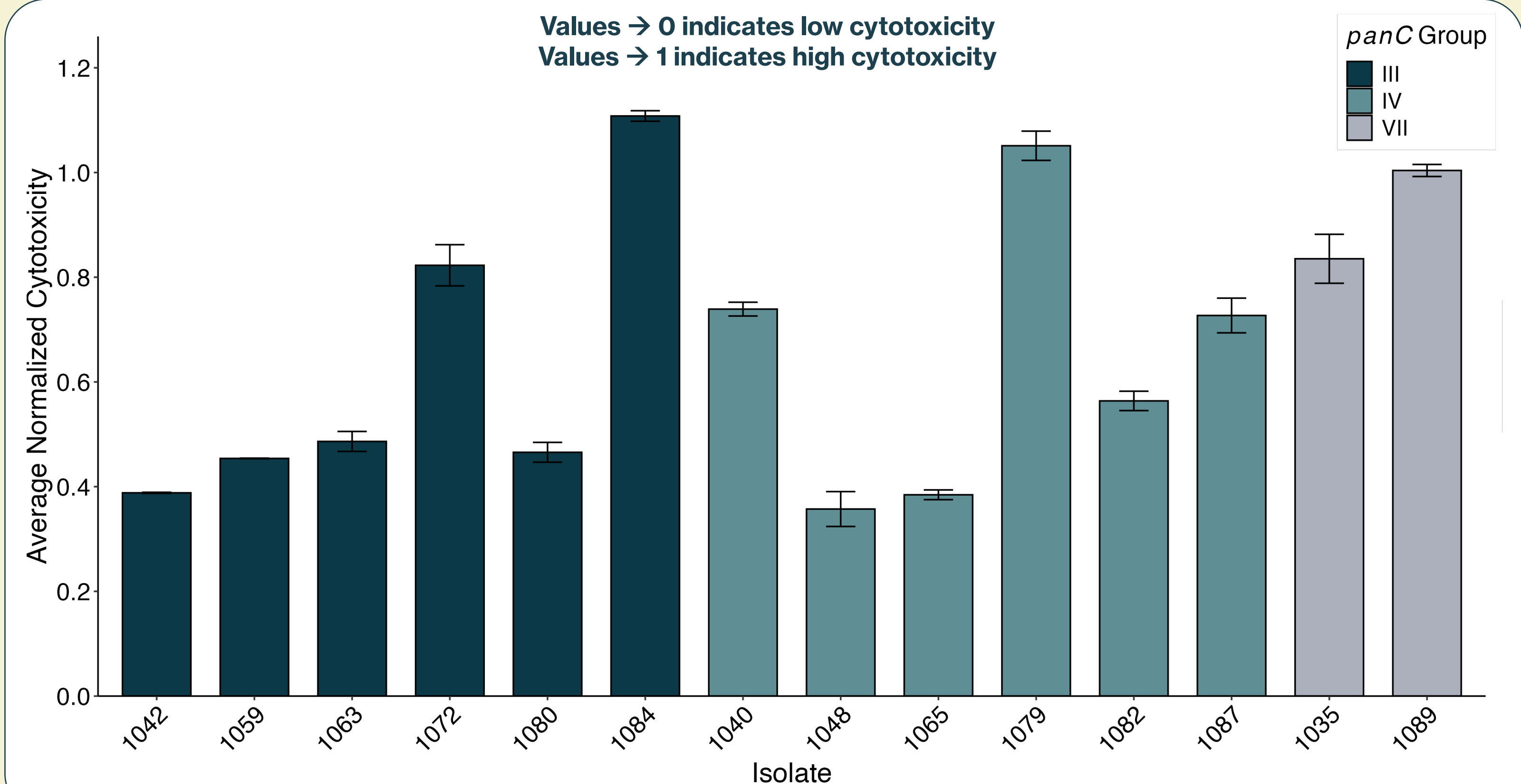
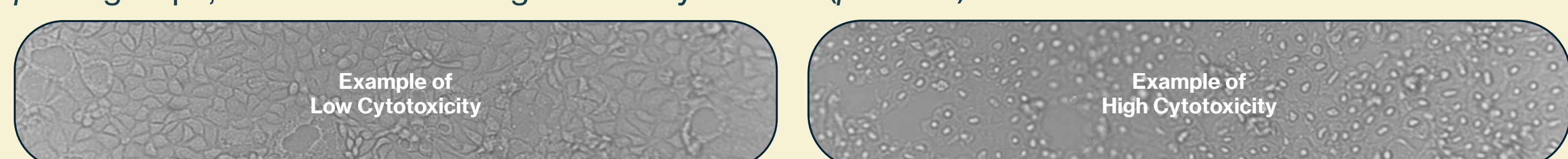


Figure 5. Data represents the average of two experiments, each with six replicates, and error bars indicate the standard deviation. Within phylogenetic (*panC*) group III, two isolates (1072, 1084) exhibited high cytotoxicity (0.82 ± 0.04 ; 1.11 ± 0.01), whereas the remaining isolates (1042, 1059, 1063, 1080) were less cytotoxic (0.45 ± 0.04). In *panC* group IV, three isolates (1040, 1079, 1087) were highly cytotoxic (0.74 ± 0.01 ; 1.05 ± 0.03 ; 0.73 ± 0.03), while others (1048, 1065, 1082) showed lower cytotoxicity (0.44 ± 0.10). Both *panC* group VII isolates (1035, 1089) were highly cytotoxic (0.84 ± 0.05 ; 1.00 ± 0.01). On average, there were no statistically significant differences in cytotoxicity among *panC* groups, as determined using a one-way ANOVA ($p = 0.111$).



All samples tested negative for *B. cereus* group species prior to enrichment, indicating <100 cells/g

Conclusions

- Recovered isolates were identified as *B. mosaicus*, *B. cereus* s. s., and *B. cytotoxicus*, all harboring virulence genes.
- High cytotoxicity was observed in some isolates from all *panC* phylogenetic groups, with variability within each group.

Future Directions

- Isolate and characterize additional *B. cereus* strains from infant food products across the United States to gain a more comprehensive understanding of the prevalence and diversity of isolates in infant formula.
- Strengthen existing exposure assessment models with phenotypic data collected in this study to better predict which *B. cereus* strains are more likely to cause illness.

References

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