Genotypic and Phenotypic Characterization of *Bacillus cereus* Isolates from Human Clinical Cases in Slovenia

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Background

The *Bacillus cereus* group consists of gram-positive, spore-forming, genetically diverse bacteria, divided into 8 phylogenetic groups based on their *panC* gene sequence. Some *B. cereus* group members that are classified as biovar Thuringiensis are used as bioinsecticides.

Diarrheal illness results from consuming food contaminated with *B. cereus*, leading to enterotoxin production in the small intestine. Emetic illness results from ingesting food with pre-formed cereulide toxin produced by *B. cereus*. In 2022, European Union member states reported 127 cases of foodborne *B. cereus* toxin outbreaks.[1]

Although foodborne illnesses from *B. cereus* are mainly associated with *B. cereus* sensu stricto and biovar Emeticus, the discovery of biovar Thuringiensis in clinical cases raises concerns.[2] Isolates from humans are underrepresented in current datasets. This work improves understanding of strain traits relevant to human illness.[3]

Objective

Characterize the genotypic and phenotypic diversity of *B. cereus* isolates recovered from human clinical cases in Slovenia.

Significance

Understanding of the relationship between *B. cereus* group isolates' genotype and foodborne illness can improve exposure and risk assessment models.

Which *B. cereus* group species are found in human clinical samples, and do they carry virulence genes?

Methods

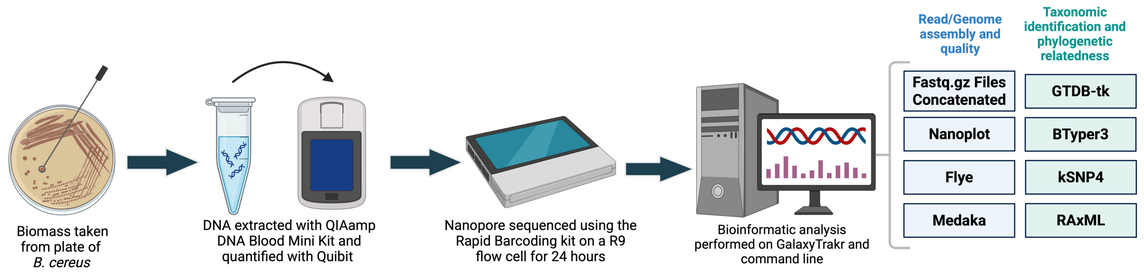

Figure 1. *B. cereus* isolates were recovered from human clinical cases by the Slovenian National Laboratory of Health, Environment, and Food between April 2024 and July 2025. Genome assembly, polishing, and quality assessment were performed using GalaxyTrakr with Flye (v 2.9.5), Medaka (v 1.3.2), and QUAST (v 5.2.0), respectively. Taxonomic classification and virulence gene identification were performed with BTyper3 (v 3.4.0), and phylogenetic grouping with kSNP4 (v 4.1.0). RAxML (Galaxy v8.2.12) was used to construct a maximum-likelihood phylogenetic tree, which was visualized in the interactive tree of life (iTOL) (v6) software.

Results

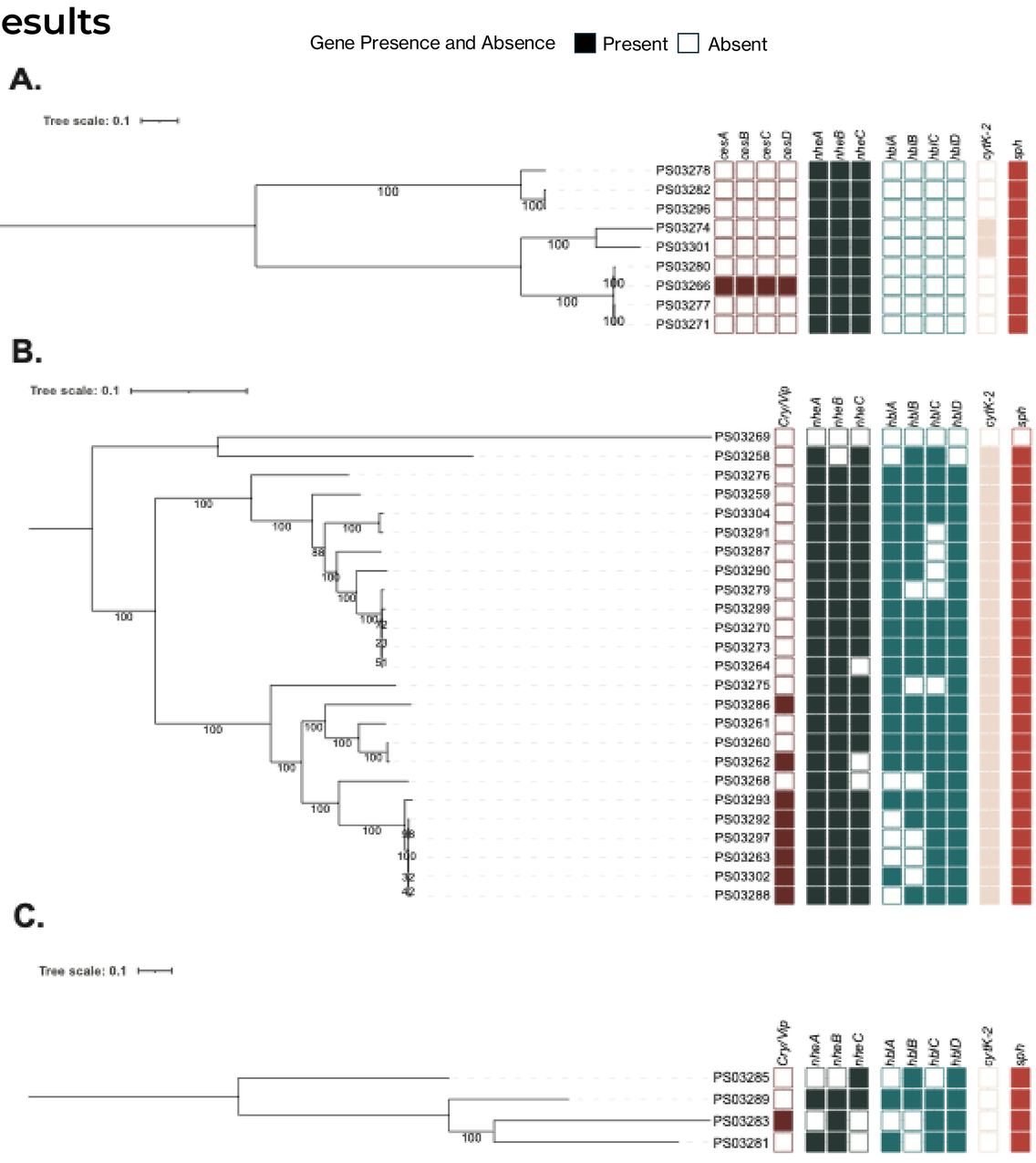

Figure 2. Maximum-likelihood phylogenetic tree of the 38 *B. cereus* group isolates recovered from human clinical samples. Isolates were classified as *B. cereus* s. s. (n=25, including 9 biovar Thuringiensis), *B. mosaicus* (n=9, including 1 biovar Emeticus), and *B. toyonensis* (n=4). BTyper3 detected *nhe* genes in 86% of all isolates, *hbl* in 65%, *cytK-2* in 60%, and *sph* in 86%, while *cesABCD* was present in only one isolate (2%).[4]

Do *B. cereus* isolates recovered from human clinical samples show toxicity toward human cells?

Methods

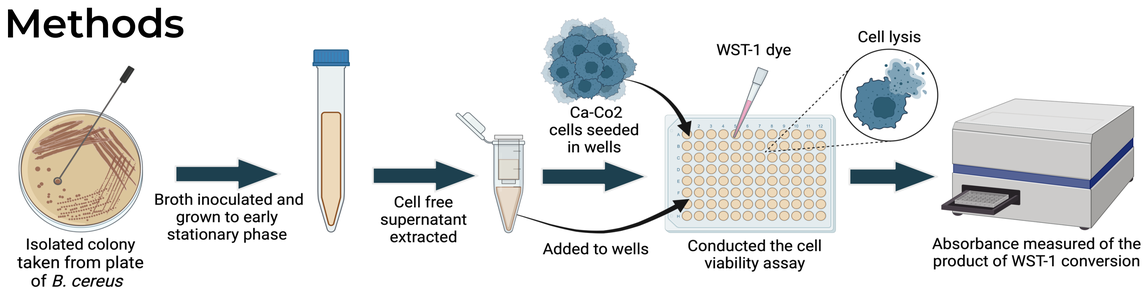

Figure 3. Isolates were grown in brain heart infusion (BHI) broth to early stationary phase at 37°C to collect cell-free supernatants. Cytotoxicity of supernatants was assessed using the WST-1 assay on Caco-2 cells. Caco-2 cells were exposed to 15% v/v of supernatants for 15 minutes before adding the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

Results

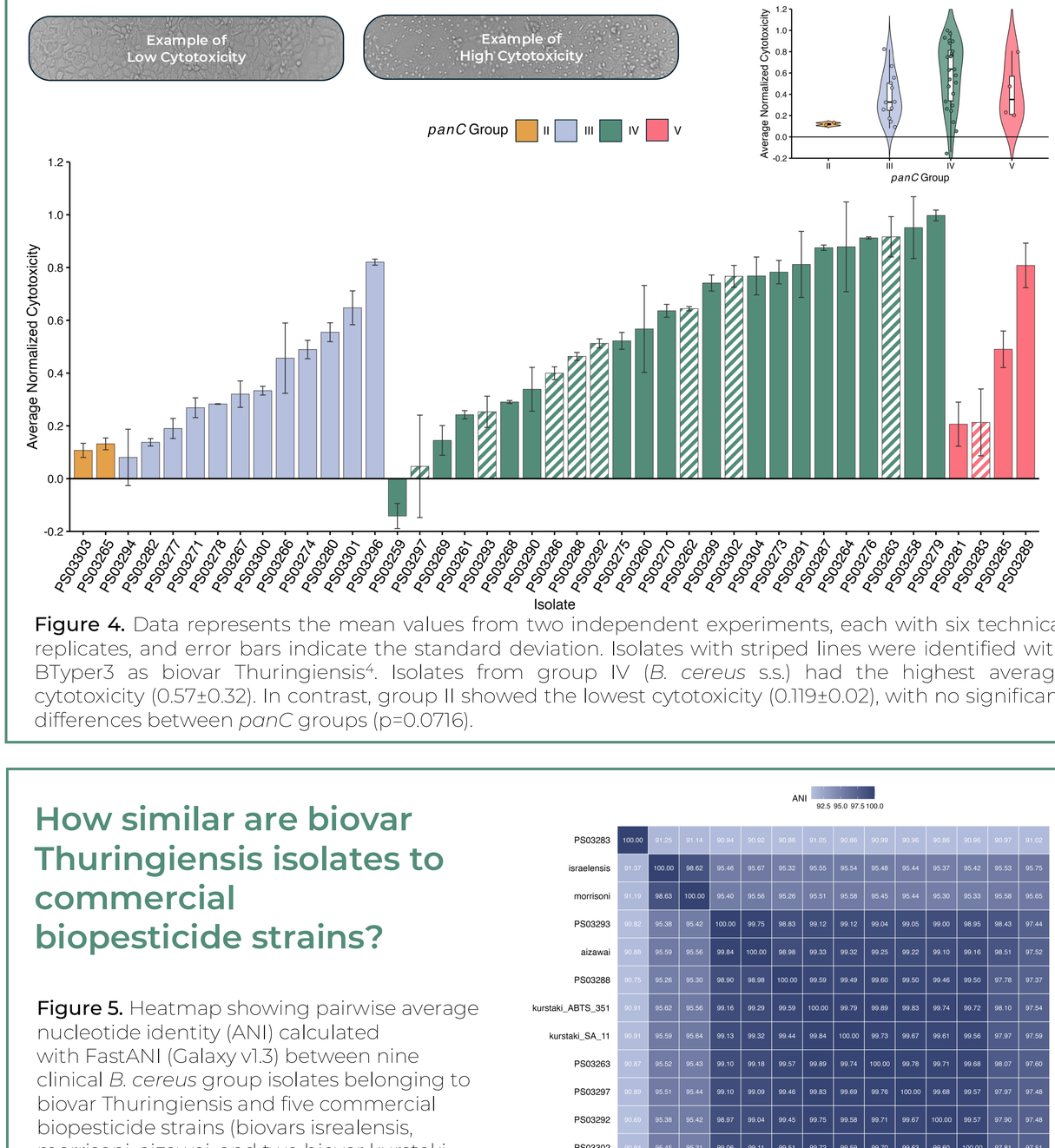

Figure 4. Data represents the mean values from two independent experiments, each with six technical replicates, and error bars indicate the standard deviation. Isolates with striped lines were identified with BTyper3 as biovar Thuringiensis[4]. Isolates from group IV (*B. cereus* s.s.) had the highest average cytotoxicity (0.57±0.32). In contrast, group II showed the lowest cytotoxicity (0.119±0.02), with no significant differences between *panC* groups (p=0.0716).

How similar are biovar Thuringiensis isolates to commercial biopesticide strains?

Figure 5. Heatmap showing pairwise average nucleotide identity (ANI) calculated with FastANI (Galaxy v1.3) between nine clinical *B. cereus* group isolates belonging to biovar Thuringiensis and five commercial biopesticide strains (biovars isrealensis, morrisoni, aizawai, and two biovar kurstaki strains). Six clinical isolates had >99% ANI with commercial biopesticide strains. Four isolates (PS03263, PS03297, PS03292, and PS03302) had high ANI to *B. thuringiensis* subsp. Kurstaki strain SA-11 and ABTS-351.

Conclusions

1. Whole-genome sequencing identified *B. cereus* s.s., *B. mosaicus*, *B. mosaicus/luti*, and *B. toyonensis* among clinical *B. cereus* group strains.
2. Cytotoxicity varied substantially among isolates, with group IV (*B. cereus* s.s.) showing high average cytotoxicity (0.57 ± 0.32) and group II the lowest (0.119 ± 0.02), but differences between *panC* groups were not significant (p = 0.0716).
3. Six biovar Thuringiensis strains were closely related (>99% ANI) to commercial biopesticide strains.

Future Directions

- 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.
- Analyze epidemiology data to assess relationships between isolates in clinical and food samples.

References

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