

Elucidating the hazardous potential of members of the *Bacillus cereus* group and developing strategies and tools for risk-based screening and control

Background:

The *Bacillus cereus* group comprises amongst others the well-known foodborne pathogen *B. cereus sensu stricto*, the newly described foodborne pathogen *B. cytotoxicus*, and *Bacillus thuringiensis*, a popular biopesticide that has been used worldwide for decades and is considered non-pathogenic to humans. Two distinct forms of foodborne illness linked to some of the members of the *B. cereus* group have been described: the emetic syndrome (an intoxication), and diarrheal syndrome (a toxico-infection).

Project 1: Creating the basis for improved risk assessment of *B. thuringiensis* occurring in food

Hypothesis: It has been shown that B. thuringiensis strains commonly carry at least one enterotoxin gene and potential links to diarrheal disease in humans have been suggested. We hypothesize that B. thuringiensis can cause diarrheal disease in humans and that biopesticide residues might be linked to rare cases of foodborne outbreaks.

Plant protection products based on *B. thuringiensis* are an important alternative to chemical pesticides and play a key role in realizing the UN's sustainable development goals. Still, there is mounting evidence suggesting that residues of *B. thuringiensis* biopesticides on foods can elicit diarrheal syndrome in humans. Although generally regarded as safe, *B. thuringiensis* is phylogenetically intermingled with the foodborne pathogen *B. cereus sensu stricto* and has been linked to foodborne outbreaks. Limited data on the pathogenicity potential of *B. thuringiensis* and the occurrence of biopesticide residues in food compromise a robust consumer risk assessment. We analyzed whole-genome sequences of 33 *B. thuringiensis* isolates from biopesticides, food, and human fecal samples linked to outbreaks. All food and outbreak-associated isolates genomically matched (≤ 6 wgSNPs; ≤ 2 cgSNPs) with one of six biopesticide strains, suggesting biopesticide products as their source. Long-read sequencing revealed a more diverse virulence gene profile than previously assumed, including a transposase-mediated disruption of the promoter region of the non-hemolytic enterotoxin gene *nhe* and a bacteriophage-mediated disruption of the sphingomyelinase gene *sph* in some biopesticide strains. Furthermore, we provide high-quality genome assemblies of seven widely used *B. thuringiensis* biopesticide strains, which will facilitate improved microbial source tracking and risk assessment of *B. thuringiensis*-based biopesticides in the future.

Conclusion: Our findings reveal the biopesticidal origin of *B. thuringiensis* in foods. Routine diagnostic differentiation of *Bt* and other *B. cereus s. l.* members and in-depth outbreak investigations are needed to enable a better understanding of the number of disease cases linked to *Bt*. In view of the collapsing taxonomic framework of the *B. cereus* group and the polyphyletic distribution of the *B. thuringiensis* characteristic *cry* and *cyt* harboring plasmids across different *panC* types, alternatives to taxonomy-driven risk assessment are direly needed. The risk associated with a strain of the *B. cereus* group should not be based on species assignment but needs to be based on strain-specific virulence and pathogenicity traits.

- Biggel et al. (2022): Whole genome sequencing reveals biopesticidal origin of *Bacillus thuringiensis* in foods. *Frontiers in Microbiology*, 12:775669. doi: 10.3389/fmicb.2021.775669
- Biggel et al. (2022): Recent paradigm shifts in the perception of the role of *Bacillus thuringiensis* in foodborne disease, *Food Microbiology*, submitted

Project 2: Elucidating the role of *B. cytotoxicus* as a foodborne pathogen

Hypothesis: B. cytotoxicus has been suggested to be an extremely cytotoxic member of the B. cereus group. We hypothesize that B. cytotoxicus commonly occurs in foodstuff sold in Switzerland and that toxicity of the organism varies widely.

The first *B. cytotoxicus*, a member of the *B. cereus* group, was isolated in the context of a severe food poisoning outbreak leading to fatal cases of diarrheal disease. Subsequent characterization of the outbreak strain led to the conclusion that this *Bacillus* strain was highly cytotoxic and eventually resulted in the description of a novel species, whose name reflects the observed toxicity: *B. cytotoxicus*. However, only a few isolates of this species have been characterized with regard to their cytotoxic potential and the role of *B. cytotoxicus* as a causative agent of food poisoning remains largely unclear. Hence, we aimed to gain further insights into the toxicity of *B. cytotoxicus*. To this end, isolates obtained from mashed potato powders and various other food matrices were characterized by toxin gene profiling and Vero cell cytotoxicity assays. While many isolates exhibited low or no toxicity towards Vero cells, few highly toxic strains were observed.

Conclusion: Our findings indicate that *B. cytotoxicus* are indeed primarily found in mashed potatoes or food items that contain potato starch. Based on our results, the cytotoxic potential of most *B. cytotoxicus* strains may be lower than initially assumed, but very few extremely toxic strains were detected.

- Burtscher et al. (2021): Further insights into the toxicity of *Bacillus cytotoxicus* based on toxin gene profiling and Vero cell cytotoxicity assays. *Toxins*, 13, 234, <https://doi.org/10.3390/toxins13040234>
- [Project ongoing, additional publication will follow](#)