Variation in *Bacillus cereus* Hemolysin Bl Transcription Associated with a SNP in Transcription Regulatory Region

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Background and Objectives

The *Bacillus cereus* group are Gram-positive, closely related spore-forming bacteria. Some strains of ***B. cereus sensu stricto (s.s.)***, also referred to as phylogenetic group IV, can secrete enterotoxins cytotoxic towards human gut epithelial cells[1].

The transcription of enterotoxins Hemolysin BL (Hbl), Non-hemolytic enterotoxin (Nhe), and Cytotoxin K (CytK) are regulated by the **PlcR-PapR** quorum-sensing system[2].

The initiation of gene transcription involves the PlcR-PapR complex binding to a **PlcR box** upstream of the virulence gene where **polymorphisms** have been linked to **changes in secretion**[3].

Objective 1
Identify single nucleotide polymorphisms (**SNPs) in PlcR boxes** upstream of enterotoxin genes ***hbl, nhe,*** and ***cytK*** in *B. cereus* isolates from group IV.

Objective 2
Evaluate if SNPs in the Hbl PlcR box sequence are associated with **changes in *hblC* gene transcription** in 20 group IV *B. cereus* isolates to better **predict enterotoxin-mediated virulence potential**.

Materials and Methods

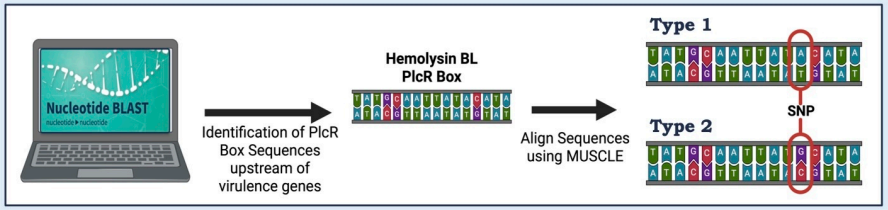

Figure 1: Whole genome sequences from 70 *B. cereus* isolates from **phylogenetic group IV** were screened for PlcR box sequences using the known sequences from the *B. cereus s.s.* type strain ATCC 14579 as the reference. Sequences were aligned using MUSCLE and SNPs were identified and recorded.

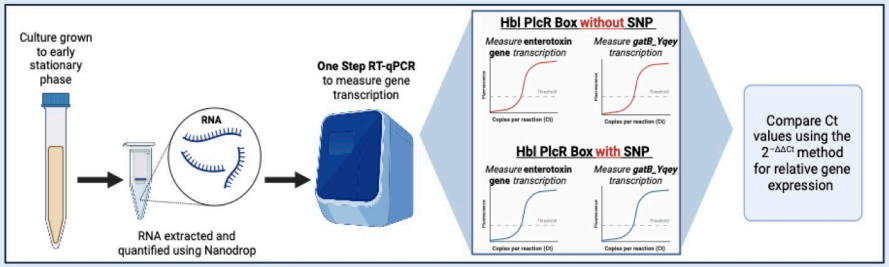

Figure 2: Isolates with identical PlcR Box sequences upstream *nhe* and *cytK* but SNPs in the *hbl* PlcR box were chosen. **10 isolates with the SNP and 10 without were selected** and grown until early stationary phase in brain heart infusion broth (BHIB). RNA was extracted and transcripts were detected using **One-Step RT-qPCR** Kit and qPCR. The the **$2^{-\Delta\Delta Ct}$ method** was used to compare Cycle threshold(CT) values between isolates with and without identified SNP in the Hbl PlcR Box.

Results

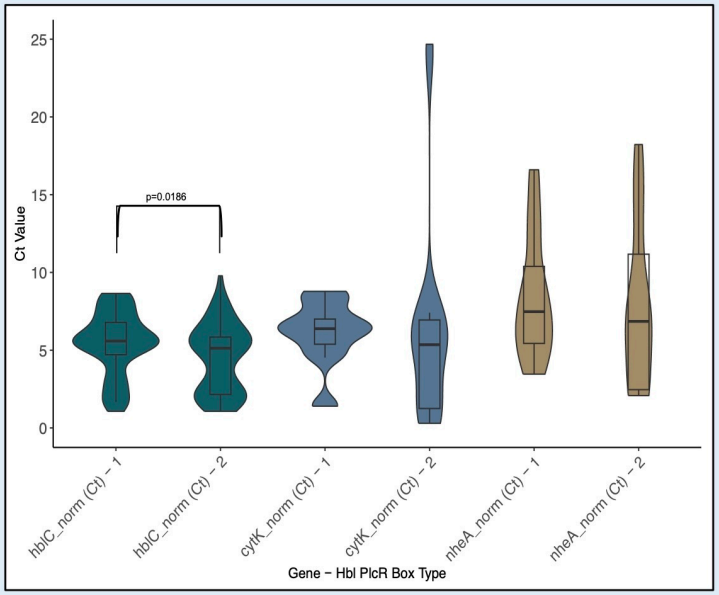

Figure 3: Violin plot for average Ct values for virulence gene transcripts in isolates without the SNP (Type 1) and with the SNP (Type 2) normalized to the housekeeping gene *gatB_Yqey*. **A SNP in the Hbl PlcR Box** -277 bp upstream from the start codon was identified **in 24 isolates (Type 2),** while 46 isolates matched the type strain *B. cereus s.s.* ATCC 14579 (Type 1). On average, there was a **significant difference (p=0.0168)** between the normalized Ct values **for *hblC* transcripts** between Type 1 and Type 2 isolates, but not for *nheA* (p=0.566) or *cytK* (p=0.911) transcripts.

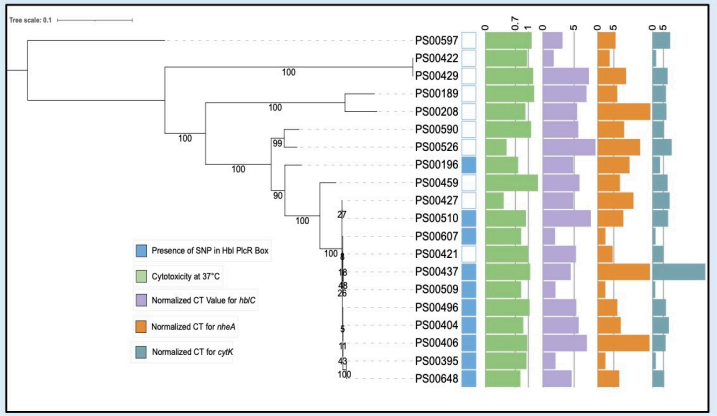

Figure 4: Phylogenetic tree of isolates with and without a SNP in the *hbl* PlcR box. The CFSAN SNP Pipeline and RAxML in GalaxyTrakr were used to build the phylogenetic tree. Cytotoxicity values were collected in previous experiments. The presence/absence of the SNP, cytotoxicity, and normalized Ct values, were plotted using the Interactive Tree of Life (iTOL).

Conclusions

(1) SNPs were identified in the PlcR-box transcriptional regulatory region of the *hbl* genes of some isolates.

(2) On average, isolates with the SNP in the PlcR-box had a 1.57-fold increase in *hblC* transcripts.

(3) There was a variability in virulence gene transcription in *B. cereus s.s.* isolates, showing a need for strain-based prediction of virulence potential.

Significance

Evaluating SNPs that are associated with *hbl* gene transcription and cytotoxicity can improve strain-specific predictions of *B. cereus* virulence potential.

Future Directions

(1) Measure transcription of other virulence factor genes like sphingomyelinase (*sph*) and phosphatidylcholine-specific phospholipase C (*plc*).

(2) Explore nonsynonymous SNPs in the *hblABCD* genes that are associated with cytotoxicity to identify sequence markers that cause changes in cytotoxicity and transcription.

References

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(3) Yokotani, Atsushi et al. *Microbiology and immunology* vol. 66,4 (2022): 157-165. doi:10.1111/1348-0421.12959

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