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Comparative Bioinformatics and Experimental Analysis of the Intergenic Regulatory Regions of Bacillus cereus hbl and nhe Enterotoxin Operons and the Impact of CodY on Virulence Heterogeneity.

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Abstract

Bacillus cereus is a food contaminant with greatly varying enteropathogenic potential. Almost all known strains harbor the genes for at least one of the three enterotoxins Nhe, Hbl, and CytK. While some strains show no cytotoxicity, others have caused outbreaks, in rare cases even with lethal outcome. The reason for these differences in cytotoxicity is unknown. To gain insight into the origin of enterotoxin expression heterogeneity in different strains, the architecture and role of 5' intergenic regions (5' IGRs) upstream of the nhe and hbl operons was investigated. In silico comparison of 142 strains of all seven phylogenetic groups of B. cereus sensu lato proved the presence of long 5' IGRs upstream of the nheABC and hblCDAB operons, which harbor recognition sites for several transcriptional regulators, including the virulence regulator PlcR, redox regulators ResD and Fnr, the nutrient-sensitive regulator CodY as well as the master regulator for biofilm formation SinR. By determining transcription start sites, unusually long 5' untranslated regions (5' UTRs) upstream of the nhe and hbl start codons were identified, which are not present upstream of cytK-1 and cytK-2. Promoter fusions lacking various parts of the nhe and hbl 5' UTR in B. cereus INRA C3 showed that the entire 331 bp 5' UTR of nhe is necessary for full promoter activity, while the presence of the complete 606 bp hbl 5' UTR lowers promoter activity. Repression was caused by a 268 bp sequence directly upstream of the hbl transcription start. Luciferase activity of reporter strains containing nhe and hbl 5' IGR lux fusions provided evidence that toxin gene transcription is upregulated by the depletion of free amino acids. Electrophoretic mobility shift assays showed that the branched-chain amino acid sensing regulator CodY binds to both nhe and hbl 5' UTR downstream of the promoter, potentially acting as a nutrient-responsive roadblock repressor of toxin gene transcription. PlcR binding sites are highly conserved among all B. cereus sensu lato strains, indicating that this regulator does not significantly contribute to the heterogeneity in virulence potentials. The CodY recognition sites are far less conserved, perhaps conferring varying strengths of CodY binding, which might modulate toxin synthesis in a strain-specific manner.

KEYWORDS:

5′ IGR; Bacillus cereus; CodY; Hbl; Nhe; enterotoxins; transcriptional regulation