

Table S1. Phenotypic and molecular characterization of all *B. cereus* group isolates included in this study.

Isolate ID	Clade ¹	<i>rpoB</i> AT ²	MLST Type	SR/RefSeq Accession	Growth at 6°C ³	Hemolytic ³	PI- PLC ^{3,4}	Lecithinase ³	NheB ^{3,5}	Hbl-L2 ^{3,5}	<i>hblA</i> ³	<i>hblC</i> ³	<i>hblD</i> ³	<i>nheA</i> ³	<i>nheB</i> ³	<i>nheC</i> ³	<i>cytK</i> ³	<i>entFM</i> ³	Cytotoxicit y Average ⁶
FSL K6-0268	IV	409	230	SRR5185034	-	+	+	+	+	-	+	+	+	+	+	+	+	0.01	
FSL K6-1030	IV	556	1191	SRR4661783	-	-	-	+	+	-	+	-	+	+	+	+	+	0.00	
FSL M8-0139	IV	424	138	SRR5185026	-	-	+	+	+	-	-	-	-	+	+	+	+	0.00	
FSL R5-0184	IV	277	927	SRR5185020	-	+	+	+	+	+	+	+	+	+	+	+	+	1.00	
FSL R5-0585	IV	278	1143	SRR5185019	-	+	+	+	+	-	+	+	+	+	+	+	+	0.01	
FSL R5-0859	IV	158	1099	SRR5185016	-	+	+	+	+	+	+	+	+	+	+	+	+	0.00	
FSL W7-1101	IV	296	1100	SRR5185011	-	+	+	+	+	+	+	+	+	+	+	+	+	0.30	
FSL W7-1334	IV	304	265	SRR5185009	-	+	+	+	+	-	+	+	+	+	+	+	-	0.00	
FSL W8-0640	IV	154	1089	SRR2541688	-	+	+	+	+	+	+	+	+	+	+	+	+	0.00	
FSL W8-0824	IV	92	24	SRR2541693	-	+	+	+	+	+	+	+	+	+	+	+	+	0.64	
ATCC 14579 ^T	IV	158	4	NC_004722.1	-	+	+	+	+	+	+	+	+	+	+	+	+	0.99	
ATCC 10792 ^T	IV	689	10	NC_005957.1	-	+	+	+	+	+	+	+	+	+	+	+	+	0.76	
FSL M8-0214	V	412	223	SRR5185025	-	+	+	+	+	+	+	+	+	+	+	+	-	0.42	
FSL H8-0482	V	129	223	SRR2541601	-	+	+	+	+	+	-	+	+	+	+	+	-	0.01	
BCT-7112 ^T	V	129	111	NC_022781.1	-	+	+	+	+	+	+	+	+	+	+	+	-	0.02	
FSL H7-0683	VI	75	222	SRR4064645	+	-	+	+	-	-	+	+	+	+	+	-	+	0.00	
FSL E2-0214	VI	531	410	SRR4661781	+	+	+	+	-	-	+	+	+	-	+	+	-	0.00	
FSL H7-0926	VI	90	667	SRR2541537	+	+	+	+	-	-	+	+	+	+	+	+	-	0.00	
FSL H8-0485	VI	132	1142	SRR5189059	+	-	+	+	+	-	-	+	+	+	+	-	+	0.00	
FSL H8-0492	VI	134	1098	SRR4661790	+	-	+	+	-	-	+	+	+	+	+	-	+	0.00	
FSL J3-0123	VI	513	1095	SRR5189058	+	+	+	+	+	-	+	+	+	+	+	-	+	0.00	
FSL M7-0109	VI	273	410	SRR5189057	+	+	+	+	+	-	+	+	+	-	+	+	-	0.00	
FSL M7-0669	VI	3	655	SRR5189065	+	+	+	+	+	-	+	+	+	-	+	+	-	0.00	

Isolate ID	Clade ¹	<i>rpoB</i> AT ²	MLST Type	SR/RefSeq Accession	Growth at 6°C ³	Hemolytic ³	PI-PLC ^{3,4}	Lecithinase ³	NheB ^{3,5}	Hbl-L2 ^{3,5}	<i>hblA</i> ³	<i>hblC</i> ³	<i>hblD</i> ³	<i>nheA</i> ³	<i>nheB</i> ³	<i>nheC</i> ³	<i>cytK</i> ³	<i>entFM</i> ³	Cytotoxicity Average ⁶
FSL M7-1219	VI	97	1092	SRR5189064	+	+	+	+	+	-	+	+	+	+	+	+	-	+	0.00
FSL R5-0708	VI	257	1144	SRR5189062	+	+	+	+	+	-	+	+	+	+	+	+	-	+	0.00
FSL W7-1108	VI	342	673	SRR5189056	+	+	+	+	+	-	+	+	+	+	+	+	-	+	0.00
DSM 2048 ^T	VI	3	116	NZ_CP009692.1	+	+	+	+	+	-	+	+	+	-	+	+	-	+	0.00
WSBC 10204 ^T	VI	3	196	NZ_CP009746.1	+	-	+	+	+	-	+	+	+	+	+	+	-	+	0.00
NVH 391-98 ^T	VII	688	930	NC_009674.1	-	+	-	+	-	-	-	-	-	-	-	-	+	+	0.25

¹Phylogenetic group determined based on concatenated 7-gene MLST sequences.

²AT refers to *rpoB* allelic type; determined based on 632 nucleotide sequence of *rpoB*.

³“+” denotes the bacterial isolate was positive for a given virulence factor, “-” denotes the virulence factor was not detected. Data presented are based on PCR amplification of target genes.

⁴PI-PLC refers to phosphoinositide phospholipase C activity as determined by plating isolates on *Bacillus cereus/Bacillus thuringiensis* chromogenic plating medium.

⁵Lecithinase activity determined by plating isolate on Bacara agar.

⁶Proportion of HeLa cells that were positive for propidium iodide staining, following a 30 minute co-incubation with supernatants (5% v/v) from bacterial cultures grown at 37°C. Results are averaged from two independent experiments.

Table S2. P-values of statistical analyses of associations between virulence factors and phylogenetic clades and cytotoxicity

Association tested ^{1,2}	P-value for overall associations ³	P-values for significant associations with specific phylogenetic clades ^{4,5,6}
Association between phylogenetic clades and virulence factors		
PI-PLC Activity	P < 0.001	I: P < 0.001
Hemolysis	P = 0.720	ND
Growth at 6°C	P < 0.001	III: P = 0.043 IV: P = 0.002 VI: P < 0.001
<i>hblCDA</i> gene presence	P < 0.001	III: P < 0.001 VI: P = 0.047
<i>nheABC</i> gene presence	P = 0.032	VII: P = 0.019
<i>entFM</i> gene presence	P < 0.001	I: P < 0.001
<i>cytK</i> gene presence	P < 0.001	I: P = 0.025 IV: P < 0.001 VI: P < 0.001
Hbl-L2 Immunoassay Detection	P < 0.001	IV: P < 0.001 VI: P = 0.005
NheB Immunoassay Detection	P < 0.001	I: P < 0.001 IV: P = 0.043
Cytotoxicity	P < 0.001	I: P = 0.001 VI: P = 0.002
Cytotoxicity versus presence/absence of selected genes		
<i>hblCDA</i>	P = 1.000	NA
<i>cytK</i>	P = 0.316	NA
<i>nheABC</i>	P = 0.422	NA
Cytotoxicity versus immunoassay detection of Hbl-L2 and NheB		
Hbl-L2	P = 0.098	NA
NheB	P = 0.140	NA

¹ Includes both *cytK-1* and *cytK-2*.

² Considered *hblCDA* or *nheABC* positive if at least one gene in the toxin gene operon was detected via PCR.

³ P-values from Fisher's exact tests, corrected using False Discovery Rate for multiple comparisons.

⁴ P-values from Fisher's exact tests, uncorrected.

⁵ ND = not determined (i.e. analysis not performed).

⁶ NA = not applicable.

Table S3. Number and proportion of PCR-positive results for toxin genes.

Clade	Gene ¹							
	<i>hblA</i>	<i>hblC</i>	<i>hblD</i>	<i>nheA</i>	<i>nheB</i>	<i>nheC</i>	<i>cytK</i>	<i>entFM</i>
I	6 (1.00)	5 (0.83)	6 (1.00)	0 (0.00)	0 (0.00)	6 (1.00)	0 (0.00)	1 (0.17)
II	2 (0.40)	3 (0.60)	3 (0.60)	5 (1.00)	5 (1.00)	5 (1.00)	2 (0.40)	5 (1.00)
III	2 (0.22)	2 (0.22)	2 (0.22)	9 (1.00)	9 (1.00)	9 (1.00)	7 (0.78)	9 (1.00)
IV	14 (0.93)	13 (0.87)	14 (0.93)	15 (1.00)	15 (1.00)	15 (1.00)	14 (0.93)	15 (1.00)
V	3 (1.00)	3 (1.00)	3 (1.00)	3 (1.00)	3 (1.00)	3 (1.00)	0 (0.00)	3 (1.00)
VI	12 (0.92)	13 (1.00)	13 (1.00)	9 (0.69)	13 (1.00)	13 (1.00)	0 (0.00)	13 (1.00)
VII	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.00)	1 (1.00)
Total	39 (0.75)	39 (0.75)	41 (0.79)	41 (0.79)	45 (0.87)	51 (0.98)	24 (0.46)	47 (0.90)

¹ Number and proportion (in parentheses) of isolates in a given clade that were PCR-positive for gene

Table S4. Number of isolates with positive results for toxin genes using WGS data, within each clade.

Clade	Gene ¹							
	<i>hblA</i>	<i>hblC</i>	<i>hblD</i>	<i>nheA</i>	<i>nheB</i>	<i>nheC</i>	<i>cytK</i>	<i>entFM</i>
I	6 (1.00)	5 (0.83)	4 (0.67)	0 (0.00)	3 (0.50)	4 (0.67)	0 (0.00)	0 (0.00)
II	2 (0.40)	3 (0.60)	3 (0.60)	5 (1.00)	5 (1.00)	5 (1.00)	2 (0.40)	5 (1.00)
III	2 (0.22)	2 (0.22)	2 (0.22)	9 (1.00)	9 (1.00)	9 (1.00)	7 (0.78)	9 (1.00)
IV	14 (0.93)	14 (0.93)	13 (0.87)	15 (1.00)	15 (1.00)	15 (1.00)	14 (0.93)	14 (0.93)
V	3 (1.00)	3 (1.00)	3 (1.00)	3 (1.00)	3 (1.00)	3 (1.00)	0 (0.00)	3 (1.00)
VI	13 (1.00)	10 (0.77)	13 (1.00)	13 (1.00)	13 (1.00)	13 (1.00)	0 (0.00)	13 (1.00)
VII	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.00)	1 (1.00)
Total	40 (0.77)	37 (0.71)	38 (0.73)	45 (0.87)	48 (0.92)	49 (0.94)	24 (0.46)	45 (0.87)

¹ Proportion of isolates in a given clade that were PCR-positive for gene