Bacillus wiedmannii sp. nov. is a new psychrotolerant and cytotoxic *Bacillus cereus* group species isolated from dairy foods and environments in the USA

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Fig. S1: Maximum likelihood tree constructed based on 632 bp long *rpoB* sequences using generalized time reversible evolutionary model (GTR) with gamma distributed sites and 1000 bootstrap repetitions in RaxML, rooted by mid-point.

Bootstrap values above >15 are displayed on branches. The bar represents 0.01 substitutions per site. The representative isolates of *B. wiedmannii* sp. nov. are shown in bold type.

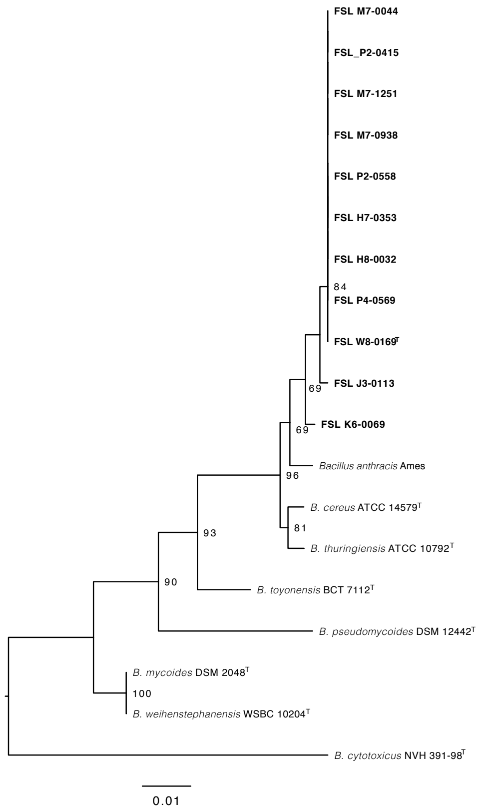

Table S1. NCBI accession numbers for 16S rRNA gene and whole genome sequences (WGS) used in phylogenetic analyses

NCBI sequence accession number	Strain	16S rRNA/WGS
NR_043774.1	*Bacillus acidiceler* CBD 119^{T}	16S rRNA
NR_041379.1	*B. panaciterrae* Gsoil 1517^{T}	16S rRNA
NR_029077.1	*Bacillus algicola* F12	16S rRNA
NR_112685.1	*Bacillus amyloliquefaciens* NBRC 15535	16S rRNA
NR_024689.1	*Bacillus atrophaeus* JCM 9070^{T}	16S rRNA
NR_026140.1	*Bacillus clausii* DSM 8716^{T}	16S rRNA
NR_026138.1	*Bacillus cohnii* DSM 6307^{T}	16S rRNA
NR_042274.1	*Bacillus foraminis* CV53^{T}	16S rRNA
NR_042905.1	*Bacillus fortis* R-6514^{T}	16S rRNA
NR_025370.1	*Bacillus fumarioli* LMG 17489^{T}	16S rRNA
NR_028624.1	*Bacillus funiculus* NAF001^{T}	16S rRNA
NR_044193.1	*Bacillus ginsengi* get14^{T}	16S rRNA
NR_025446.1	*Bacillus halodurans* DSM 497^{T}	16S rRNA
NR_040848.1	*Bacillus hemicellulosilyticus* C-11^{T}	16S rRNA
NR_025626.1	*Bacillus humi* LMG 22167^{T}	16S rRNA
NR_029022.1	*Bacillus indicus* Sd/3^{T}	16S rRNA
NR_025060.1	*Bacillus jeotgali* YKJ-10^{T}	16S rRNA
NR_074923.1	*Bacillus licheniformis* ATCC 14580^{T}	16S rRNA
NR_025511.1	*Bacillus luciferensis* LMG 18422^{T}	16S rRNA
KJ569088.1	*Bacillus megaterium* IAM 13418^{T}	16S rRNA
NR_040985.1	*Bacillus methanolicus* NCIMB 13113^{T}	16S rRNA
AJ542512.1	*Bacillus novalis* LMG 21837^{T}	16S rRNA
NR_026145.1	*Bacillus pseudalcaliphilus* DSM 8725^{T}	16S rRNA
NR_026139.1	*Bacillus pseudofirmus* DSM 8715^{T}	16S rRNA
NR_043242.1	*Bacillus pumilus* ATCC 7061^{T}	16S rRNA
KJ812207.1	*Bacillus subtilis* subs. *subtilis* DSM 10^{T}	16S rRNA
NR_025595.1	*Bacillus gelatini* LMG 21880^{T}	16S rRNA
NR_040794.1	*Geobacillus stearothermophilus* IFO12550^{T}	16S rRNA
NC_004722.1	*Bacillus cereus* ATCC 14579^{T}	WGS
NC_003997.3	*Bacillus anthracis* Ames	WGS
NZ_CM000753.1	*Bacillus thuringiensis* ATCC 10792^{T}	WGS
NZ_CP009746.1	*Bacillus weihenstephanensis* WSBC 10204^{T}	WGS
NZ_CM000745.1	*Bacillus pseudomycoides* DSM 12442^{T}	WGS
NZ_CM000742.1	*Bacillus mycoides* ATCC 6462^{T}	WGS
NC_009674.1	*Bacillus cytotoxicus* NVH 391-98^{T}	WGS
CP006863.1	*Bacillus toyonensis* BCT-7112^{T}	WGS
KU198626/SRX1297474	*Bacillus wiedmannii* sp. nov. FSL W8-0169^{T}	16S rRNA/WGS

Table S2. Predicted DNA-DNA hybridization (DDH) between *B. wiedmannii* sp. nov. type strain FSL W8-0169^T and representative strains of other *B. cereus* group species

DDH represents predicted DNA-DNA Hybridization values calculated using formula 2 of Genome-to-Genome Distance Calculator (GGDC 2.1; Meier-Kolthoff et al., 2013), which is recommended for draft genome analysis.

Isolate	DDH[a]	Model C.I.[b]	Bootstrap C.I.[b]	Distance	Prob.[c] DDH >= 70%
B. anthracis Ames	50.9	48.2 - 53.5%	50.8 - 50.9%	0.0697	21.52
B. cereus ATCC 14579^T	44	41.5 - 46.6%	44 - 44%	0.0871	6.74
B. cytotoxicus NVH 391-98^T	25.5	23.2 - 28%	25.5 - 25.5%	0.1703	0.01
B. mycoides DSM 2048^T	40.5	38 - 43%	40.5 - 40.5%	0.0978	3.06
B. pseudomycoides DSM 12442^T	27	24.7 - 29.5%	27 - 27.1%	0.1598	0.03
B. thuringiensis ATCC 10792^T	44	41.4 - 46.5%	44 - 44%	0.0872	6.68
B. toyonensis BCT-7112^T	44	41.5 - 46.6%	44 - 44%	0.087	6.75
B. weihenstephanensis WSBC 10204^T	40.4	37.9 - 42.9%	40.4 - 40.4%	0.0981	3
"Bacillus gaemokensis" KCTC 13318	26.3	24 - 28.8%	26.3 - 26.3%	0.1645	0.02
"B. manliponensis" JCM 15802	22.4	20.1 - 24.8%	22.4 - 22.4%	0.1959	0
"B. bingmayongensis" FJAT-13831	26.9	24.5 - 29.4%	26.9 - 26.9%	0.1608	0.03
FSL J3-0113	66.3	63.4 - 69.2%	66.3 - 66.4%	0.0414	70.68
FSL H7-0353	88.1	85.6 - 90.2%	88 - 88.1%	0.0142	95.09
FSL H8-0032	88	85.5 - 90.2%	88 - 88.1%	0.0143	95.09
FSL M7-0044	95.1	93.5 - 96.3%	95.1 - 95.1%	0.0066	97.22
FSL M7-0938	94.5	92.8 - 95.8%	94.5 - 94.5%	0.0072	97.08
FSL M7-1251	88.8	86.3 - 90.8%	88.8 - 88.8%	0.0135	95.36
FSL P2-0415	89.2	86.8 - 91.2%	89.2 - 89.2%	0.013	95.51
FSL P2-0558	89.7	87.4 - 91.7%	89.7 - 89.7%	0.0124	95.7
FSL P4-0569	88.5	86 - 90.6%	88.5 - 88.5%	0.0138	95.25
FSL K6-0069	57.9	55.1 - 60.7%	57.9 - 57.9%	0.0553	45.24

[a] DDH represents predicted DNA-DNA Hybridization values calculated using formula 2, which is appropriate for draft genome analysis, as it does not depend on the genome length.

[b] C.I. is estimated confidence interval.

[c] Prob. is probability of DDH being equal or above 70%, which is set as a species cut-off

Table S3. Results of phenotypic characterization of all *Bacillus wiedmannii* sp. nov. strains included in this study. Only carbohydrates for which acid production was observed for at least one of the strains, are reported. A "+" indicates the isolate was positive for a given characteristic, and "-" indicates the isolate was negative for a given characteristic. The *B. cereus* ATCC 14579 was included as a control.

			General Characteristics														
Isolate ID	**Source**	**Cytotoxicity**[a]	**Growth Temperature Range**	**Growth Range of pH**	**Maximum % NaCl (w/v)**	**Oxidase**	**Catalase**	**Casein Hydrolysis**	**Starch Hydrolysis**	**NHE Duopath**	**HBL Duopath**	**PI-PLC activity**	**Hemolysis 35°C**	**Lecithinase activity**	**Anaerobic growth 30°C**	**Motility 30°C**	**Nitrate Reductio**
FSL H7-0353	Pasteurized milk	+	5-43°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	-	-
FSL H8-0032	Pasteurized milk	+	5-43°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	+	-
FSL P2-0415	Raw milk	+	10-43°C	pH 5-10	2%	-	+	+	+	+	+	+	+	+	+	+	+
FSL P2-0558	Raw milk	+	5-43°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	+	+
FSL P4-0569	Raw milk	+	5-40°C	pH 5-10	2%	-	+	+	+	+	-	+	+	+	+	+	+
FSL M7-0044	Raw milk	+	10-43°C	pH 5-10	2%	-	+	+	+	+	+	+	+	+	+	+	+
FSL M7-0938	Raw milk	+	10-40°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	+	+
FSL M7-1251	Raw milk	+	10-40°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	+	+
FSL W8-0169	Raw milk	+	5-43°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	+	+
FSL J3-0113	Pasteurized milk	+	10-40°C	pH 5-10	2%	-	+	+	+	+	-	+	+	+	+	+	+
FSL K6-0069	Raw milk	+	10-40°C	pH 5-10	5%	-	+	+	+	+	-	+	+	+	+	+	-
ATCC 14579	Air in a cow shed	+	10-45°C	pH 5-10	5%	-	+	+	+	+	+	+	+	+	+	+	+

Table S3 continued.

Isolate ID	API 20E Biochemical Characterizations										
	ONPG	ADH	LDC	ODC	CIT	H2S	Urea	TDA	Indole	VP	Gelatinase
FSL H7-0353	-	-	-	-	+	-	-	-	-	+	+
FSL H8-0032	-	-	-	-	-	-	-	-	-	+	+
FSL P2-0415	-	-	-	-	+	-	-	-	-	+	+
FSL P2-0558	-	-	-	-	+	-	-	-	-	+	+
FSL P4-0569	-	-	-	-	-	-	-	-	-	+	+
FSL M7-0044	-	-	-	-	-	-	-	-	-	+	+
FSL M7-0938	-	-	-	-	-	-	-	-	-	+	+
FSL M7-1251	-	-	-	-	+	-	-	-	-	+	+
FSL W8-0169	-	-	-	-	+	-	-	-	-	+	+
FSL J3-0113	-	-	-	-	-	-	-	-	-	+	+
FSL K6-0069	-	-	-	-	+	-	-	-	-	+	+
ATCC 14579	-	+	-	-	-	-	-	-	-	+	+

Table S3 continued.

	50CH Biochemical Characterizations[b]															
Isolate ID	**D-ribose**	**D-glucose**	**D-fructose**	**D-mannose**	**N-acetyl glucosamine**	**Amygdalin**	**Arbutin**	**Esculin ferric citrate**	**salicin**	**D-cellobiose**	**D-maltose**	**D-Saccharose**	**D-trehalose**	**Amidon**	**Glycogen**	**Potassium gluconate**
FSL H7-0353	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
FSL H8-0032	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
FSL P2-0415	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-
FSL P2-0558	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
FSL P4-0569	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
FSL M7-0044	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
FSL M7-0938	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
FSL M7-1251	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
FSL W8-0169	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
FSL J3-0113	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	-
FSL K6-0069	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
ATCC 14579	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-