Supporting information------

A cellular logic circuit for the detection of bacterial pore-forming toxins

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Content list

Experimental Section

B. cereus toxin production

The wildtype *B. cereus* reference strains for Nhe, Hbl and CytK productions were designated MHI 1491, MHI 1505 and MHI 1307 in this work (1, 2). MHI means *B. cereus* strain collection of the Chair of Hygiene and Technology of Milk, LMU Munich. Caseinhydrolysat-Glucose-Yeast (CGY) medium plus 1% glucose was used for the toxin production according to the previous publication (3). Briefly, a single colony of *B. cereus* on the overnight cultured blood agar was inoculated into 20 mL CGY medium containing 1% glucose, then cultured in water bath for 17 h at 32 °C, subsequently transferred 0.1 mL of the culture liquid into a new 20 mL CGY medium containing 1% glucose for another 6 h incubation at 32 °C. 200 µL 0.1 M EDTA was added to the final concentration 1 mM before the harvest of toxin supernatants. The mixture was collected by centrifugation at 3 000 g for 20 min, at 4 °C, then passed through 0.22 µm Millipore filter to get bacteria-free supernatants for the further use.

Vero cells and culture condition

Vero cell line was purchased from the European Collection of Cell Cultures (ECACC). The culture condition Vero cells were recommended by the suppliers and incubated at 37 °C with 7% CO_2 (4). Minimum Essential Medium (MEM) Eagle was used for Vero cells, supplemented additional nutritional factors such as 1% fetal bovine serum (FBS), 1% sodium pyruvate and penicillin-streptomycin for the inhibition of bacterial growth.

Cytotoxicity detection assays

Cytotoxic effects of the different supernatants from *B. cereus* were performed on Vero cells using simultaneous incubation method with three different methods for detection as shown in Scheme 1a, WST assay (water-soluble tetrozolium salt, Roche Diagnostics), LDH assay (lactate dehydrogenase, Roche Diagnostics) and PI assay (propidium iodide, Fluka). The schemes of simultaneous incubation method for each assay are shown in Fig. S2. Briefly, the serially diluted toxin supernatants and Vero cells (1×10^4 cells/well) were seeded on 96-well microplates with the final culture medium volume 200 µL per well, and incubated at 37 °C with 7% CO_2 atmosphere for 18 h, then the morphology of Vero cells in the presence and absence of toxins were checked under inverted microscopy *in situ* (Biozero, Keyence), Fig. S1. Although the three mentioned above include the same procedure for cytotoxicity assays using simultaneous method by mixing different toxins with Vero cells and cultured for 24 h, the quantification of cellular viability are based on different principles. LDH which is a stable cytoplasmic enzyme present in all cells, were rapidly released into the cell culture medium through the pore formation on cell membrane with PFTs treatment. After centrifugation, LDH in the cell-free supernatants was transferred to a new 96-well microplate and subsequently was used for catalyzing its specific substrate with a dramatic color change in solution detected at 490 nm, Fig. S2a. WST assay is based on the presence of WST reduced by the activity of mitochondria of viable cells and results in color

changes (from red to yellow) for colorimetric detection as well at 450 nm Fig. S2b, (5). However, PI measurement is detected by fluorescence changes. Because PI can penetrate the damaged plasma membrane and binds to DNA in the nucleus with a large Stokes shift of the fluorescent probe, which can be measured at the excitation and emission wavelengths of 535 nm and 617 nm, respectively, Fig. S2c. The dose dependent manners were used to evaluate the 50% inhibitory concentrations (IC50) of the tested toxins by linear interpolation. The cytotoxic titers were defined as the highest serial dilution of the tested toxins that resulted in the 50% cells lost their viability, comparing with the parallel control.

PCR and ELISA

The *nhe, hbl* and *cytk* genes in each strain were detected according to the previous reports (2). The presence of each component of Nhe and Hbl complexes were deciphered by different monoclonal antibodies (mAb) in two types of ELISA (indirect and sandwich ELISAs), as shown in Fig. S5a. NheA (mAb 1A8), Hbl L1 (1E9) and Hbl B (1B8) were detected by indirect ELISAs, however, NheB (2B11 and 1E11), NheC (3D6 and 1E11) and Hbl L2 (1A12 and 8B12) were detected by sandwich ELISAs (6, 7). Subsequently, the horseradish peroxidase (HRP) conjugates were used to catalyze the TMB substrates (3,3',5,5'-tetramethylbenzidine) and the reaction was stopped by 1 M H_2SO_4 for colorimetric detection at 450 nm. Due to the lack of proper antibodies against CytK, so there was no available ELISA for this component in this work.

Table

	Strain (MHI)	Gene			Nhe			Hbl			Cellular Circuit
		nhe	*hbl*	*cytk*	A	B	C	L1	L2	B	
	1491	+			+	+	+				1
Ref.	1505	+	+		+	+	+	+	+	+	1
	1307	+		+							1
1	1475	+	+	+	+	+	+	+	+	+	1
2	1489	+			+	+	+				1
3	1490	+	+	+	+	+	+	+	+	+	1
4	1501	+	+		+	+	+	+	+	+	1
5	1503	+			+	+	+				1
6	1513	+	+	+	+	+	+	+	+	+	1
7	1522	+		+	+	+	+				1
8	1556	+	+								0
9	1647	+			+						0
10	1669	+	+	+	+	+	+	+	+	+	1
11	1672	+			+	+					0
12	1676	+		+							1
13	1678	+	+		+	+	+	+	+	+	1
14	1700	+									0
15	1761	+				+	+				0
16	2964	+			+	+	+				1
17	2968	+		+		+	+				0
18	2969	+	+		+	+	+	+	+	+	1
19	2971	+		+	+	+	+				1
20	3016	+	+	+	+	+	+	+	+	+	1
	Total	**20**	**9**	**9**	**15**	**15**	**14**	**8**	**8**	**8**	**14**

Table S1 Comparison of the presence of PFT productions of 20 *B. cereus* strains isolated from food related samples at genetic, proteinic and cellular levels. *nhe*,

hbl and cytk genes were detected by PCR; the proteins of NheA, B and C, Hbl L1, L2 and B were detected by different forms of ELISA with specific monoclonal antibodies against each components; results of cellular circuit were based on the cytotoxicty assay of Vero cells.

Note: Ref., the reference strains of *B. cereus*; "+", positive detection.

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Figures

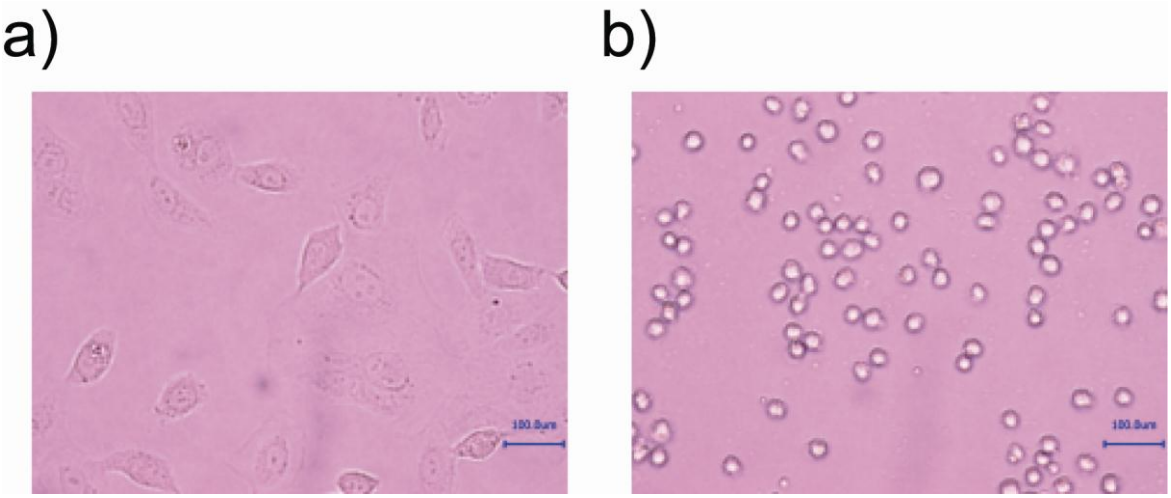


Figure S1 Morphologies of Vero cells in the absence (a) and presence (b) of the complete Nhe complex were shown as a model under an inverted microcopy *in situ*. The rose-colored background is due to the intrinsic color of MEM Eagle medium.

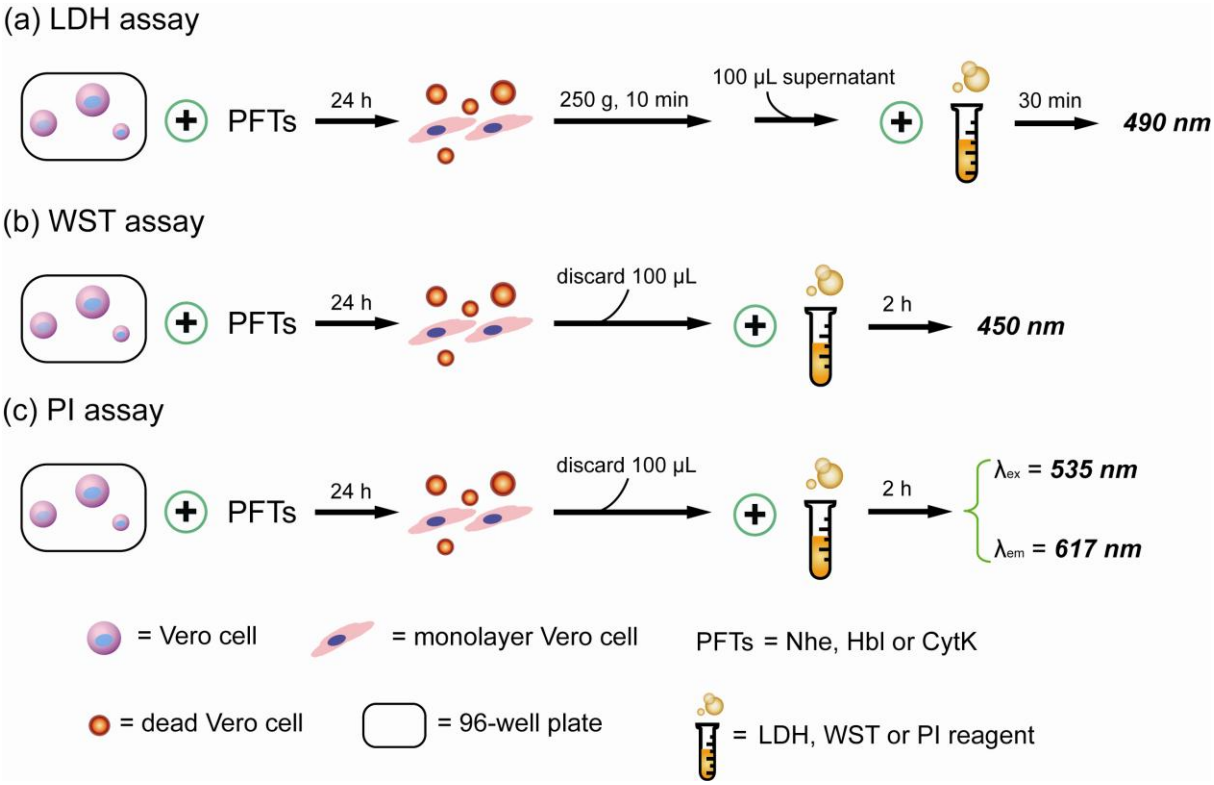


Figure S2 Schematic representation of the simultaneous methods used for cytotoxicity assays in the present work, (a) LDH assay, (b) WST assay and (c) PI assay.

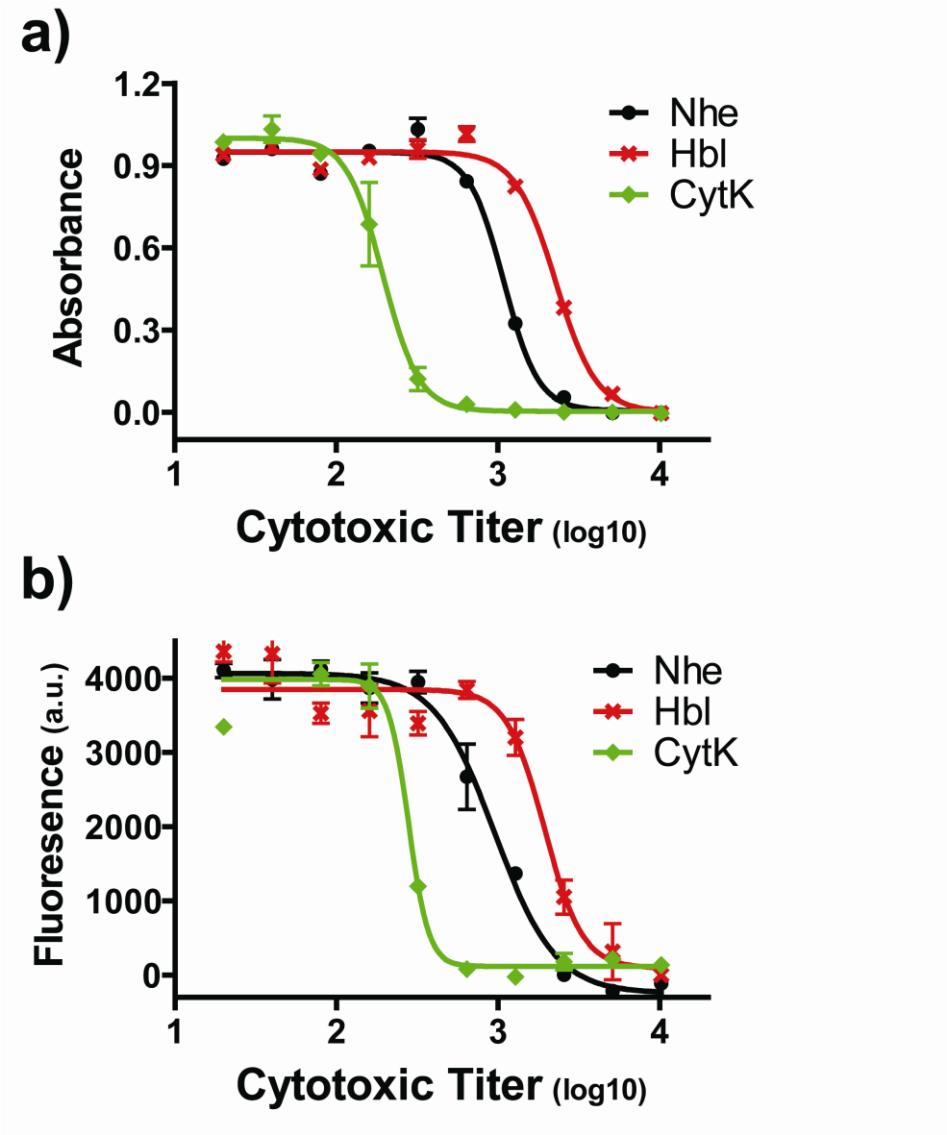


Figure S3 Standard curves of the reference strains with Nhe, Hbl and CytK productions for the cytotoxicity of Vero cells based on LDH assays (a) and PI assay (b).

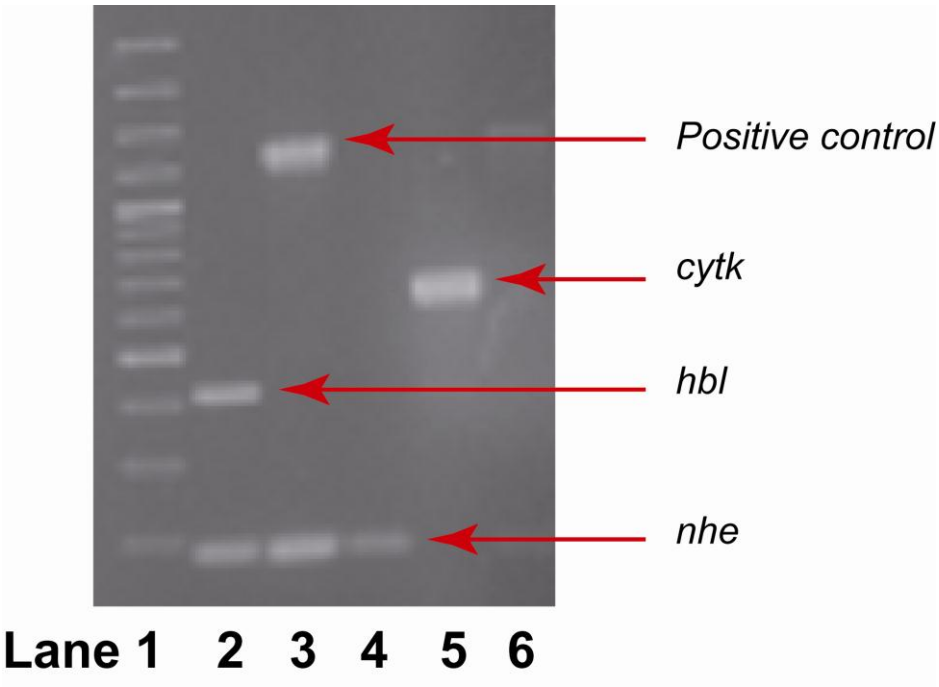
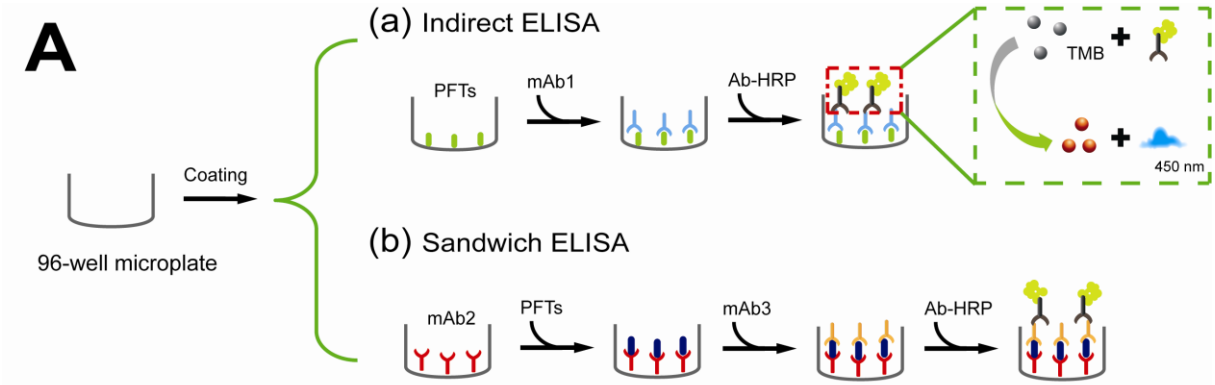


Figure S4 The presence of *nhe*, *hbl* and *cytk* genes were detected by PCR in *B. cereus* reference strains. Lane 1, DNA marker; Lane 2, MHI 1505 (*hbl* positive); Lane 4, MHI 1491 (*nhe* positive) and Lane 5, MHI 1307 (*cytk* positive); Lane 3 and Lane 6 were positive and negative controls, respectively. Due to the gene variant encoding Nhe in MHI 1307, *nhe* gene was not detected in the current PCR protocol for the generally designed primers.



B

mAb	Nhe			Hbl		
	A	B	C	L1	L2	B
1A8	+					
1E11		+	+			
2B11		+				
3D6			+			
1E9				+		
1A12					+	
8B12					+	
1B8						+

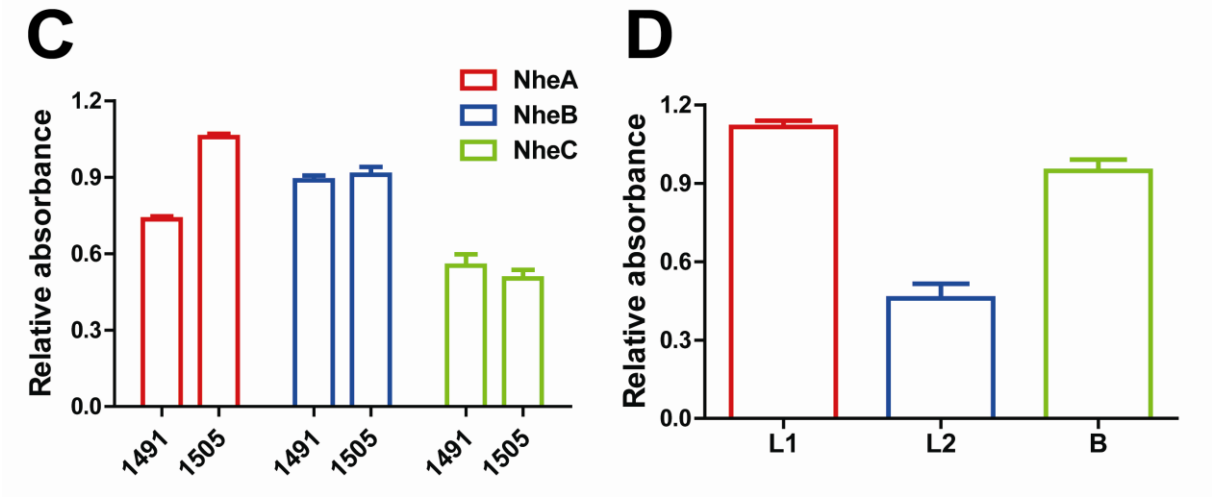


Figure S5 (A) schematic representation of two formats of ELISAs for the detection of different components in Nhe and Hbl complexes from *B. cereus*, (a) indirect ELISA and (b) sandwich ELISA; (B) diverse monoclonal antibodies (mAbs) involved for the specific binding to NheA (1A8), Hbl L1 (1E9) and Hbl B (1B8) with indirect ELISAs, NheB (2B11 and 1E11), NheC (3D6 and 1E11)

and Hbl L2 (1A12 and 8B12) with sandwich ELISA; (C) the presence of NheA, B and C in the reference strains of MHI 1491 (for Nhe production) and MHI 1505 (for Hbl production); and (D) the presence of Hbl in MHI 1505.

References

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