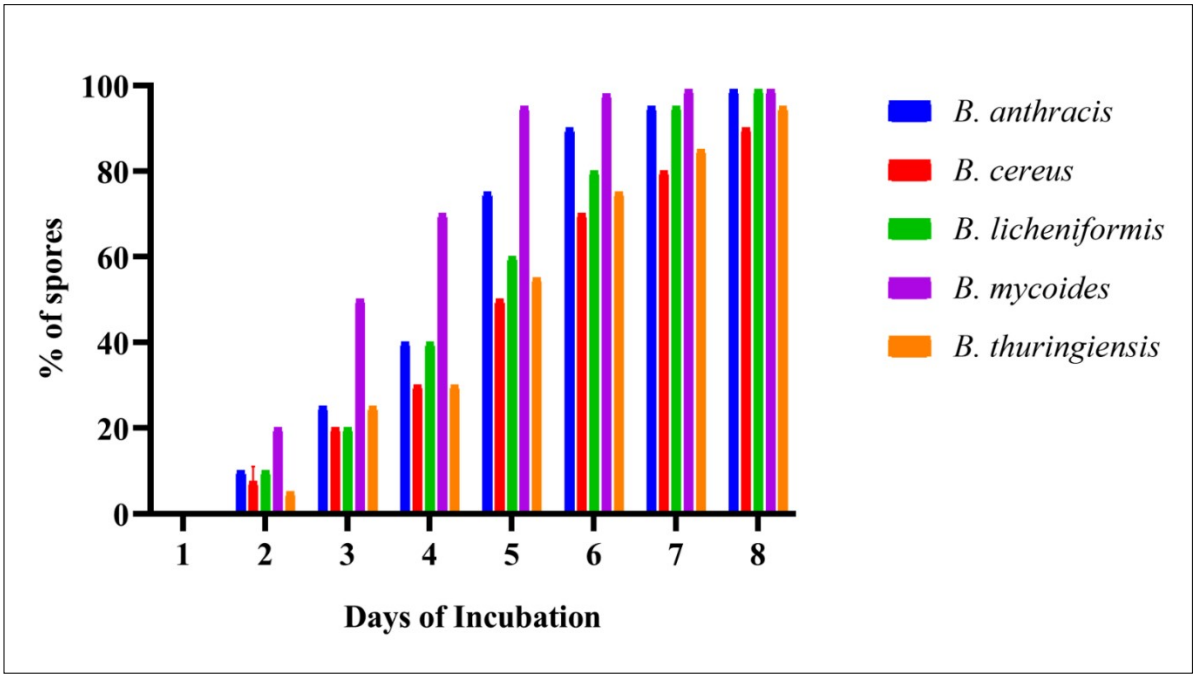
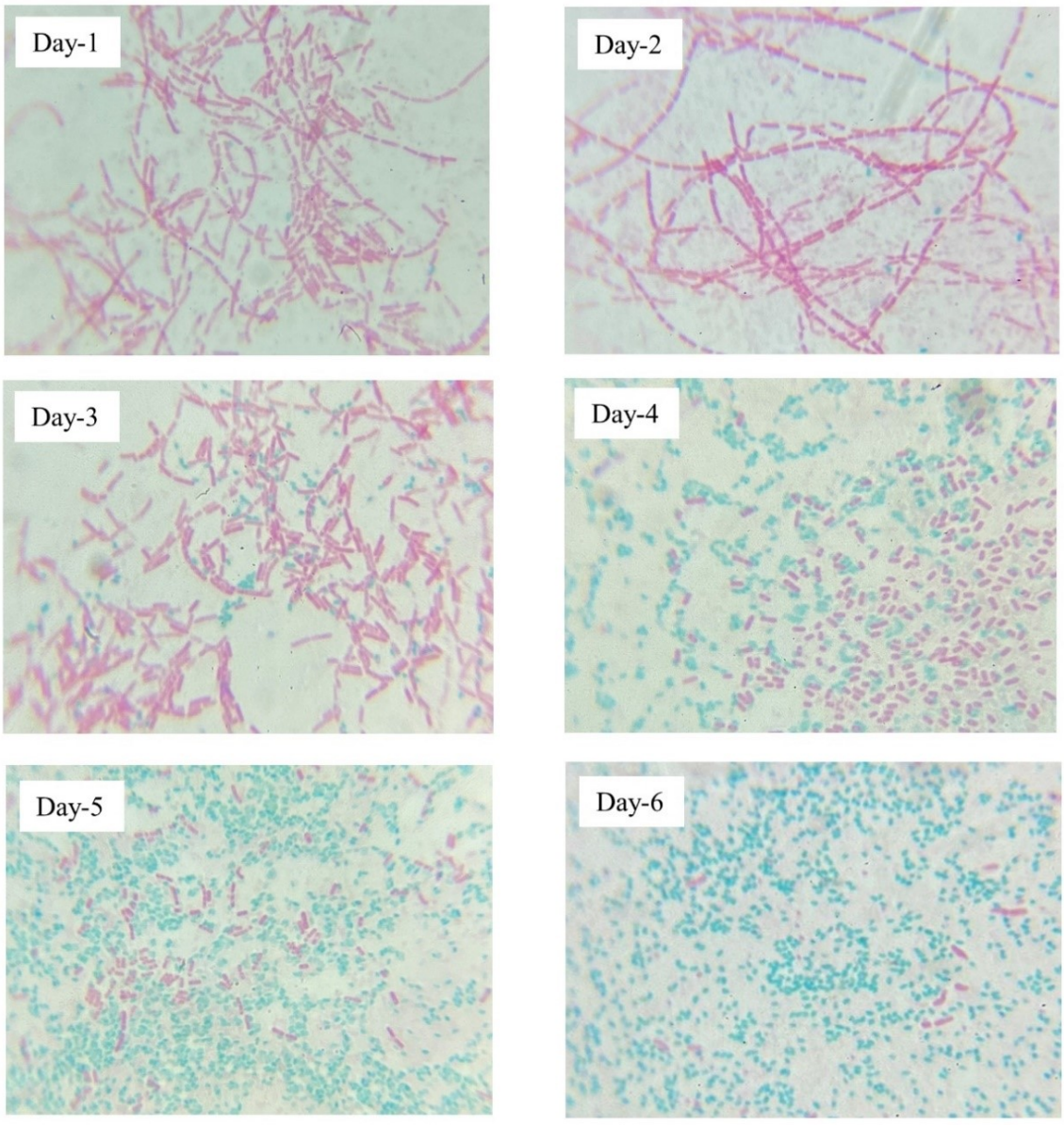
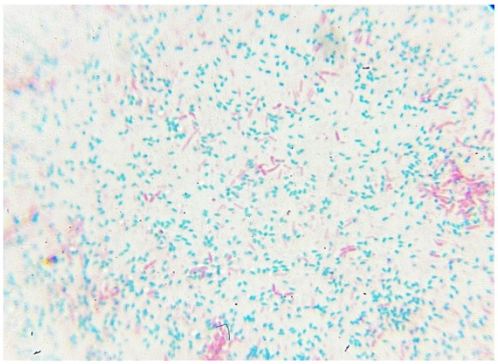


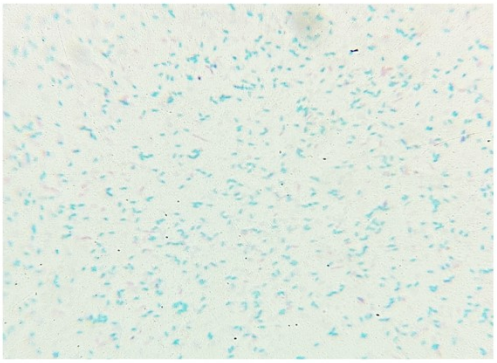
Supplementary Fig. 1 Sporulation percentage of different *Bacillus* spp. at different time-points



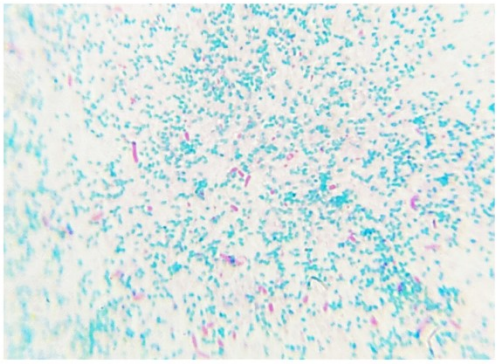
Supplementary Fig. 2a *B. anthracis* spore preparation (Schaffer and Fulton Spore Staining)



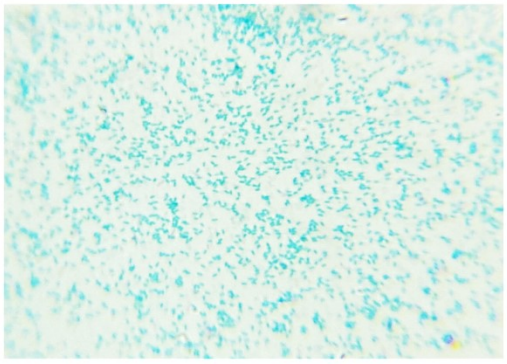
B. cereus



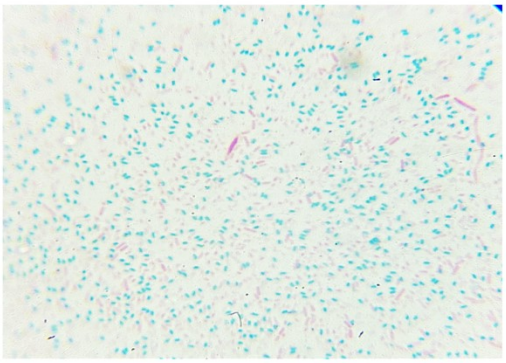
B. licheniformis



B. anthracis

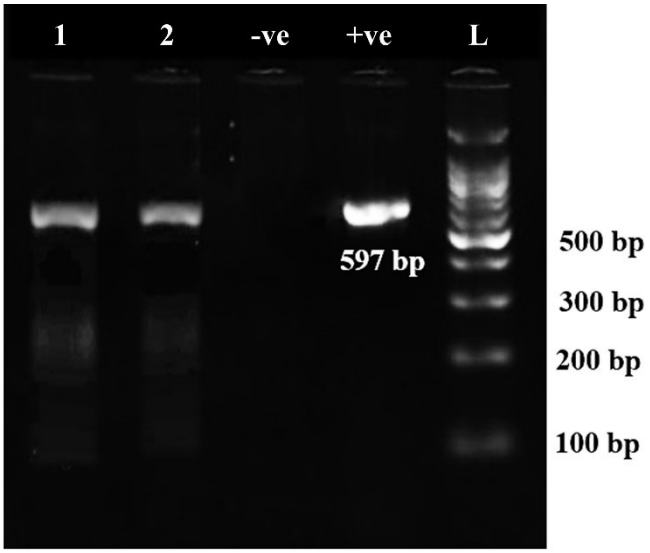


B. mycoides

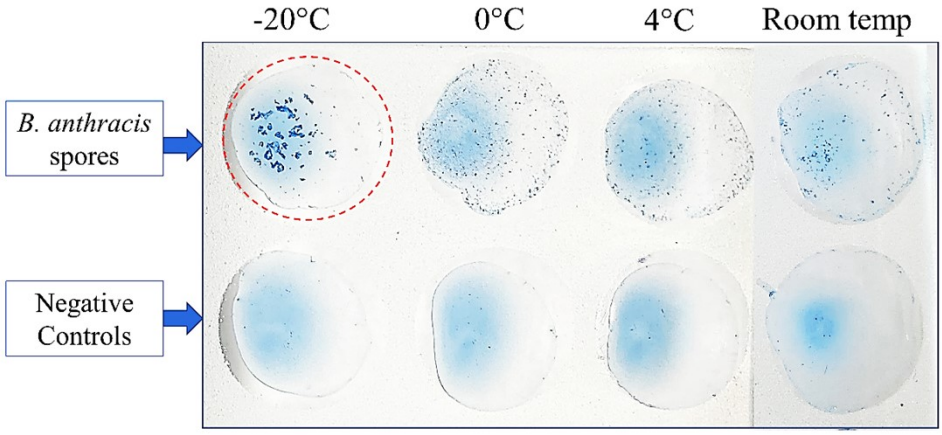


B. thuringiensis

Supplementary Fig. 2b Spores of different *Bacillus* spp. after 5-6 days of incubation (100X magnified)

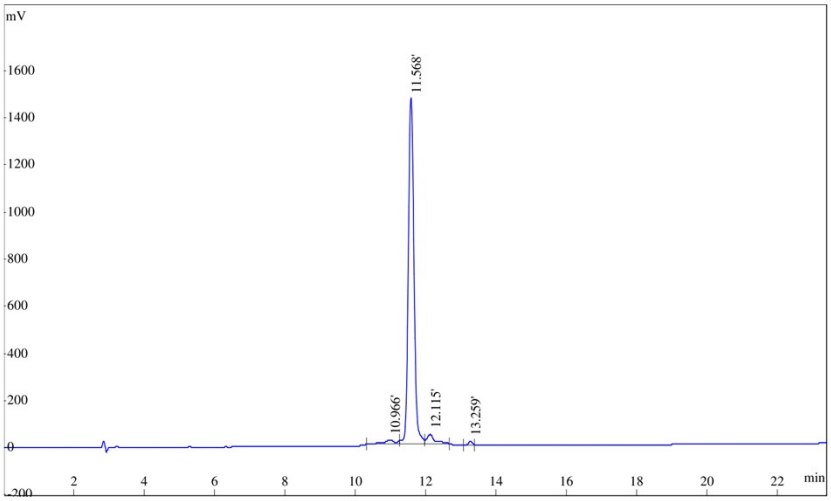


Supplementary Fig. 3a PCR targeting the *pag* gene with DNA extracted from inactivated and live spores. 1- live spores. 2- UV-inactivated spores. -ve – Negative control. +ve- Positive control.



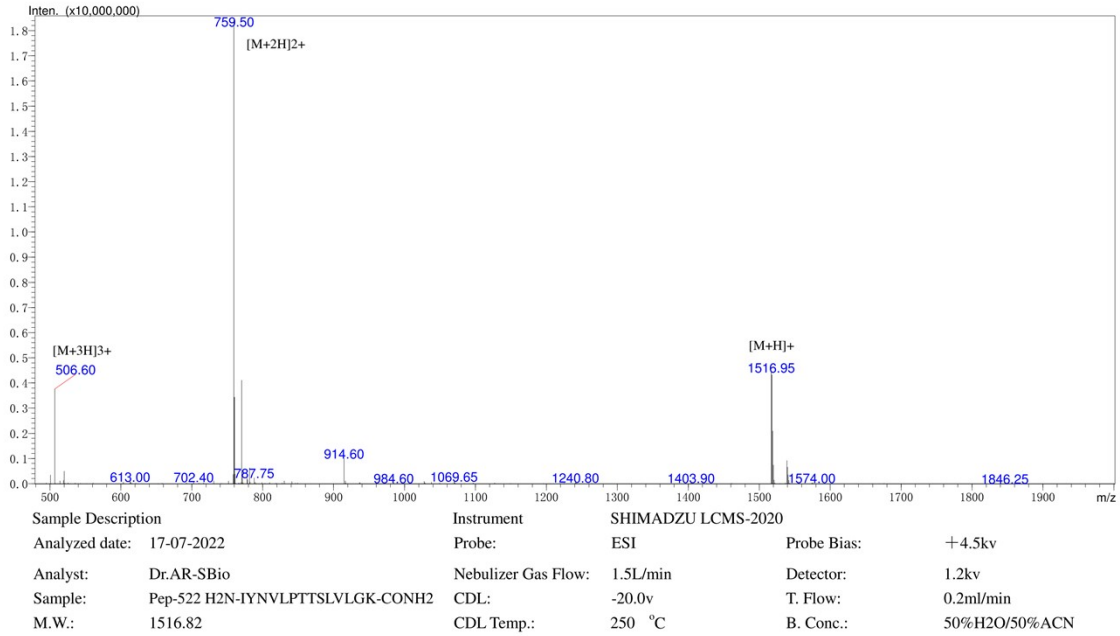
Supplementary Fig. 3b Agglutination pattern of inactivated *B. anthracis* spores stored at different temperatures (After 2 weeks of storage)

Sample:	Pep-522 H2N-IYNVLPTTSLVLGK-CONH2		Analyzed date: 16-07-2022
Analyst:	Dr.RS-SBio		
Column:	Kromasil-C18, 4.6*250mm, 5µm		
Solvent A	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile		
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water		
Gradient:		A	B
	0.0min	24%	76%
	25.0min	49%	51%
	25.1min	100%	0%
	30.0min		Stop
Volume:	10µl		
Wavelength:	220nm		
Flow rate:	1.0ml/min		



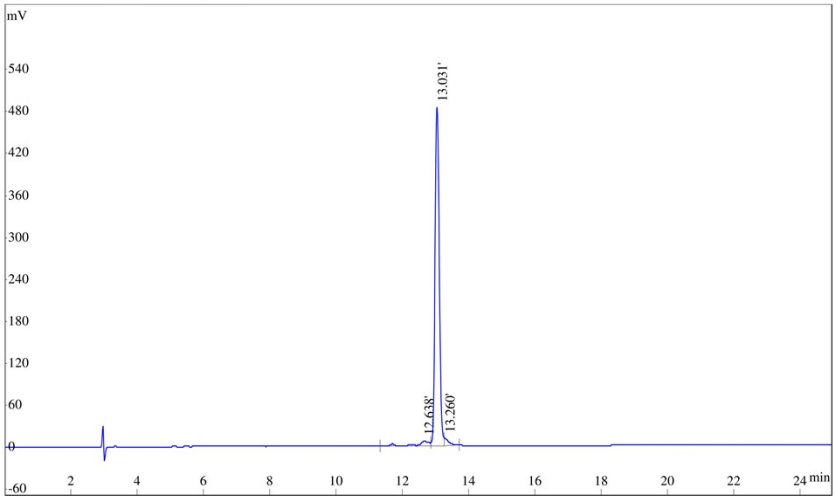
Rank	Time	Conc.	Area	Height
1	10.966	2.665	440952	16966
2	11.568	92.68	15333472	1464288
3	12.115	3.903	645647	40098
4	13.259	0.7508	124209	15089
Total		100	16544280	1536441

Supplementary Fig. 4 HPLC report of PA-1 peptide



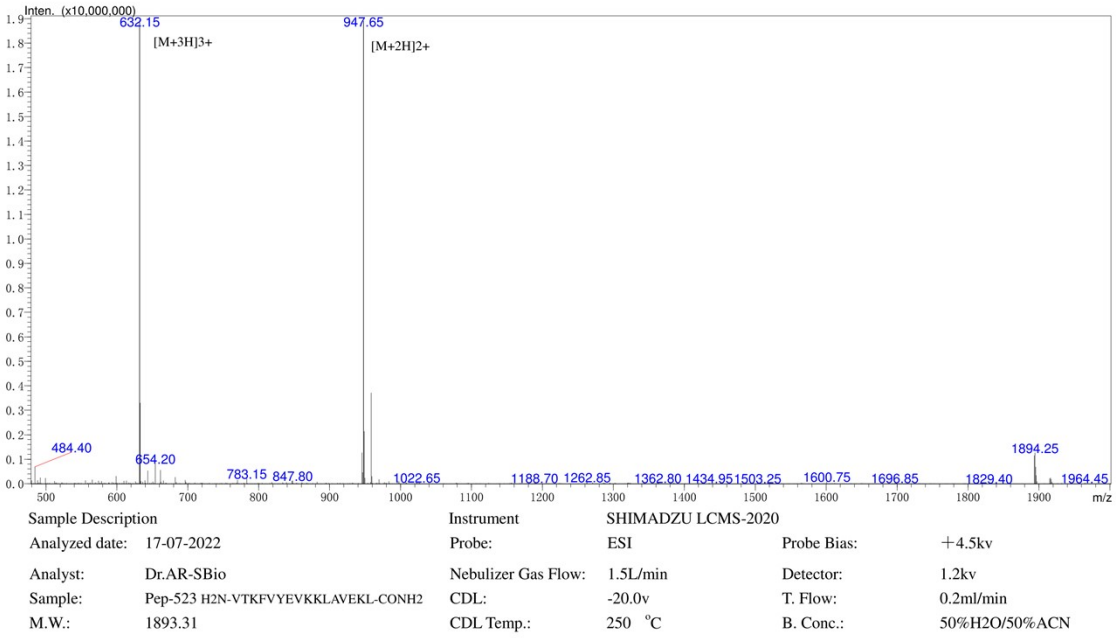
Supplementary Fig. 5 Mass spectrophotometry report of PA-1 peptide

Sample:	Pep-523 H2N-VTKFVYEVKKLAVEKL-CONH2		Analyzed date: 16-07-2022
Analyst:	Dr.RS-SBio		
Column:	Kromasil-C18, 4.6*250mm, 5μm		
Solvent A	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile		
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water		
Gradient:		A	B
	0.0min	24%	76%
	25.0min	49%	51%
	25.1min	100%	0%
	30.0min		Stop
Volume:	10μl		
Wavelength:	220nm		
Flow rate:	1.0ml/min		



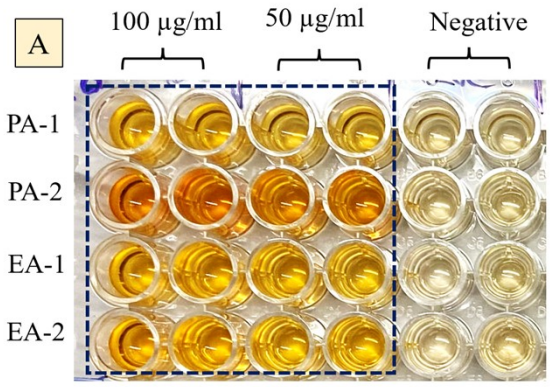
Rank	Time	Conc.	Area	Height
1	12.638	3.624	151447	6043
2	13.031	94.23	3938412	481815
3	13.260	2.145	89629	10588
Total		100	4179488	498446

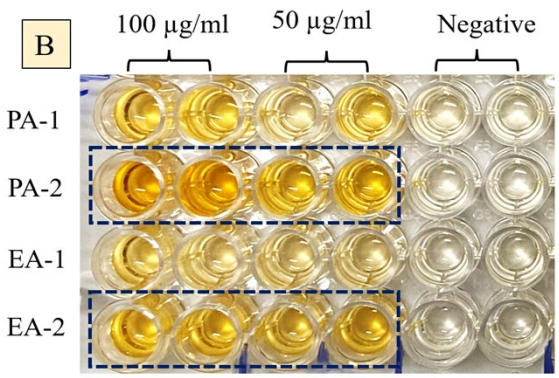
Supplementary Fig. 6 HPLC report of EA-1 peptide

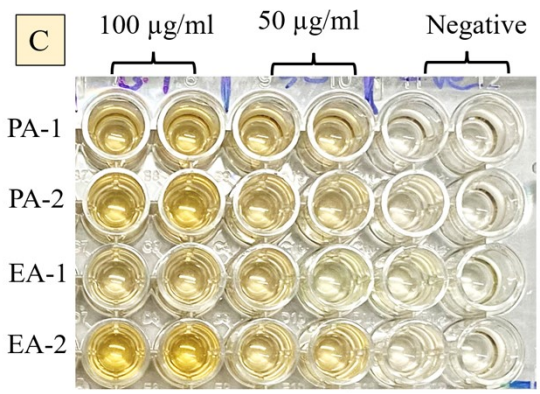


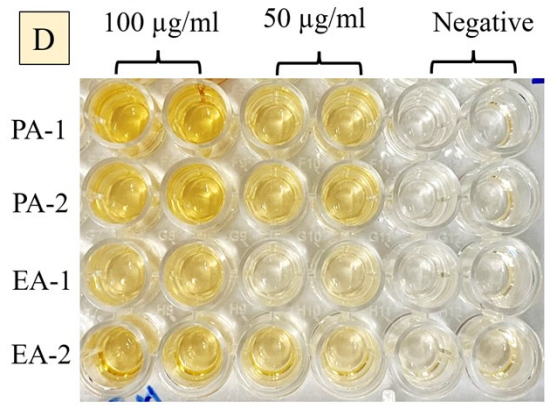
Sample Description		Instrument	SHIMADZU LCMS-2020		
Analyzed date:	17-07-2022	Probe:	ESI	Probe Bias:	+4.5kv
Analyst:	Dr.AR-SBio	Nebulizer Gas Flow:	1.5L/min	Detector:	1.2kv
Sample:	Pep-523 H2N-VTKFVYEVKKLAVEKL-CONH2	CDL:	-20.0v	T. Flow:	0.2ml/min
M.W.:	1893.31	CDL Temp.:	250 °C	B. Conc.:	50%H2O/50%ACN

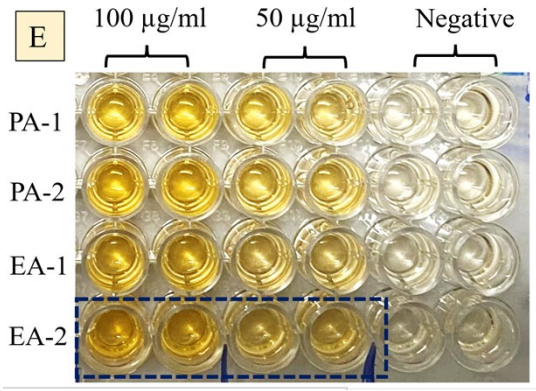
Supplementary Fig. 7 Mass spectrophotometry report of EA-1 peptide


A
100 µg/ml
50 µg/ml
Negative
PA-1
PA-2
EA-1
EA-2

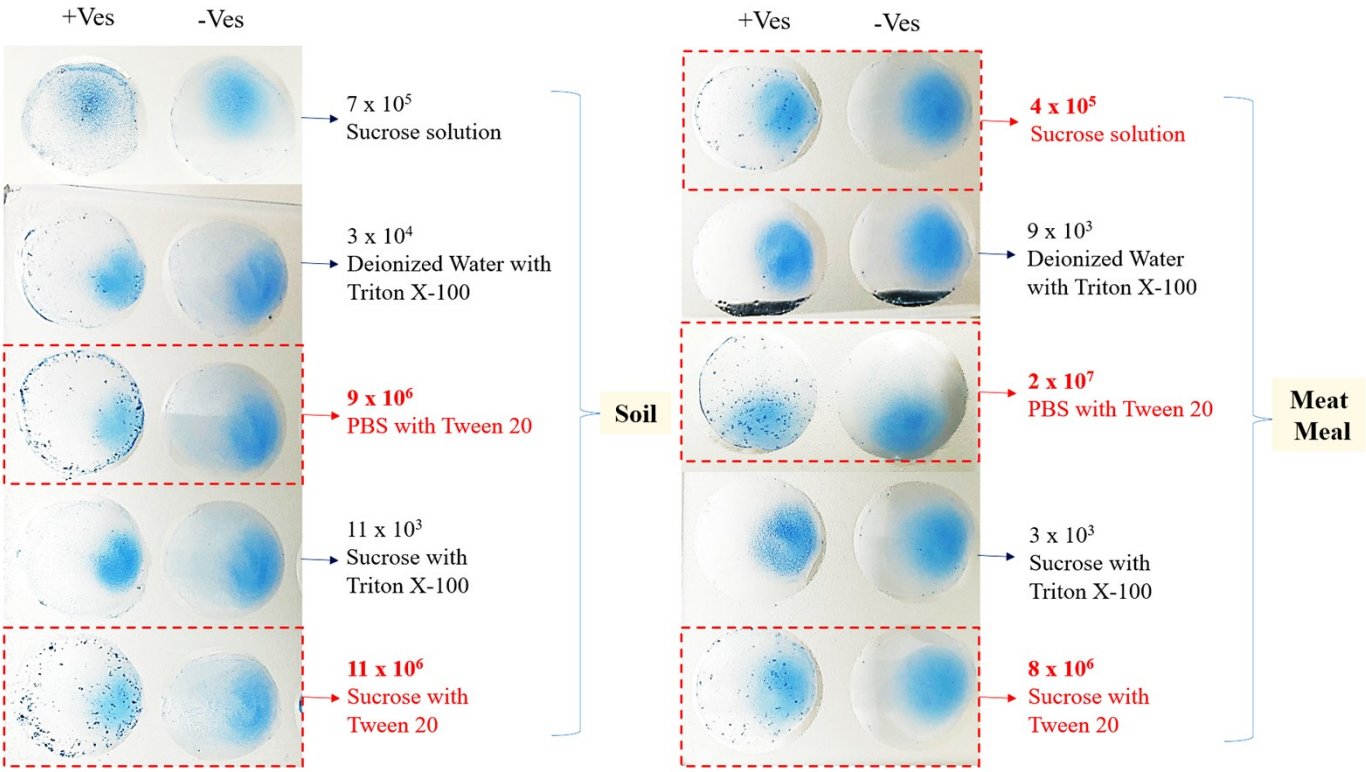

B
100 µg/ml
50 µg/ml
Negative
PA-1
PA-2
EA-1
EA-2


C
100 µg/ml
50 µg/ml
Negative
PA-1
PA-2
EA-1
EA-2


D
100 µg/ml
50 µg/ml
Negative
PA-1
PA-2
EA-1
EA-2


E
100 µg/ml
50 µg/ml
Negative
PA-1
PA-2
EA-1
EA-2

Supplementary Fig. 8 Specificity of the peptide-derived IgY antibodies using in-house-developed indirect ELISA. A- Coating plates with inactivated spores of *B. anthracis*. B- Coating plates with inactivated spores of *B. cereus*. C- Coating plates with inactivated spores of *B. thuringiensis*. D- Coating plates with inactivated spores of *B. licheniformis*. E- Coating plates with inactivated spores of *B. mycoides*.



Supplementary Fig. 9 Performance of LAT with the spores recovered through the GABRI technique using different carrier media

Supplementary Table 1 Details of the primers used in this study

Target Gene	Primer sequence	Product Size (bp)	Reference
pag (Protective antigen)	F: 5'-TCCTAACACTAACGAAGTCG-3'	596	[6, 40]
	R: 5'-CAGGTAGAAGGATATACGGT-3		

Supplementary Table 2 Absorbance (OD) and cut-off values of in-house designed indirect ELISA

Bacillus spp.	Peptide	Mean absorbance at 592 nm			Cut-off Value
		50 µg/ml	100 µg/ml	–ve control	
B. anthracis	**PA-1**	0.208	0.300	0.109	**0.219**
	PA-2	0.477	0.655	0.093	**0.189**
	EA-1	0.326	0.234	0.094	**0.201**
	EA-2	0.354	0.241	0.081	**0.172**
B. cereus	**PA-1**	0.140	0.180	0.091	**0.190**
	PA-2	0.210	0.363	0.078	**0.158**
	EA-1	0.146	0.125	0.088	**0.177**
	EA-2	0.219	0.193	0.070	**0.142**
B. thuringiensis	**PA-1**	0.110	0.118	0.067	**0.142**
	PA-2	0.093	0.121	0.067	**0.146**
	EA-1	0.096	0.089	0.067	**0.140**
	EA-2	0.103	0.087	0.054	**0.116**
B. mycoides	**PA-1**	0.155	0.187	0.093	**0.188**
	PA-2	0.147	0.177	0.089	**0.180**
	EA-1	0.190	0.140	0.099	**0.204**
	EA-2	0.201	0.132	0.080	**0.161**
B. licheniformis	**PA-1**	0.158	0.175	0.090	**0.183**
	PA-2	0.144	0.170	0.089	**0.180**
	EA-1	0.146	0.108	0.097	**0.201**
	EA-2	0.1640	0.125	0.097	**0.190**

Supplementary Table 3 Cost of production of the LAT assay

S. No.	Parameters	Concentration used for 100 reactions	Amount (₹)
1.	Cost of antibody (Peptide and IgY/IgG antibody raising)	1ml (200 µg/ml)	295/-
2.	Cost of latex beads	500 µL	800/-
3.	Cost of buffer (50 mM MES)	6.5 ml	190/-
4.	Cost of other reagents (PBS, Glycine, Sodium azide)	1X PBS, 0.1% Glycine, 0.1% Sodium azide	50/-
5.	Cost of Bovine serum Albumin (BSA)	1%	10/-
6.	Cost of EDAC buffer	50 mg	115/-
7.	Labor cost	500/- ×3 days	1500/-
8.	Electricity charges	@ 20 units	200/-
9.	Equipment cost	-	500/-
		Total cost/100 reactions	**3660/-**
		Total cost/reaction	**36.60/- (US $0.42)**