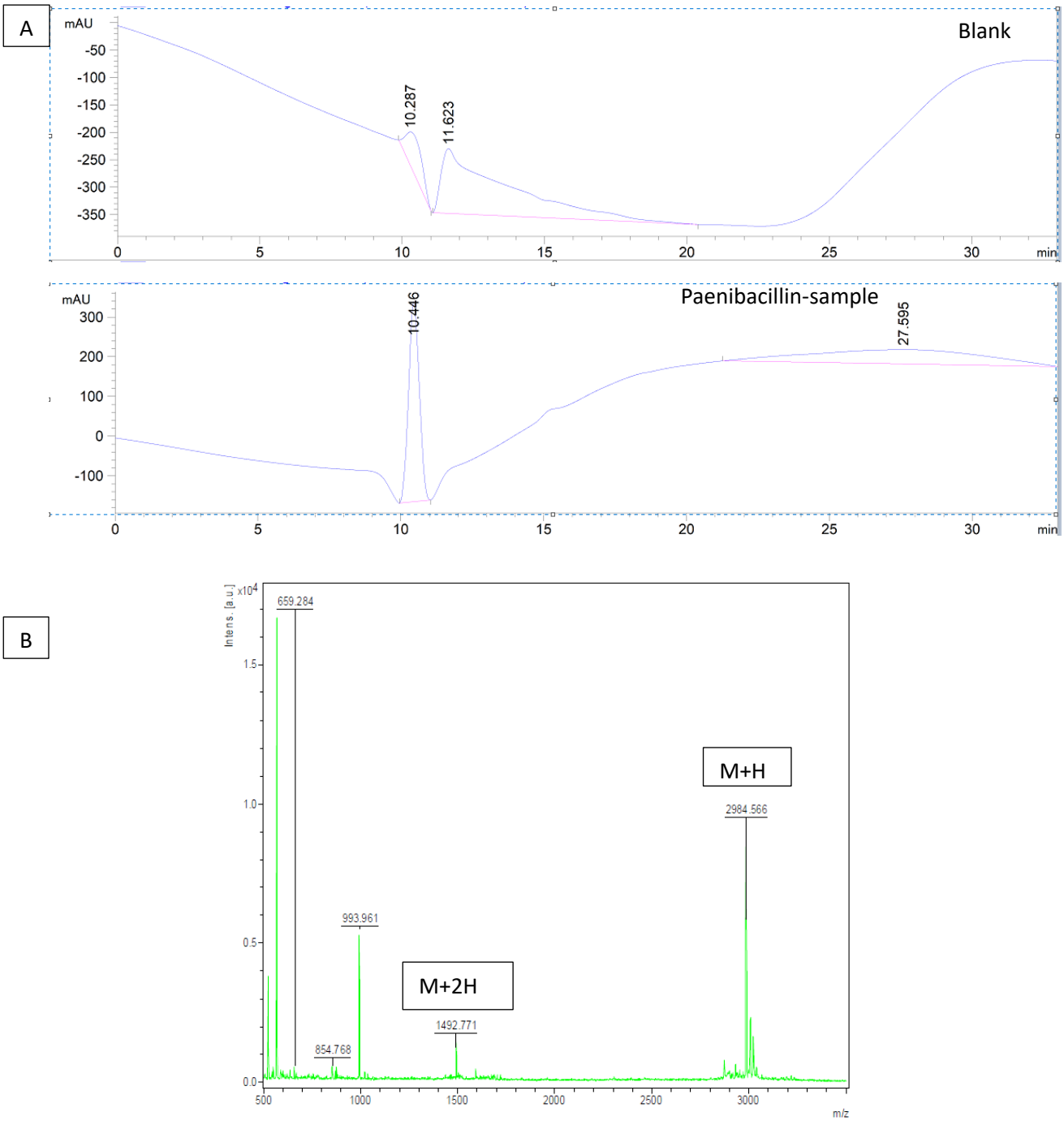
Supplementary file S1

1. Comparison of competitive paenibacillin ELISA to conventional assay

Supplementary Tabel S1: Comparison of the paenibacillin competitive ELISA and conventional bioassay for paenibacillin detection:

Assay criteria	Competitive ELISA	Conventional bioassay
Limit of detection of paenibacillin	15.6 ng/mL	20 µg/mL
Specificity	Highly specific (antibody-based)	cannot discriminate between co-produced antimicrobials
Detection agents	Specific polyclonal antibodies	Indicator bacterial strain
Repeatability	Repeatable due to low coefficient of variation among samples	Variable with the physiological status of the indicator strain
Sensitivity	Sensitive to low paenibacillin levels (nanograms)	Detect paenibacillin at microgram levels
Recovery from different matrices	78-91%	Prone to interference by media composition (e.g., acids).
Advantages	• Paenibacillin detection occurs in a timely manner. • Facilitates discovery of paenibacillin or similar peptides using the specific polyclonal antibodies generated. • Enables investigation of peptide biosynthesis and localization inside the cell. • Accurate quantification of paenibacillin at low levels.	• confirms the presence of antimicrobial compounds in the tested preparations.
Disadvantages	• The assay development is expensive and resource intensive.	• Assay results vary with the growth status of the indicator strain. • Selection of the indicator strains is a laborious process. • Th assay completion is time consuming. • Assay interpretation can be confounded by the presence of multiple antimicrobial compounds in the test samples.

2. Paenibacillin purity check using HPLC using area under the curve and excluding blank-related peaks and confirming the peptide molecular mass using MALDI-TOF.



Supplementary Figure S1. HPLC chromatograph (A) and MALDI-TOF analysis (B) for molecular mass confirmation of purified paenibacillin used for rabbit immunization.

3. ELISA specificity

The competitive ELISA has been conducted as detailed in the manuscript. Different antimicrobial peptides at the defined concentrations shown below were mixed with the anti-paenibacillin pAbs and incubated for one hour at 37°C. Anti-paenibacillin pAbs were mixed with water instead for the positive control. Portions of the mixture were added to pre-coated and blocked wells and the bound anti-paenibacillin pAbs were measured by adding goat anti-rabbit IgG-HRP and the signals were measured at 450 nm as described in the manuscript.

Supplementary Table S2: Reactivity of different antimicrobial peptides with anti-paenibacillin polyclonal antibodies as determined by the competitive ELISA.

Samples mixed with the polyclonal antibodies	**OD_{450}**
Antimicrobial-free control (Water)	0.309 ±0.038
Nisin (500 IU/mL)	0.263±0.004
Vancomycin (4 µg/mL)	0.242±0.004
Polymyxin (16 µg/mL)	0.268±0.005
Blank (No polyclonal antibodies nor paenibacillin)	0.053±0.001

Supplementary file S2

1. Primer sequences in the current study

Primer sequences, designed in this study, for paenibacillin biosynthetic genes used for the Real-Time PCR in the current study. The paenibacillin biosynthetic gene cluster (GenBank accession: JQ728481.1) from *Paenibacillus polymyxa* OSY-DF was used to design the primers using NCBI primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/; accessed on 10 August 2020).

Paenibacillin biosynthetic genes	Gene function	Primer sequences (5'-3')	PCR products (bp)
paeA	Paenibacillin prepropeptide	F: GCTATGAGGCCAGTGAGCTT R: TGGAACAAGAACCGGTACAGA	111
paeP	Peptidase	F: CGATTACGTCGGGCATGGTA R: CATTGGCGCAGTCAACCAAA	260
paeB	Dehydratase	F: GCCGGTTCAATTCGTGCTTT R: TCGAGGGGCTGGAGACATAA	224
paeC	Cyclase	F: ATGCAGGCTTGCTCCTGATT R: ACACCTGCACATCCATCGAG	190
paeI	Putative immunity protein	F: ACAGCCGAATGTGTCGGAAT R: TTTCCGCCCTCTCTTCAACC	204
paeT	Transporter	F: TTACACTGATTTCCGCCGCT R: GAGGAGCAGAGAAGACACGG	161
agrA	Response regulator	F: CGACGCTCTGTCTGCTTTCT R: AGTGCGATGACCACGAAAAC	151
agrB	Accessory gene regulator	F: CCATACCCAGAGACTCAAGGC R: ACCCGAAACAAATCCCTCCTC	77
agrC	Histidine kinase	F: TGGACGAGGGGATCAGAACT R: CAACTCCACGCTTCTTTGCC	186
agrD	Autoinducing peptide	F: CACGGTCATCTGTCTCCGAT R: TTTTGTGGGGAACACCCGTT	238
paeN	Putative acetylase	F: TGTGGTGCCTTACGGATTGG R: ACGCAAAGAGCTGAAATCGC	147
16S rRNA	Housekeeping gene	F: CTGCTTAGAGTGCCCAGCTT R: ACGCGAAGAACCTTACCAGG	189