

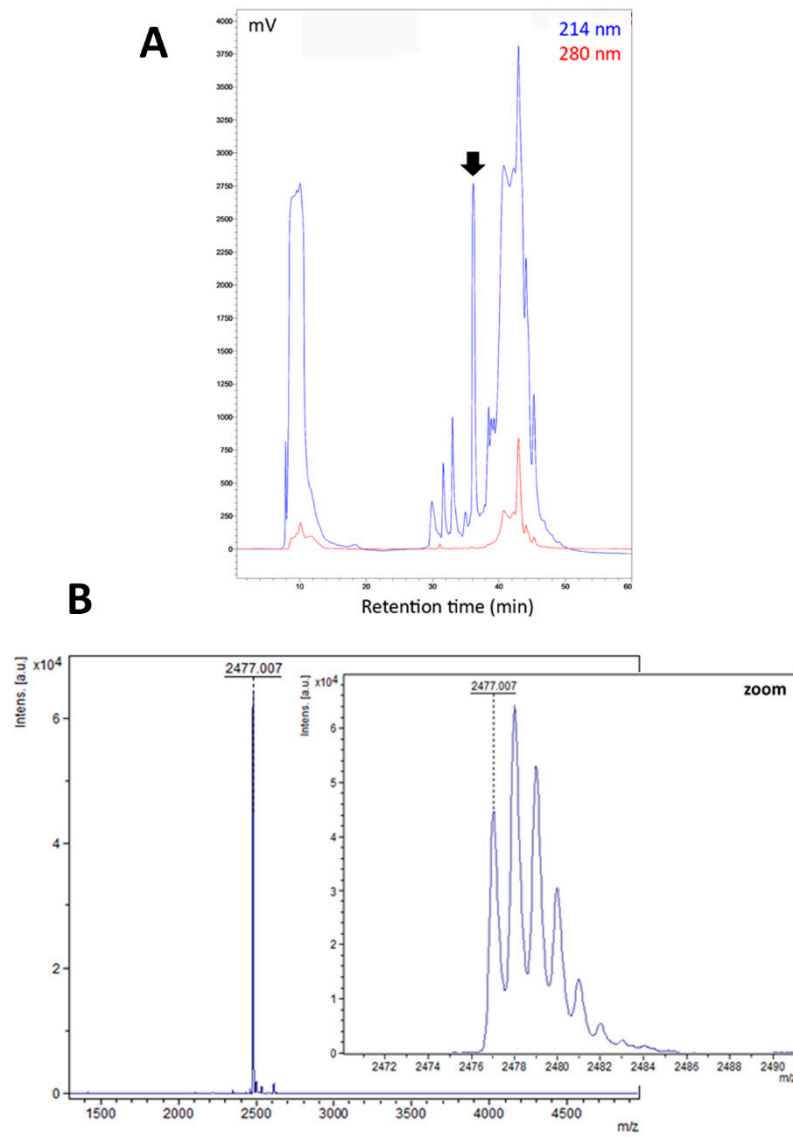
## *Supplementary Materials*

### **Genomic insights into bacterial resistance to proline-rich antimicrobial peptide Bac7**

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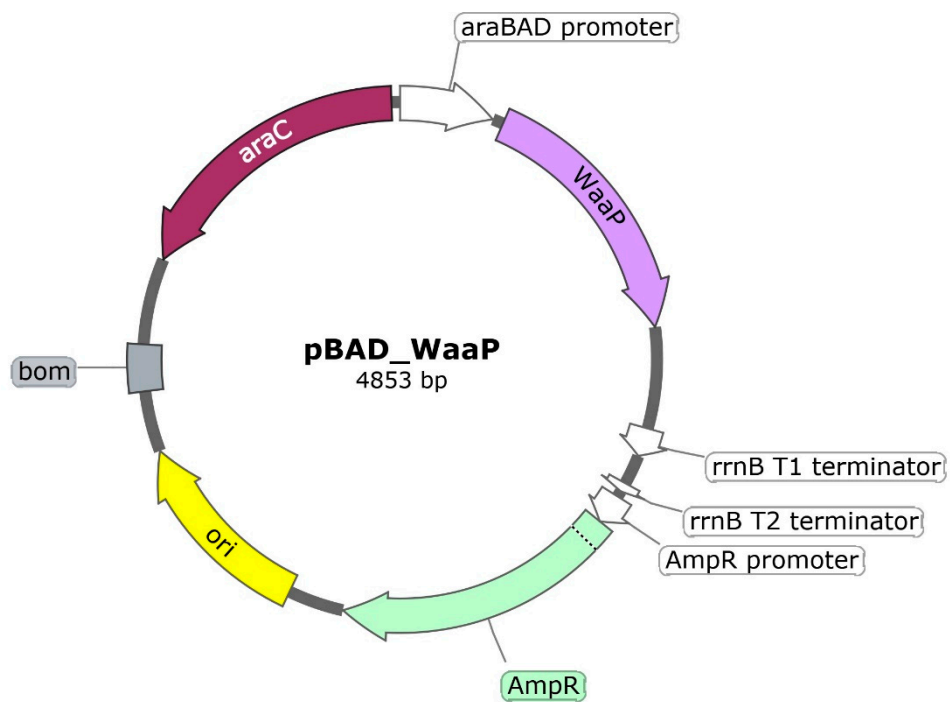
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**Figure S1.** Purification of the recombinant pexiganan. **(A)** Reverse-phase high-performance liquid chromatography (RP-HPLC) purification of the recombinant pexiganan. The fraction of the target peptide is marked with an arrow. **(B)** MALDI-TOF mass spectrometry analysis of the recombinant pexiganan. The experimental  $[M + H]^+$  monoisotopic mass is presented in the picture.

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
amikacin	aminoglycoside	No resistance	blaCTX-M-15 (blaCTX-M-15_AY044436)
tigecycline	tetracycline	No resistance	
tobramycin	aminoglycoside	No resistance	
cefepime	beta-lactam	Resistant	
chloramphenicol	amphenicol	No resistance	blaCTX-M-15 (blaCTX-M-15_AY044436)
piperacillin+tazobactam	beta-lactam	No resistance	
cefoxitin	beta-lactam	No resistance	
ampicillin	beta-lactam	Resistant	
ampicillin+clavulanic acid	beta-lactam	No resistance	blaCTX-M-15 (blaCTX-M-15_AY044436)
cefotaxime	beta-lactam	Resistant	
ciprofloxacin	quinolone	Resistant	
colistin	polymyxin	No resistance	
sulfamethoxazole	folate pathway antagonist	Resistant	sul1 (sul1_U12338)
imipenem	beta-lactam	No resistance	gyrA (p.S83L), gyrA (p.D87N)
trimethoprim	folate pathway antagonist	No resistance	
nalidixic acid	quinolone	Resistant	
ertapenem	beta-lactam	No resistance	
tetracycline	tetracycline	No resistance	blaCTX-M-15 (blaCTX-M-15_AY044436)
fosfomycin	fosfomycin	No resistance	
ceftazidime	beta-lactam	Resistant	
temocillin	beta-lactam	No resistance	
gentamicin	aminoglycoside	No resistance	mph(A) (mph(A)_D16251)
meropenem	beta-lactam	No resistance	
azithromycin	macrolide	Resistant	

**Figure S2.** Identification of antibiotic resistance genes in *E. coli* MDR 1057 strain performed using ResFinder 4.1 (<https://cge.food.dtu.dk/services/ResFinder/>).



**Figure S3.** pBAD-based plasmid vector for the expression of *waaP* under the tightly regulated arabinose promoter. Map was visualized with SnapGene software.

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**Figure S4.** A fragment of *E. coli* MDR 1057 genome sequence showing intergenic *menE-pmrD* region. The found SNP mutation is highlighted with yellow color. Start and termination codons in the corresponding genes are marked with green and red color, respectively.

**Table S1.** Oligonucleotide primers used in this study

Name	Sequence 5'→3'	Description
Pex-f	GC <u>CAGATCT</u> CATATGGGGATCGGCAAATTCCTTAAAGAAAGCGAAGAAGTTCGGGAAAGCC	Synthesis of DNA fragment encoding pexiganan followed by insertion into pET expression plasmid
Pex-r	GCGAATTCCTTATTTCTTCAGAATTTTCACAAAGGCTTCCCGAACTTCT	
Sbm-f	GTCGAAACAATTCTTATGGTCAG	Amplification of <i>sbmA</i> gene of <i>E. coli</i> (TA-cloning followed by Sanger sequencing)
Sbm-r	GCGAAGATAGAGGATTGACGCG	
Waa-f	GCCCATGGTTGAACTTAAAGAGCCGTTTG	Amplification of <i>waaP</i> gene of <i>E. coli</i> to insert into pBAD plasmid by NcoI/EcoRI sites (underlined)
Waa-r	CGGAATTCCTTATAATCCTTTGCGTTGTGTTTCGC	

**Table S2.** Amino acid sequences and molecular masses of AMPs used in this study

Peptide	Origin	Sequence	Molecular mass, Da		Source
			Calculated [M+H] <sup>+</sup> value <sup>1</sup>	Measured value <sup>2</sup>	
Bac7 <sub>1-22</sub>	Rec	RRIRPRPPRLPRPRRPLPFPR	2783.72	2783.89	[1]
PR-39 <sub>1-22</sub>	Rec	RRRPRPPYLPRRPPPPFFPRL	2765.61	2765.35	[1]
mini-ChBac7.5N $\alpha$	Rec	RRLRPRRRLPRPRRPRRPR	2894.81	2894.05	[2]
VicBac	Synt	RRIRRPRLPRPRVPRPRIPPRIPRVLPPPRVPFPRFPR*	4815.98	4815.70	[1]
AA139	Rec	GFCWYVCARRNGARVCYRRCN	2549.17	2550.07	[3]
Tachyplesin-1	Rec	KWCFRVCYRGICYRRCR	2264.08	2263.73	[4]
Protegrin-1	Rec	RGGRLCYCRRRFCVCVGR	2156.07	2156.01	[5]
Thanatin	Rec	GSKKPVPIIYCNRRRTGKCQRL	2415.32	2415.36	[6]
Pexiganan	Rec	GIGKFLKKAKKFGKAFVKILKK	2476.61	2477.01	This study <sup>3</sup>
ChMAP-28	Rec	GRFKRFRKKLKRLWHKVGPFVGPILHY	3364.00	3364.22	[2]
Melittin	Synt	GIGAVLKVLTTGLPALISWIKRKRQQ	2846.74	2846.60	[1]
LL-37	Synt	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES	4491.58	4491.40	[1]

Rec – recombinant peptide

Synt – synthetic peptide

\* C-terminal amidation

<sup>1</sup> according to ExPASy Isotopident tool

<sup>2</sup> monoisotopic m/z were measured using MALDI-TOF MS

<sup>3</sup> final yield of pexiganan was 6.7 mg per liter of the culture medium.

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