Supplementary file 1

Identification and characterization of antibacterial peptides produced by *Lactobacillus plantarum* 1407

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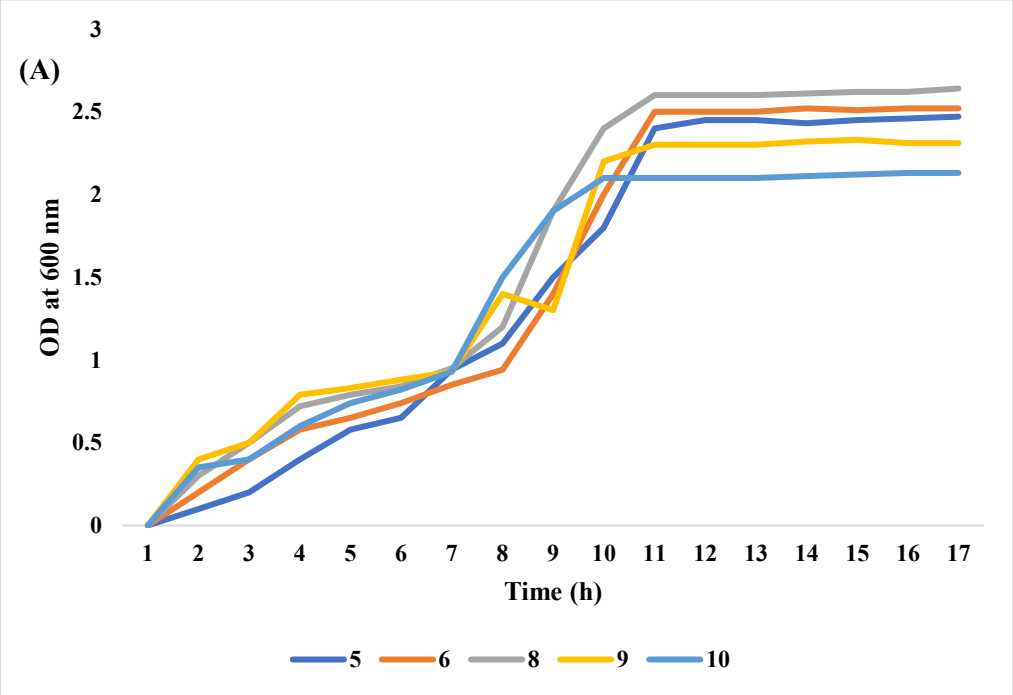


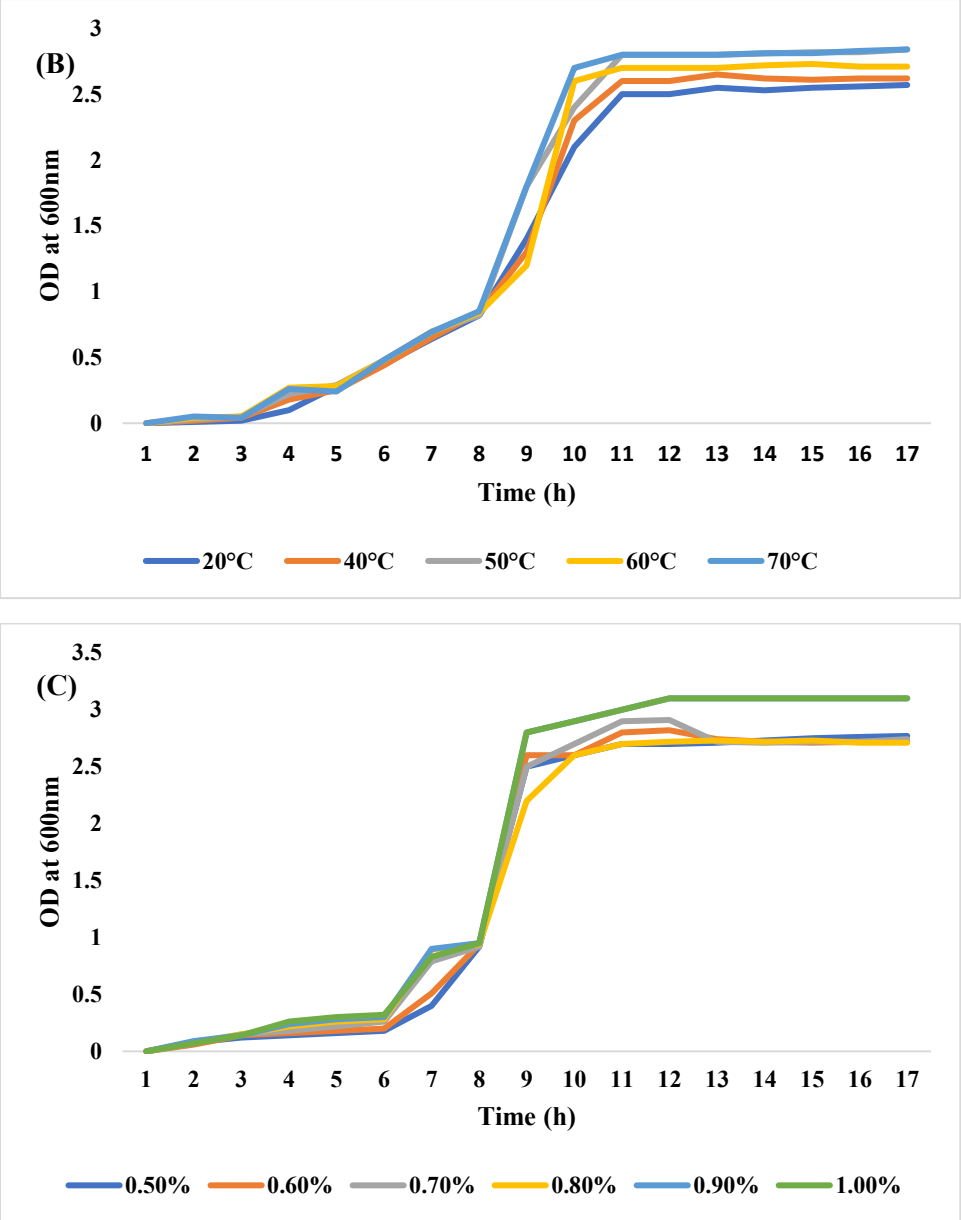


Fig .S1. Growth curve demonstrating the influence of (A) pH, (B) temperature and (C) salt concentration on the growth of *L.plantarum* 1407

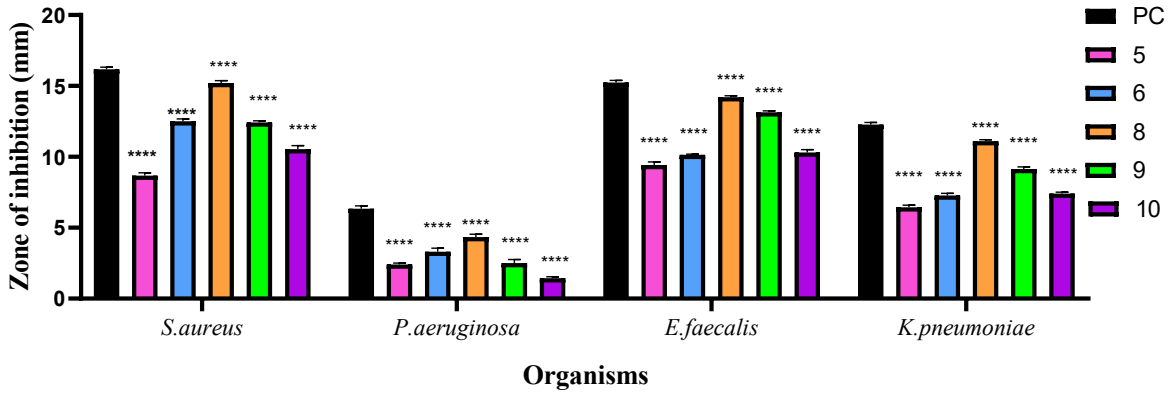


Fig .S2. Effect of initial pH on growth of *L.plantarum* 1407. Data are representative of three independent experiments and are plotted as mean ± SD (n=3); ****p < 0.0001

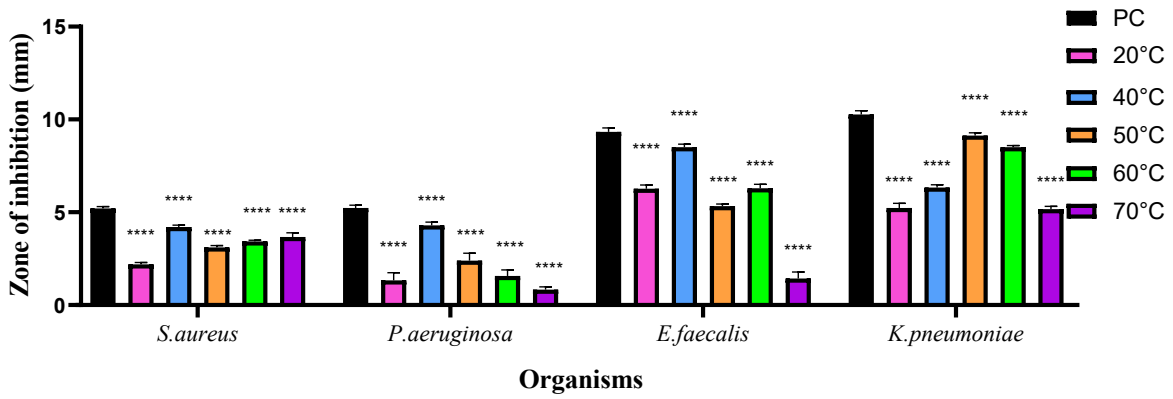


Fig .S3. Effect of incubation temperature on growth of *L.plantarum* 1407. Data are representative of three independent experiments and are plotted as mean ± SD (n=3); ****p < 0.0001

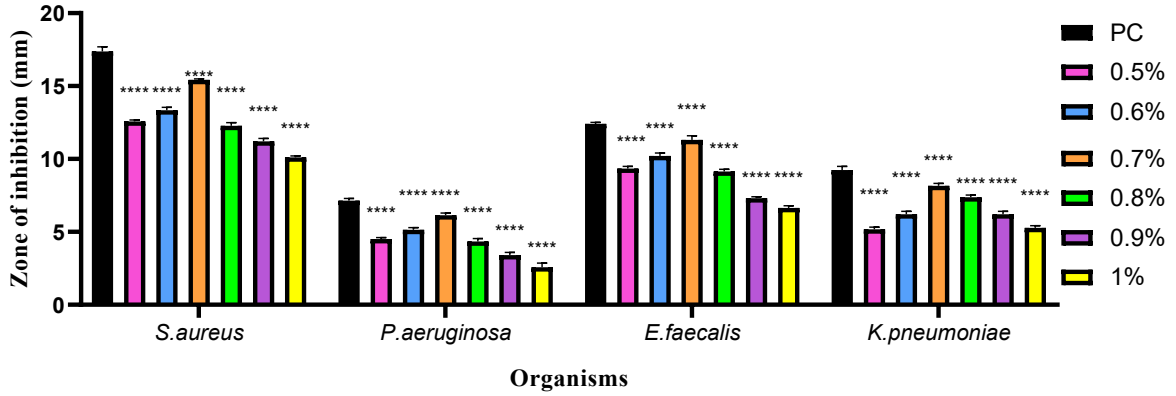
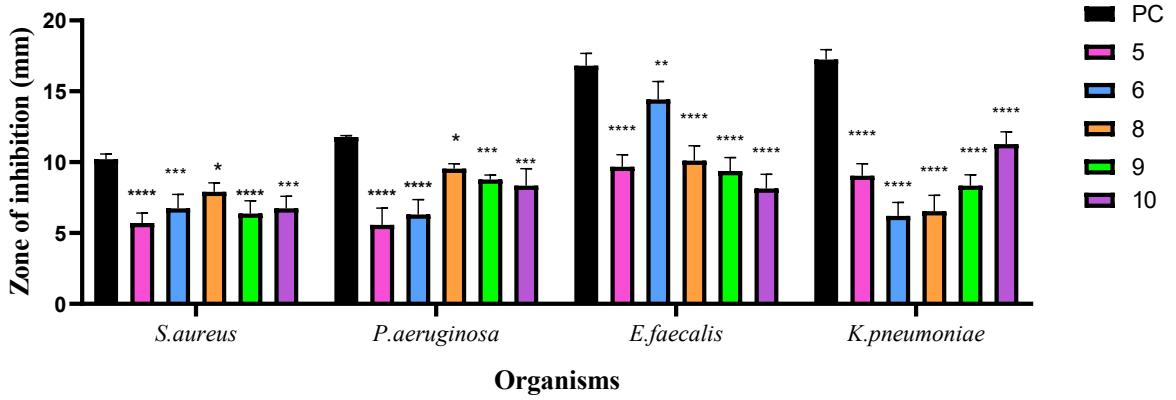


Fig.S4. Effect of salt concentration on growth of *L.plantarum* 1407. Data are representative of three independent experiments and are plotted as mean ± SD (*n*=3); ****$p < 0.0001$

(A)



(B)

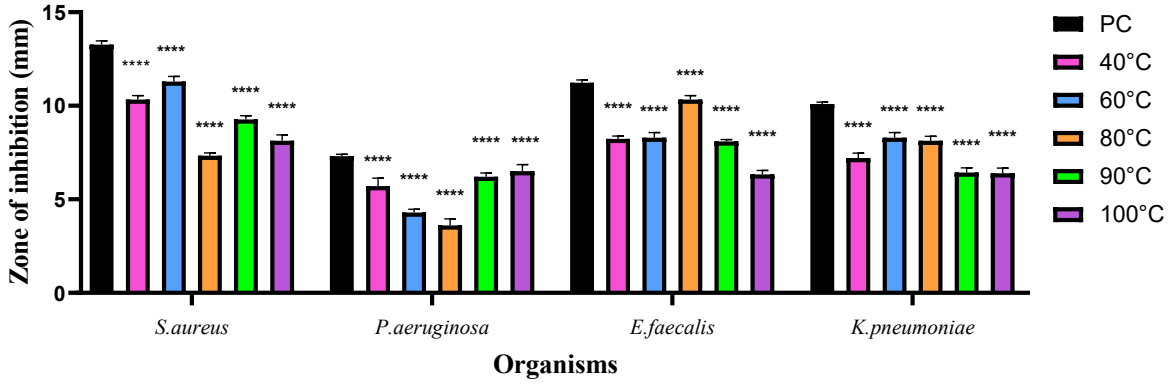
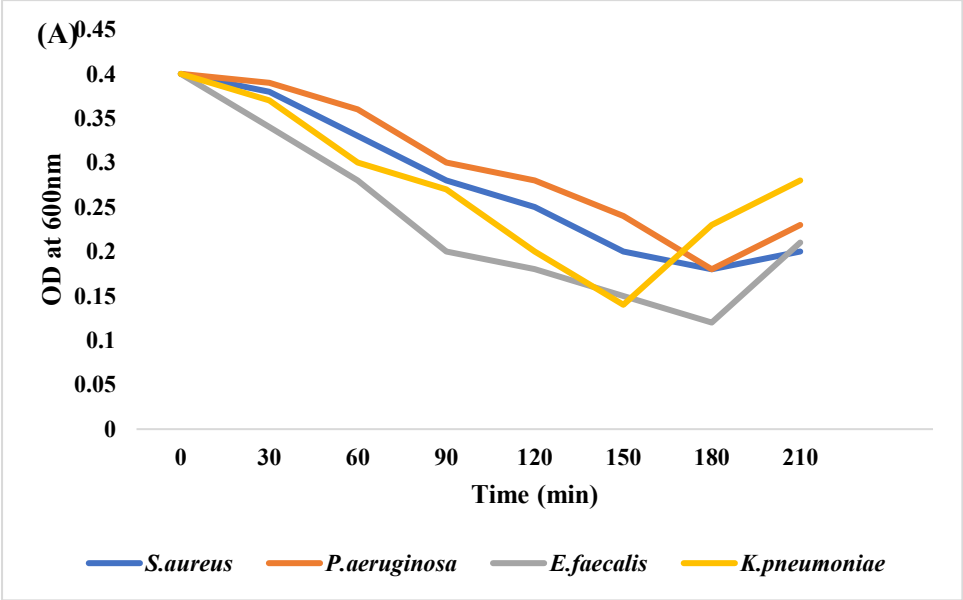
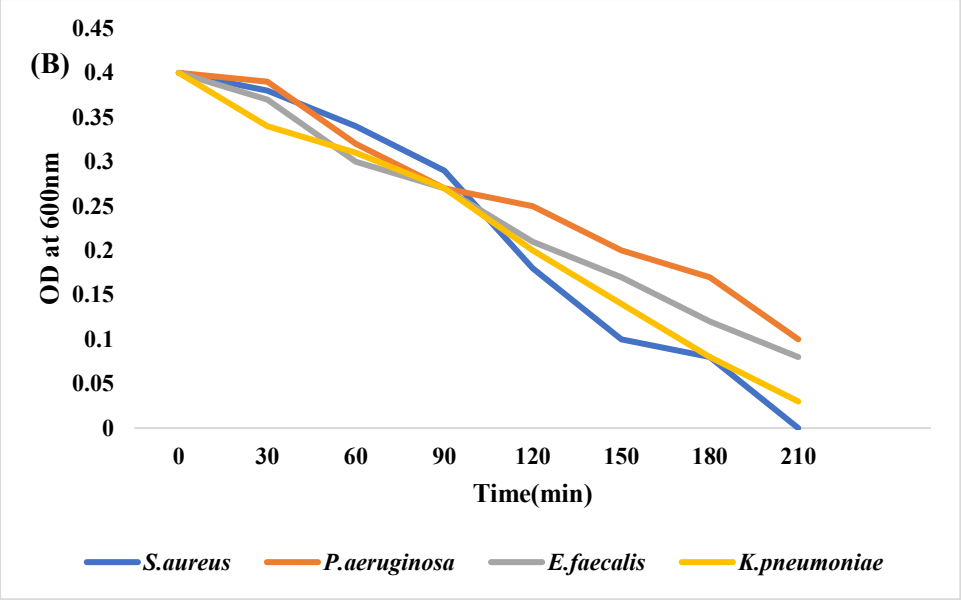
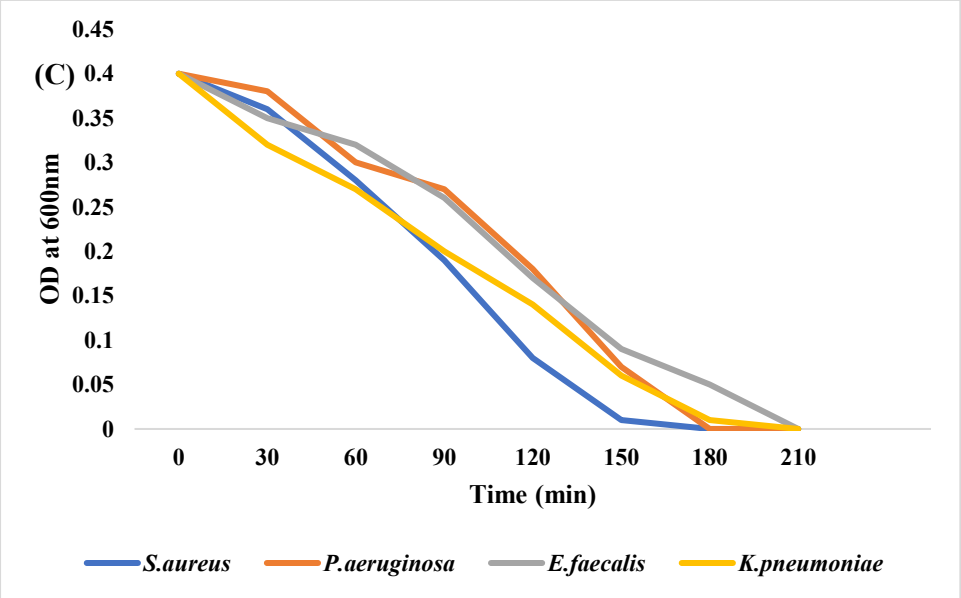


Fig.S5. Effect of (A) pH and (B) temperature on the antibacterial activity of CFS. Data are representative of three independent experiments and are plotted as mean ± SD (n=3); * p<0.5, *** p<0.001, ****p < 0.0001




(B)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD at 600nm
0
30
60
90
120
150
180
210
Time(min)
S.aureus
P.aeruginosa
E.faecalis
K.pneumoniae


(C)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD at 600nm
0
30
60
90
120
150
180
210
Time (min)
S.aureus
P.aeruginosa
E.faecalis
K.pneumoniae

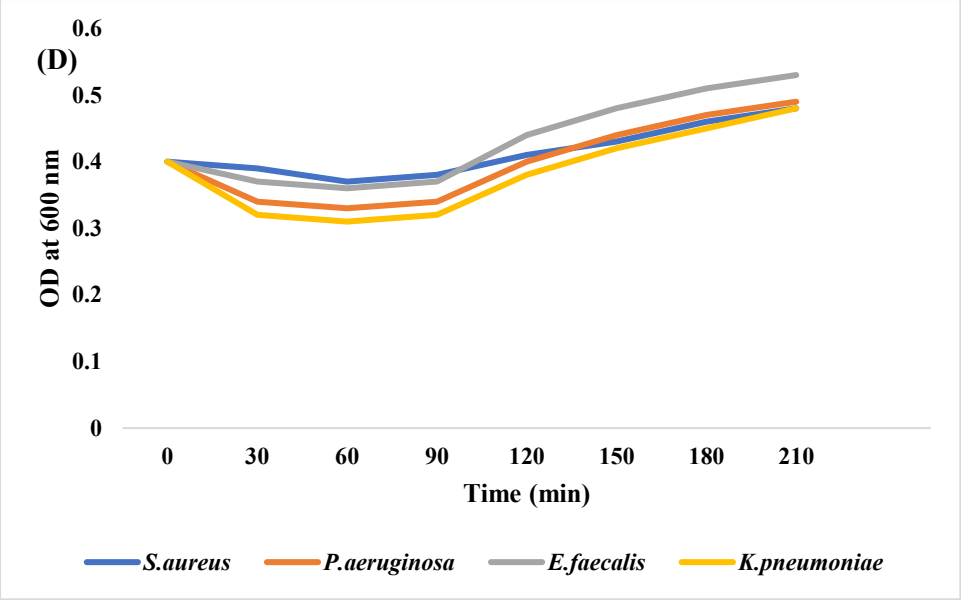


Fig .S6. Killing activity curve of (A) F1 (B) F2 (C) F3 (D) F4 fractions against Gram positive (*S.aureus,E.faecalis*) and Gram negative (*P.aeruginosa, K.pneumoniae*) organims.

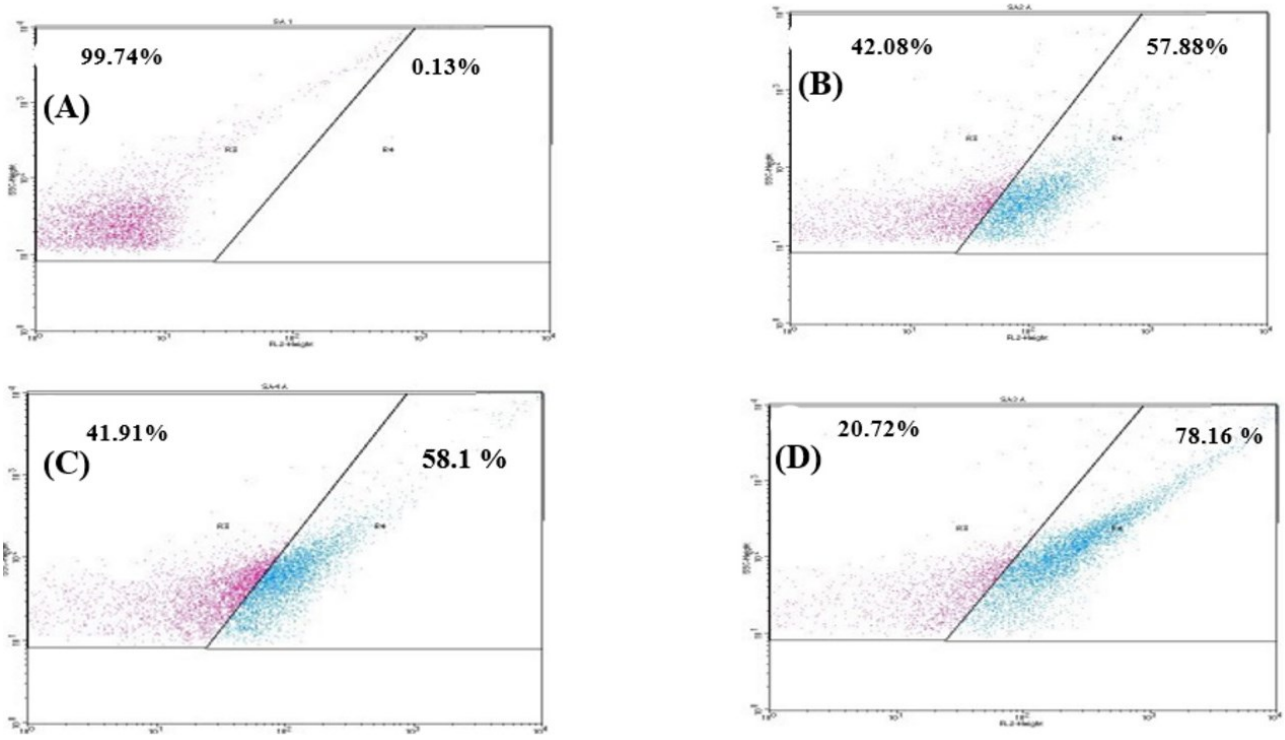


Fig.S7.Membrane integrity analysis F3 fraction peptides treated *S. aureus* using flow cytometry (A)Untreated cells (Control) (B) Cells treated with F3 fraction peptides for 20 min (C) Cells treated with F3 fraction peptides for 1hr (D) Cells treated with F3 fraction peptides for 2 hr. (R3= percentage of live cells; R4: Percentage of dead cells).

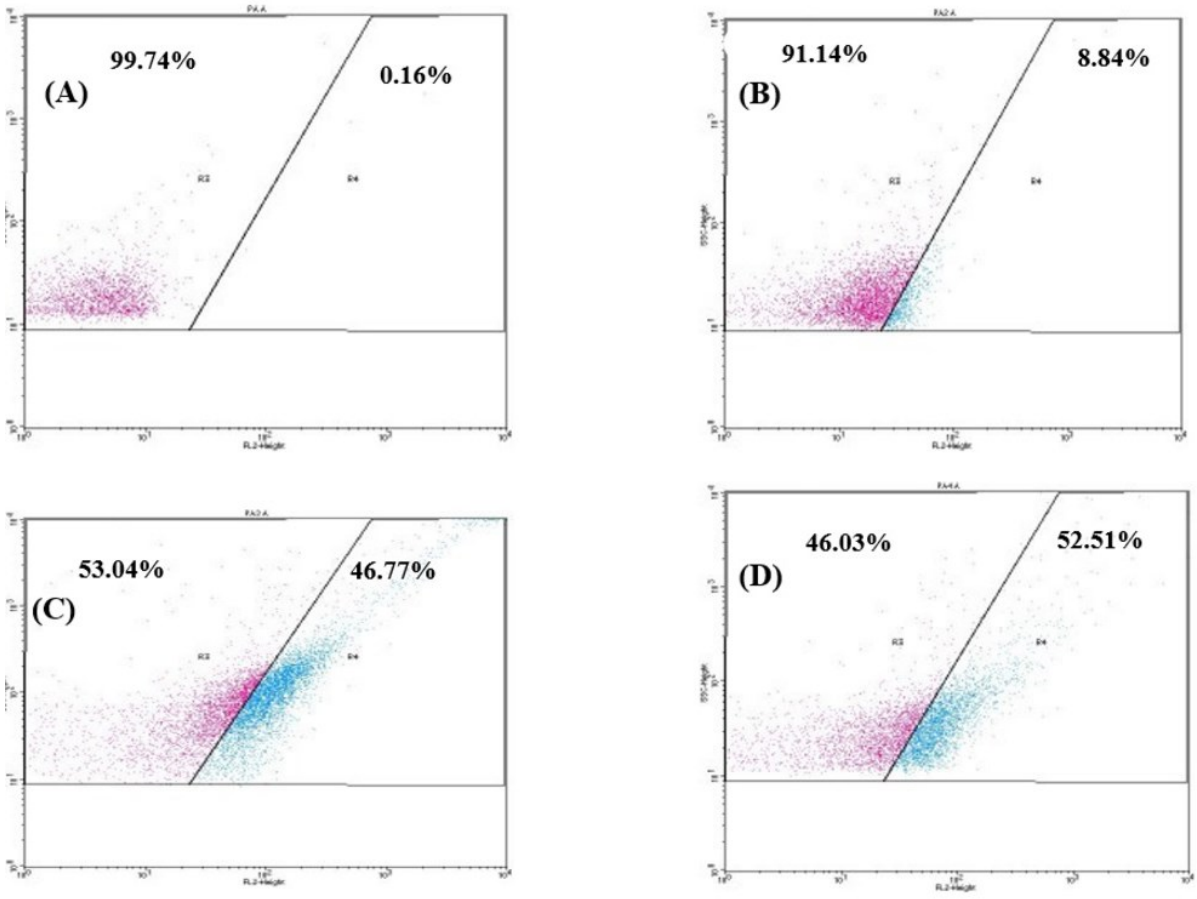


Fig .S8. Membrane integrity analysis of F3 fraction peptides treated *P.aeruginosa* using flow cytometry (A)Untreated cells (Control) (B) Cells treated with F3 fraction peptides for 20 min (C) Cells treated with F3 fraction peptides for 1 hr (D) Cells treated with F3 fraction peptides for 2 hr. (R3= percentage of live cells; R4: Percentage of dead cells).

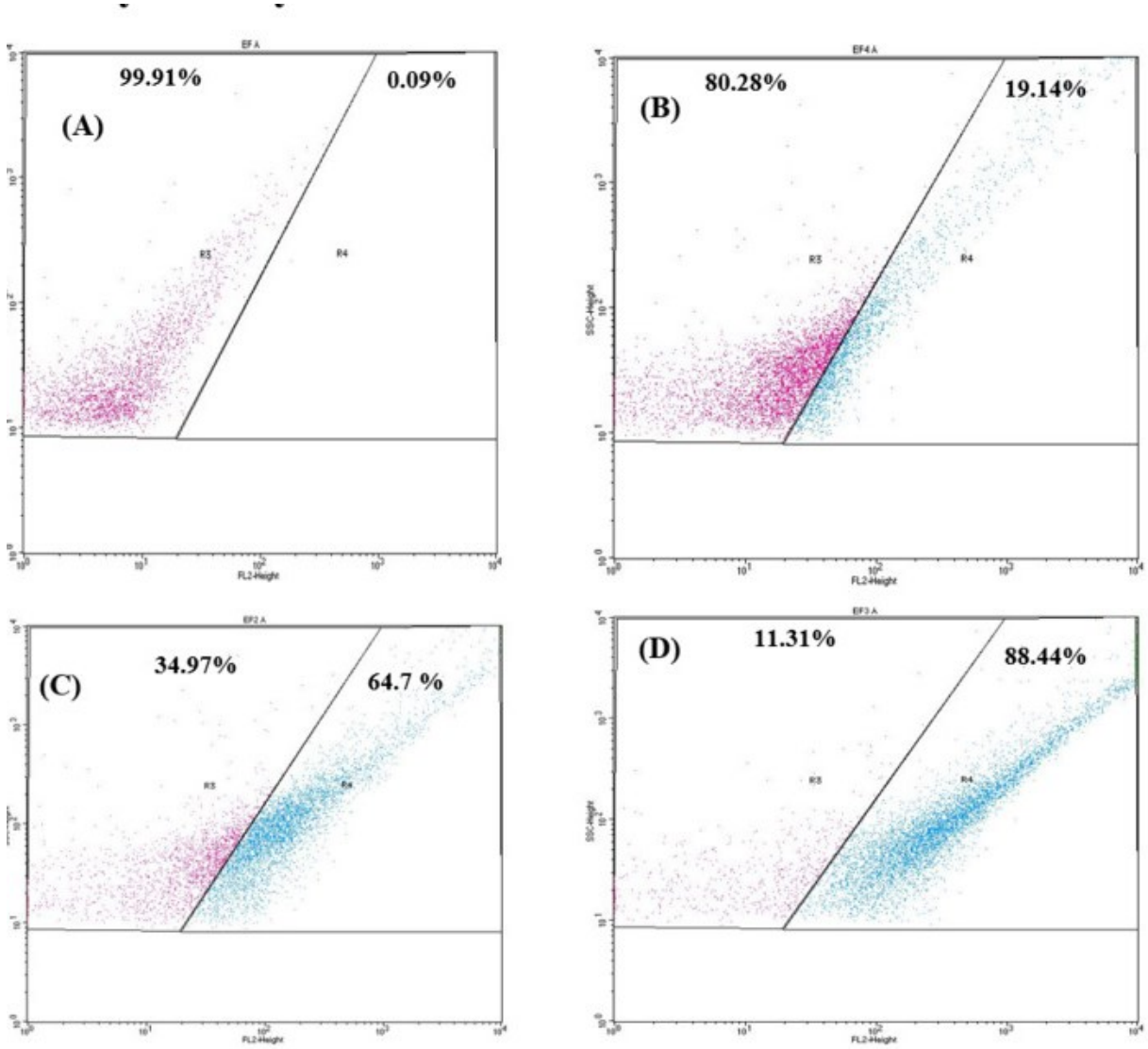


Fig .S9. Membrane integrity analysis of F3 fraction peptides treated *E.faecalis* using flow cytometry (A)Untreated cells (Control) (B) Cells treated with peptides for 20 min (C) Cells treated F3 fraction peptides for 1hr (D) Cells treated with F3 fraction peptides for 2 hr. (R3= percentage of live cells; R4: Percentage of dead cells).

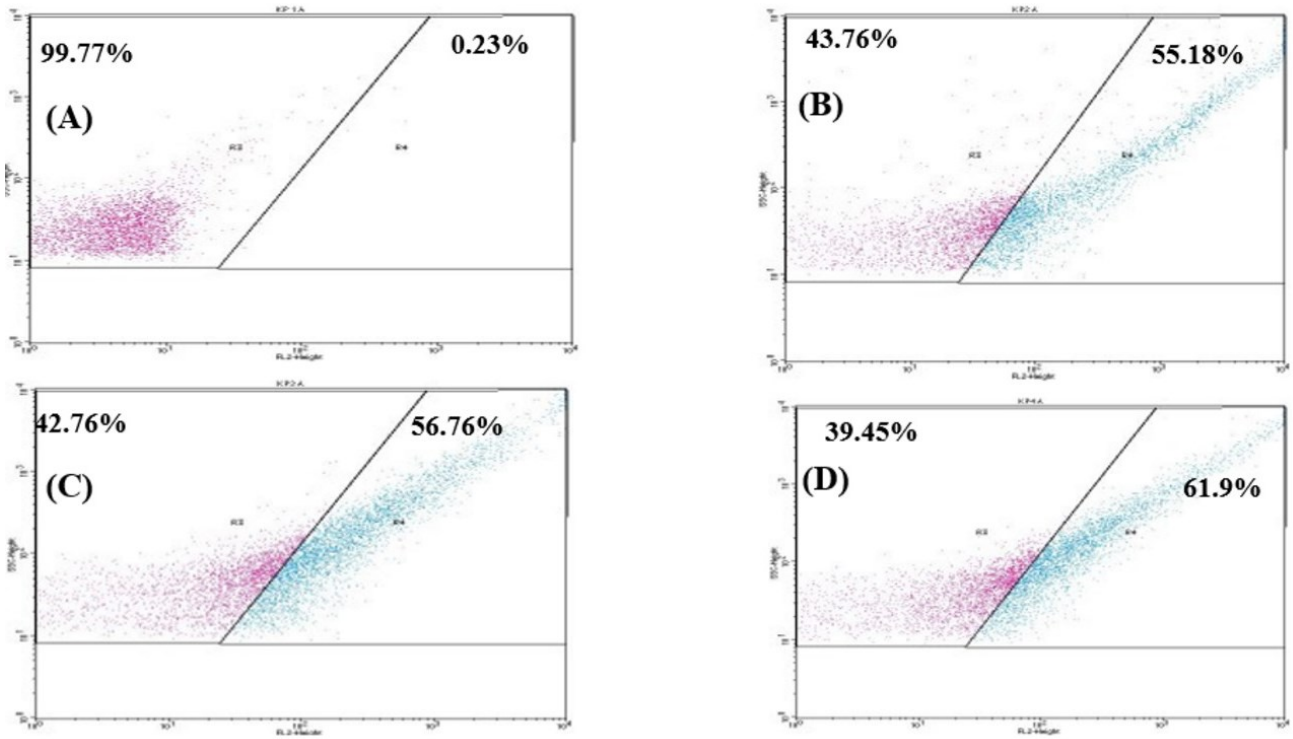


Fig .S10. Membrane integrity analysis of F3 fraction peptides treated *K. pneumoniae* using flow cytometry (A)Untreated cells (Control) (B) Cells treated with F3 fraction peptides for 20 min (C) Cells treated with F3 fraction peptides for 1hr. (D) Cells treated with F3 fraction peptides for 2hr. (R3= percentage of live cells; R4: Percentage of dead cells).

Table .S1.Summary of purification of the peptides from *L.plantarum* 1407.

Purification steps	Volume (ml)	Activity (AU/ml)	Total Activity (AU)	Protein (mg/ml)	Total protein (mg)	Specific activity (AU/mg protein)	Yield (%)	Purification Fold
CFS	200	700	140000	20.8	4160	33.65	100	1
Ammonium Precipitation (60%)	9	10300	92700	12.4	111.6	830.6	66	24
Ultrafiltration (Retentate)	2	19200	38400	7.7	15.4	2493.5	27	74.1
FPLC (F3 fraction)	0.2	35878.9	7175.79	2.24	0.448	3203.48	5.1	95.2

Table. S2. Enzyme stability test of the peptide fractions

Contents	Inhibition Zone (mm)				Residue Activity (%) [a]			
	Org 1 [b]	***Org 2*** [c]	***Org 3*** [d]	***Org 4*** [e]	***Org 1*** [b]	***Org 2*** [c]	***Org 3*** [d]	***Org 4*** [e]
F1 fraction peptides without proteinase	8.64	4.1	9.69	10.57	100	100	100	100
F1 fraction peptides treated with proteinase K (1mg/ml)	1.2	0.2	1.9	2.0	9.5	5.3	66.0	53.8
F2 fraction peptides without proteinase	8.36	2.5	7.4	6.8	100	100	100	100
F2 fraction peptides treated with proteinase K (1mg/ml)	0	0	0	0	0	0	0	0
F3 fraction peptides without proteinase	13.8	10.8	13.7	12.6	100	100	100	100
F3 fraction peptides treated with proteinase K (1mg/ml)	11.08	7.05	10.98	9.88	69.09	35.34	68.73	64.21
F4 fraction peptides without proteinase K	1.2	0.5	4.8	4.2	100	100	100	100
F4 fraction peptides treated with proteinase K (1mg/ml)	0	0	0	0	0	0	0	0

[a] Residue Activity = ((Inhibition zone of treated sample -5)/ (inhibition zone of untreated sample – 5)) X 100

[b] Org 1 = *S.aureus*; [c] Org 2 = *P.aeruginosa;* [d] Org 3 = *E.faecalis*; [e] Org 4 = *K.pneumoniae*

Table .S3.Applications of heat resistant antibacterial peptides

Industry	**Peptide**	**Heat resistance property above 100°C**	**Application/Treatment**
Food & Processing Engineering	Nisin	Polymer packaging materials incorporated with antimicrobial peptides are synthesized at 100-120 °C.	As antimicrobial active packaging material.[1]
	Polylisine	Polylisine and dextran have been conjugated by the Maillard reaction from 140°C to 165 °C to enhance the emulsifying and	To extend the shelf-life of products like sushi, boiled rice and meat [1]

		antibacterial activities.	
Cosmeceuticals	Pentapeptide-3 (GPRPA)	Preparation of creams with peptides involves heating the components at 100°C and the heat stability is necessary to ensure the antibacterial activity at the target site.	Decreases skin roughness and wrinkles [2]
	hexapeptide 11 (FVAPFP)		Improves skin firmness[2].
Pharmaceutical Industry	Gramicidin	Commercial gramicidin is prepared at a temperature of 100°C.	Effective against skin diseases caused by Gram-positive bacteria [3]
	Daptomycin	Daptomycin peptide retains antibacterial activity after topical thermal treatment of the implant surface above 100°C	To treat implant related Gram-positive bacterial infections[3]
	Lactoferricin B and Protegrin-1	These peptides withstand high temperatures (121 °C) during the manufacturing process of the lens.	Used as coating material in lenses to prevent bacterial infections in eye [5]

References:

1. Santos JCP, Sousa RCS, Otoni CG, Moraes ARFe, Souza VGL, Medeiros EAA, et al. Nisin and other antimicrobial peptides: Production, mechanisms of action, and application in active food packaging. Innovative Food Science and Emerging Technologies 2018; 48: 179-94.
2. Dini I, Mancusi A. Food Peptides for the Nutricosmetic Industry. Antioxidants (Basel) 2023; 12. doi: 10.3390/antiox12040788.

3. Huan Y, Kong Q, Mou H, Yi H. Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. Front Microbiol 2020; 11: 582779. doi: 10.3389/fmicb.2020.582779.
4. Lei J, Sun L, Huang S, Zhu C, Li P, He J, et al. The antimicrobial peptides and their potential clinical applications. Am J Transl Res 2019; 11: 3919-31.