

High-level heterologous production and Functional Secretion by recombinant *Pichia pastoris* of the shortest proline-rich antibacterial honeybee peptide Apidaecin

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TABLE S1. Microorganisms and plasmids used in this study

Strain or plasmid	Relevant characteristic	Source or reference
Bacterial strain		
<i>E.coli</i> JM109	Host strain; F <i>endA1 recA1</i> <i>gyrA96 thi hsdR17</i> ($r_K^- m_K^+$) <i>relA1 supE44</i> Δ (<i>lac-proAB</i>) F' <i>traD36 proAB lacIZ</i> Δ M15	Promega
Yeast (<i>P. pastoris</i>) strains		
SMD1168	Host strain; protease A mutant strain for selection on the MD medium; <i>his4</i> , <i>pep4</i> , Mut ⁺	Invitrogen
C	<i>P. pastoris</i> SMD1168 derivative carrying pPIC9K; His ⁺	This work
AP26	<i>P. pastoris</i> SMD1168 derivative carrying pPICAP; His ⁺	This work
Plasmids		
pPIC9K	<i>P. pastoris</i> 9-kb protein expression and secretion vector carrying a methanol-inducible promoter (PAOX1), 5'AOX1 region, MF α 1 _s ; His ⁺	Invitrogen
pPICAP	pPIC9K derivative carrying apidaecin fused in frame to MF α 1 _s ; His ⁺	This work

TABLE S2. Mediums used in this study

MD	BMGY	BMGluY	YDFM ³⁴	BMM	BMMY	BSM
1.34%	1.34%	1.34%	11.83	1.34%	1.34%	26.7 ml/L
YNB	YNB	YNB	g/L	YNB	YNB	Phosphoric acid, 85%
4 x 10-5%	4 x 10-5% biotin	4 x 10-5% biotin	2.29 g/L K ₂ HPO ₄	4 x 10-5% biotin	4 x 10-5% biotin	0.93 g/L Calcium sulfate
2% Glucos	1% glycerol	2% Glucose	1 g/L	0.5% methanol	0.5% methanol	18.2 g/L Potassium

e			MgSO ₄	(ever 24h)	(ever 24h)	sulfate
-	100 mM potassium phosphate, pH 6.0	100 mM potassium phosphate, pH 6.0	10 g/L NH ₄ SO ₄	100 mM potassium phosphate, pH 6.0	100 mM potassium phosphate, pH 6.0	14.9 g/L Magnesium sulfate-7H ₂ O
-	1% yeast extract	1% yeast extract	0.33mg/L CaCl ₂	-	1% yeast extract	4.13 g/L Potassium hydroxide
-	2% tryptone	2% tryptone	1 g NaCl	-	2% tryptone	40.0 g/L Glycerol
-	-	-	1g KCl	-	-	4.35 ml/L PTM
-	-	-	3% Glucose	-	-	-
-	-	-	4.35 ml/L PTM	-	-	-

PTM is the trace salts mix, which contains 6.0 g Cupric sulfate-5H₂O; 0.08 g Sodium iodide; 3.0 g Manganese sulfate-H₂O; 0.2 g Sodium molybdate-2H₂O; 0.02 g Boric Acid; 0.5 g Cobalt chloride; 20.0 g Zinc chloride; 65.0 g Ferrous sulfate-7H₂O; 0.2 g Biotin and 5.0 ml Sulfuric Acid per liter.

TABLE S3. The antimicrobial activity (AU/ml) from supernatants of APmu4 and AP26 incubated with 100ug of chemical synthetic apidaecin in different time

Incubation time at 30°C	AP26 96h	AP26 96h+100ug AP	APmu4 96h	APmu4 96h+100ug AP
0h	32,470 ± 481	1,291,233 ± 35114	154,382 ± 605	1,436,145 ± 45182
24h	26,950 ± 325	901,213 ± 9628	122,589 ± 472	976,981 ± 11573

TABLE S4. The production antimicrobial activity (AU/ml) of apidaecin from supernatants of *P. pastoris* APmu4 in different medium

	BMGY/BMMY ^a	BMGluY/BMMY
0h ^b	ND ^c	ND
24h	1033 ± 92	1351 ± 118
48h	23,519 ± 525	45,382 ± 539
72h	341,196 ± 998	735,611 ± 1068

96h	164,583 ± 794	549,282 ± 1104
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^a Strain was grown in BMGY or BMGluY and inuction in BMMY at 30°C.

^b Gene expression was induced at time zero.

^c ND is no activity detected.

TABLE S5. The production antimicrobial activity (AU/ml) of apidaecin from supernatants of *P. pastoris* APmu4 and AP26

	APmu4	AP26
0h^b	ND	ND
24h	1351 ± 118	1283 ± 108
48h	45,382 ± 539	24,589 ± 472
72h	735,611 ± 1068	261,910 ± 996
96h	549,282 ± 1104	75,331 ± 528

TABLE S6. Pilot-scale fermentation of apidaecin

	Cell wet weight (g/L)	Antimicrobial activity (AU/ml)
Batch phase	92 ± 8	ND ^a
Glucose feeding phase	156 ± 13	ND
Methanol induction 24h	195 ± 11	3785 ± 211
Methanol induction 48h	238 ± 24	852,104 ± 1589
Methanol induction 72h	272 ± 18	5,740,361 ± 38520

^a ND is no activity detected.

TABLE S7. Purification of recombinant apidaecin produced by pilot-scale fermentation

Purification stage	Volume (ml)	Total A₂₅₄	Total activity (10³ AU)	Specific activity (AU/A₂₅₄)
Culture supernatant	100	438.5	5740	13,090
0.22um hollow-fiber membranes	100	424	5716	13,481
10kDa hollow-fiber membranes	83	28.6	3527	123,322
1kDa hollow-fiber membrane	10	220.5	3182	14,431

Anion exchange chromatography	3	5.2	2568	493,846
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