Antibacterial properties of a recombinant antimicrobial peptide MP1102 against *Staphylococcus aureus* and *Clostridium perfringens in vitro* and *in vivo*

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Abstract

Antimicrobial peptide MP1102 (N9Q, L13V, and R14K), a new derivative of NZ2114, was designed and expressed in *Pichia pastoris* X-33. The yield of recombinant MP1102 (rMP1102) was 197.1 mg/l and its purity was about 96.4%. rMP1102 exhibited stronger potent activity *in vitro* against Gram-positive bacteria than native NZ2114, especially the methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium perfringens*. The minimum inhibitory concentrations (MICs) of rMP1102 against 20 clinical isolates of MRSA and *C. perfringens* were 0.004 - 0.23 μM and 0.05- 1.33 μM, respectively, and their minimum bactericidal concentrations (MBCs) were 0.007 - 0.46 μM and 0.9 - 7.28 μM, respectively. In addition rMP1102 had high stability over a wide pH range of 2.0 and 10.0 and high thermal stability from 20 to 80 °C, and remarkable resistance to pepsin. It showed also no hemolytic activity and cytotoxicity toward mammalian cells. The fractional inhibitory concentration index (FICI) indicated an additive or synergic effect between rMP1102 and bacitracin zinc, nisin, vancomycin, virginiamycin, aureomycin, and ampicillin against *C. perfringens* (FICI= 0.3125 – 1.0) respectively. Antibacterial activity of rMP1102 against *S. aureus* and *C. perfringens* was further assessed in a mouse thigh infection model. A dose of 5 - 20 mg/kg rMP1102 could kill over 90% of tested *S. aureus* ATCC43300 cells within 12 h, indicating that it had higher activity than vancomycin. Meanwhile, a decrease of 0.70 and 1.03 log10 CFU/g *C. perfringens* CVCC61 was observed after 24 h treatment with 10 and 20 mg/kg of rMP1102, respectively. Moreover, rMP1102 improved the survival rate of the mice infected *C. perfringens* CVCC61, reduced the serum tumor necrosis factor (TNF)- a level (prevention or/and treatment).

Results

The Characteristics of rMP1102

Table 1 Physical and chemical parameters of MP1102 and NZ2114

Peptide	Amino acid sequence	Net charge	Isoelectric point	Hydrophobic moment	α-Helix index	Instability index
NZ2114	GFGCNGPWNEDDLRCHNHCKSIKGYKGGYCAKGGFVCKCY	4	8.6	0.48	16.7	25.49
MP1102	GFGCNGPWQEDDVKCHNHCKSIKGYKGGYCAKGGFVCKCY	4	8.6	0.56	33.3	21.68

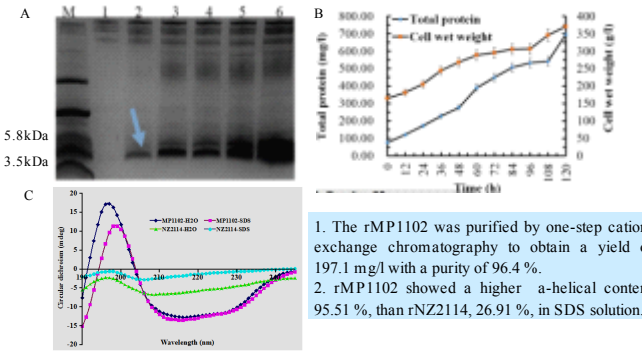

Fig. 1 Expression and characteristics of rMP1102 in P. pastoris X-33. A: Tricine-SDS-PAGE analysis of fermentation supernatants in High-density fermentation.M:10 μl protein molecular weight marker.1-6: a total of 5 μl fermentation supernatants taken at 0, 24, 48, 72, 96,120 h. B: Time curve of the total secreted protein level and cell wet weight. C: CD spectra of the secondary structure of rMP1102 and rNZ2114.

1. The rMP1102 was purified by one-step cation exchange chromatography to obtain a yield of 197.1 mg/l with a purity of 96.4 %.
2. rMP1102 showed a higher a-helical content, 95.51 %, than rNZ2114, 26.91 %, in SDS solution.

Antibacterial activity of rMP1102 *in vitro*

1. rMP1102 exhibited stronger potent activity *in vitro* against Gram-positive bacteria than native NZ2114, especially MRSA and *C. perfringens*.
2. rMP1102 showed a synergistic or additive effect (FICI= 0.3125 – 1.0) with antibiotics against *C. perfringens* type C

Table 2 The antimicrobial activity of rMP1102 against *C. Perfringens* type C

Antibacterial agents	MIC (μM)			MBC (μM)		
	CVCC61	CVCC2032	CVCC1163	CVCC61	CVCC2032	CVCC1163
MP1102	0.91	0.91	0.91	0.91	0.91	0.91
Plectasin	3.64	3.64	3.64	3.64	3.64	3.64
NZ2114	0.91	1.82	1.82	0.91	1.82	1.82
MP1106	7.28	7.28	7.28	7.28	7.28	7.28
Bacitracin zinc	1.33	0.665	1.33	2.66	1.33	2.66
Nisin	0.57	1.14	0.29	0.57	1.14	0.57
Vancomycin	0.34	0.34	0.34	0.68	0.68	0.34
Virginiamycin	0.48	0.96	0.48	0.96	0.96	0.48
Aureomycin	4.18	16.74	2.09	4.18	16.74	2.09
Ampicillin	0.05	0.1	0.1	0.05	0.2	0.1

Table 3 Effects of rMP1102 in combination with antibiotics against *C. perfringens* type C

Combination	Variety	*C.perfringens* CVCC61				*C.perfringens* CVCC2032				*C.perfringens* CVCC1163			
		MICa	MICc	FIC	FICI	MICa	MICc	FIC	FICI	MICa	MICc	FIC	FICI
MP1102-Bacitracin zinc	MP1102	0.91	0.455	0.50	0.75[b]	0.91	0.455	0.5	0.75[b]	0.91	0.455	0.5	1[b]
	Bacitracin zinc	1.33	0.3325	0.25		0.665	0.1675	0.25		1.33	0.665	0.5	
MP1102-Nisin	MP1102	0.91	0.2275	0.25	0.3125[a]	0.91	0.455	0.5	1[b]	0.91	0.455	0.5	0.75[b]
	Nisin	0.57	0.0356	0.0625		1.14	0.57	0.5		0.29	0.0725	0.25	
MP1102-Ampicillin	MP1102	0.91	0.2275	0.25	0.5[a]	0.91	0.455	0.5	0.75[b]	0.91	0.455	0.5	0.625[b]
	Ampicillin	0.05	0.0125	0.25		0.2	0.05	0.25		0.1	0.0125	0.125	
MP1102-Vancomycin	MP1102	0.91	0.2275	0.25	0.75[b]	0.91	0.455	0.5	0.75[b]	0.91	0.455	0.5	0.75[b]
	Vancomycin	0.34	0.17	0.50		0.34	0.17	0.25		0.34	0.085	0.25	
MP1102-Virginiamycin	MP1102	0.91	0.455	0.50	0.75[b]	0.91	0.1138	0.125	0.625[b]	0.91	0.1138	0.125	0.625[b]
	Virginiamycin	0.48	0.12	0.25		0.96	0.48	0.5		0.48	0.24	0.5	
MP1102-Aureomycin	MP1102	0.91	0.2275	0.25	0.375[a]	0.91	0.455	0.5	0.75[b]	0.91	0.2275	0.25	0.75[b]
	Aureomycin	4.18	0.5225	0.125		16.74	4.185	0.25		2.09	1.045	0.5	

a: synergic effect ; b: additive effect

Table 4 The antimicrobial activity of rMP1102 to *S. aureus* and cMRSA strains

Strains	MIC (μM)				MBC (μM)			
	Ampicillin	Vancomycin	NZ2114	MP1102	Ampicillin	Vancomycin	NZ2114	MP1102
S.aureus								
ATCC25923	5.4	0.7	0.028	0.028	10.8	1.4	0.06	0.06
ATCC29213	10.8	5.7	0.11	0.06	21.5	5.7	0.11	0.06
ATCC6538	1.3	1.4	0.11	0.11	2.7	1.4	0.11	0.11
ATCC43300	172	2.8	0.9	0.06	> 1723	2.8	0.9	0.06
Clinical strains of MRSA	from the Dep t of Food Sci and the Bor Luh Food Safety Center, Shanghai Jiao Tong Univ (SJU)							
SJU20009	11.46	-	0.11	0.004	45.84	-	0.11	0.008
SJU20010	22.92	-	0.11	0.004	91.69	-	0.9	0.014
SJU20013	183.38	-	0.9	0.014	366.76	-	0.11	0.028
SJU20015	91.69	-	0.11	0.007	366.76	-	0.11	0.028
SJU20018	366.76	-	0.11	0.007	> 366.76	-	1.8	0.028
SJU20023	366.76	-	0.9	0.007	> 366.76	-	0.23	0.007
SJU20024	45.84	-	0.23	0.007	91.69	-	0.23	0.007
SJU20027	183.38	-	0.23	0.007	> 366.76	-	0.45	0.46
SJU20032	45.84	-	0.45	0.23	183.38	-	0.45	0.014
SJU20033	183.38	-	0.11	0.007	366.76	-	0.11	0.028
SJU20035	91.69	-	0.11	0.014	366.76	-	0.11	0.056
SJU20040	91.69	-	0.11	0.014	366.76	-	0.11	0.016
SJU20045	91.69	-	0.11	0.004	366.76	-	0.11	0.028
SJU20047	183.38	-	0.11	0.007	> 366.76	-	0.11	0.014
SJU20049	366.76	-	0.11	0.007	> 366.76	-	0.45	0.22
SJU20055	183.38	-	0.23	0.11	366.76	-	0.11	0.016
SJU20056	183.38	-	0.11	0.004	366.76	-	0.23	0.44
SJU20057	366.76	-	0.23	0.11	> 366.76	-	0.45	0.23
SJU20064	183.38	-	0.45	0.23	366.76	-	0.23	0.016
SJU08P157	11.46	-	0.23	0.004	45.84	-	0.91	0.014

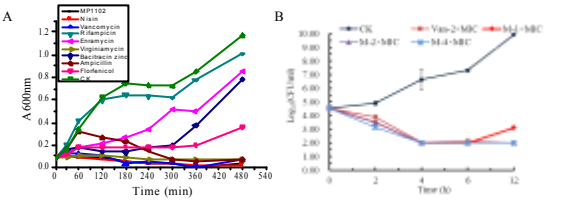

Fig. 2 Time-kill curves *in vitro* of rMP1102 or various antibiotics with known cellular targets. A: Growth kinetic of *C. perfringens* CVCC61 exposed to 2× MIC peptide or antibiotics. B: Time-kill curves of rMP1102 against *S. aureus* ATCC43300. CK: S. aureus ATCC43300 was incubated in the medium alone; M×MIC: *S. aureus* was incubated with 1×, 2×, or 4× MIC rMP1102; Van-2 × MIC: *S. aureus* was incubated with 2× MIC vancomycin.

The treatment with rMP1102 resulted in a sustained reduction in the bacterial counts.

Antibacterial activity of rMP1102 *in vivo*

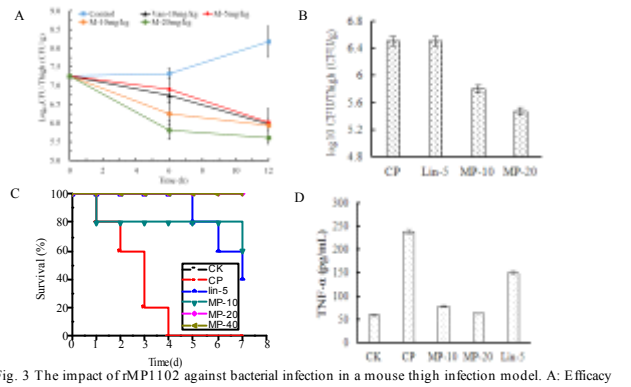

Fig. 3 The impact of rMP1102 against bacterial infection in a mouse thigh infection model. A: Efficacy of rMP1102 against *S. aureus* ATCC43300. Control: the group treated with saline; Van-10 mg/kg: the group treated with 10 mg/kg single subcutaneous doses of vancomycin; M-5, 10 and 20mg/kg: the groups treated with 5, 10 and 20 mg/kg single subcutaneous doses of rMP1102, respectively. B: Effect of rMP1102 on the bacterial colony number in thigh tissue of mouse infected by *C. perfigens* CVCC61 after 12h . Survival (C) and release of TNF- a (D) of mice infected by *C. perfigens* CVCC61 with treatment. Control: the group treated with only saline; Lin-5: the group treated with 10 mg/kg single tail intravenous dose of lincomycin ; MP-10, 20 and 40: the groups treated with 10, 20 and 40 mg/kg single tail intravenous dose of rMP1102, respectively.

rMP1102 reduced the bacterial count in infected thigh muscle tissue, and improved the survival rate of the mice infected *C. perfringens*.

Conclusion and References

These results indicated that it is feasible for large-scale production of rMP1102 by the yeast expression system, and rMP1102 is a promising new antimicrobial agent for *S. aureus* and *C. perfringens*.

1. Zhang Yong, et al. In vitro and in vivo characterization of a new recombinant antimicrobial peptide, MP1102, against methicillin-resistant *Staphylococcus aureus*. *Appl Microbiol Biotechnol* 99. 15 (2015): 6255-6266.
2. Zong Lifen, et al. Mechanism of action of a novel recombinant peptide, MP1102, against *Clostridium perfringens* type C. *Appl Microbiol Biotechnol* 100. 11 (2016): 5045-5057.