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Antibacterial activity of Bac7(1-35) and BMAP-27 against biofilm-embedded *A. baumannii*

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Introduction

A. baumannii (Ab) has recently emerged as a leading cause of nosocomial infections, mainly affecting debilitated individuals staying in Intensive Care Units. *Ab* infections often represent a serious therapeutic issue, due to the high frequency of multidrug-resistant strains. Additionally, most *Ab* isolates form biofilms, that may further hinder antimicrobial therapy. Biofilms are communities of bacteria attached to a surface and/or to each other and embedded in an extracellular matrix of polymeric material, whose composition varies depending on the bacterial species and on the strain.

Aim of the study

The development of new antimicrobials effective against both planktonic and biofilm-grown *Ab* would be extremely useful. In this study, we tested two antimicrobial peptides (AMPs), namely Bac7(1-35) and BMAP-27 (Fig. 1), against a panel of *Ab* clinical isolates differing in lineage and biofilm-forming ability.

Table 1. AMPs activity against planktonic and sessile *A. baumannii*

Ab strain	Bac-7(1-35)		BMAP-27		Biofilm production[c]
	MIC[a] (μM)	BIC[b] (μM)	MIC[a] (μM)	BIC[b] (μM)	
420	4	32	4	32	scarce
7B	4	64	4	64	scarce
39A	2	>64	4	>64	good
56A	4	>64	4	>64	good
105B	2	>64	4	>64	good
215B	2	>64	4	>64	excellent
3B	4	>64	4	>64	excellent
141A	2	>64	4	>64	excellent

[a]MICs were assessed by the broth micro-diluition method following the CLSI specifications[3]. [b]BICs were measured as described by Moskovitz[4]. [c]Biofilm production was assessed by the colorimetric crystal violet-based method[5] and categorized as follows: scarce: OD<0,300; good: 0,300<OD<0,600; excellent: 0,600<OD<0,950.

Table 2. AMPs activity against planktonic *A. baumannii* in the presence of a crude matrix preparation

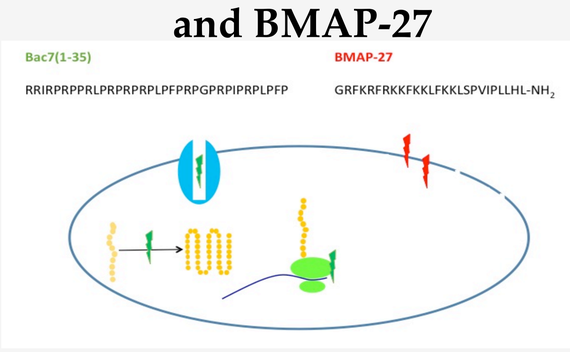
strain	Crude matrix extracted from Ab 3B	Bac-7(1-35) (μM)	BMAP-27 (μM)	Tobramycin (μg/ml)	Ciprofloxacin (μg/ml)	Colistin (μg/ml)
3B	-	4	4	1	2	1
3B	0,1 mg/ml	4	8	2	2	2
3B	1 mg/ml	32	64	8	2	32
420	-	4	4	>512	256	0,5
420	0,1 mg/ml	8	8	ND	ND	4
420	1 mg/ml	32	64	ND	ND	32

Matrix was extracted from *Ab* 3B biofilm grown on cellulose membranes deposited on Mueller-Hinton agar medium, using the method described by Bales *et al*.[6]

References

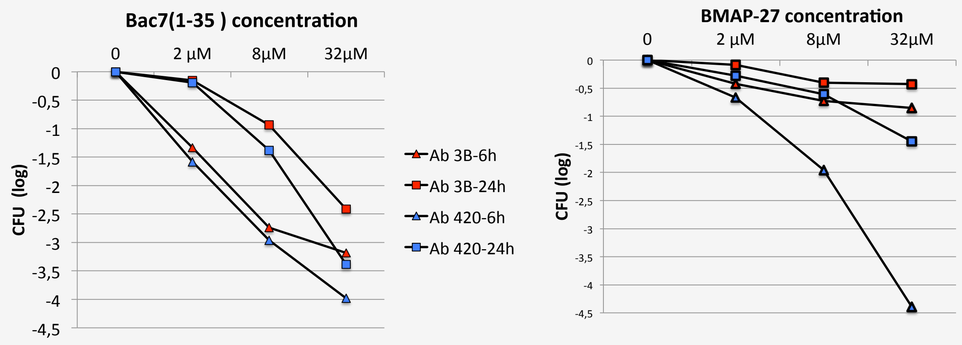
1. Scocchi *et al*., **2011**. Proline-rich antimicrobial peptides: Converging to a non-lytic mechanism of action. *Cell Mol. Life Sci. 68*, 2317–2330.
2. Tomasinsig *et al*., **2010**, Role of cathelicidin peptides in bovine host defense and healing. *Probiotics Antimicrob. Proteins 2*, 12–20.
3. CLSI. **2012**. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute.
4. Moskowitz *et al*., **2004**. Clinically feasible biofilm susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *J. Clin. Microbiol*. *42*, 1915–1922.
5. Stepanovic *et al*., **2007**. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS* 115*:* 891-899.
6. Bales *et al.*, **2013**. Purification and characterization of biofilm-associated EPS exopolysaccharides from ESKAPE Organisms and Other Pathogens. PLoS One 8:e67950.

Fig. 1. Structure and mechanism of action of Bac7(1-35) and BMAP-27



Bac7(1-35) and BMAP-27 are bovine AMPs belonging to the cathelicidin family. Bac7(1-35) is the 1–35 fragment of the Pro-rich extended bactenecin Bac7, which can penetrate into susceptible Gram-negative bacteria and inactivate cytoplasmic targets[1], while BMAP-27 is a membranolytic peptide with a helical active form[2].

Fig. 2. Killing activity of AMPs on *A. baumannii* biofilm-embedded cells



Peptides were added at the indicated concentrations to *Ab* biofilm that had been developed for 6 or 24 hours. Bacterial counts at time 0 were about 10^7. Values are the mean of two independent experiments performed in duplicate.

Results

- The Biofilm Inhibitory Concentrations (BICs) were up to 32 times higher than the Minimal Inhibitory Concentrations (MICs) of planktonic cells. Scarce biofilm producers showed lower BICs, suggesting that lower susceptibility to AMPs is related to the production of an abundant biofilm layer (Tab. 1).

- Killing of biofilm embedded cells was studied on strains 3B and 420, which are an excellent and a scarce biofilm producer, respectively. Both peptides were more active against the scarce producer *Ab* 420 (Fig. 2). Indeed, BMAP-27 was almost inactive against the excellent biofilm producer *Ab* 3B.

- Both peptides were more active against a newly formed (6 hours) biofilm than against a well established (24 hours) one. This result suggests that biofilm maturation plays an important role, perhaps through matrix production (Fig. 2).

- To test the latter hypothesis, MIC assays were repeated in the presence of a crude matrix preparation. Matrix addition caused a 8-16x increase of the MICs of both peptides (Tab. 2). The same effect could be observed for the MIC values of the antibiotic colistin and, to a lesser extent, of tobramycin, but not of ciprofloxacin, the smallest antimicrobial molecule tested.

Conclusions

Both peptides have a good activity against planktonic *Ab*, but biofilm-grown bacteria show a significant decrease of susceptibility.

It appears likely that the *A. baumannii* biofilm matrix, at least the one produced by strain *Ab* 3B, decreases or delays access of peptides and larger antimicrobials to bacterial cells.