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Antibacterial and anti-biofilm activity of branched peptides derived from antimicrobial peptides

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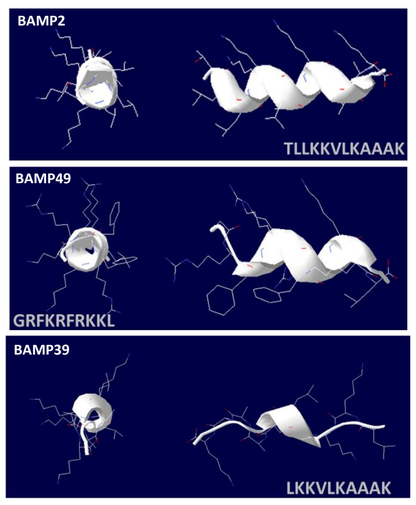
Background

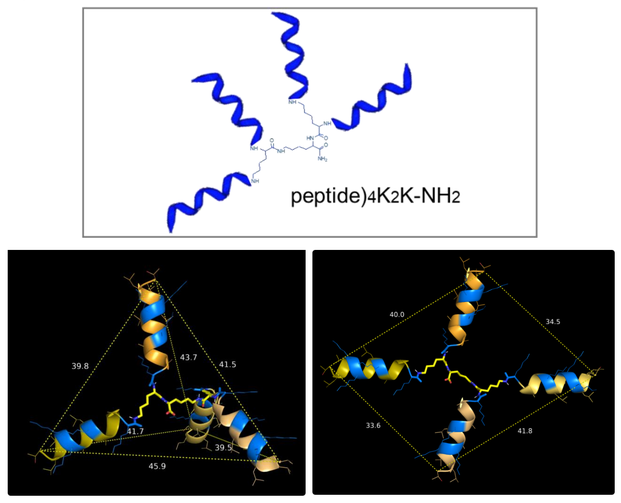
Antibiotic resistance is a global public health issue that reduces the effectiveness of conventional antibiotics. Overuse of antibiotics led to the development of multi-drug-resistant microorganisms. Developing new anti-infective agents is crucial to protect the efficacy of standard antibiotics and reduce the risk of antibiotic resistance. Antimicrobial peptides (AMPs) are short, cationic, and amphipathic molecules found in all living organisms, belonging to the innate immune system. AMPs can potentially replace antibiotics having a wide range of activity against Gram-positive and Gram-negative bacteria, fungi, protozoa, and viruses. Moreover, they show immunomodulatory and antibiofilm activity, and they are less likely to induce resistance mechanisms compared to antibiotics (*Uddin SJ et al. 2021*). The synthesis of AMPs in branched structure not only improves the resistance profile to protease degradation but also generates the possibility to form polyvalent interactions increasing the binding avidity *(Falciani et al. 2007).*

Tetra-branched antimicrobial peptides (BAMPs)

A small library of **tetra-branched peptides** was solid-phase synthesized starting from 9-10-residue linear sequences derived from natural AMPs.

The prediction of the linear analogues' tertiary structure with APPTEST protocol (*Brendan Timmons et al. 2021)* revealed that **BAMP2** displayed an extensive amphipathic alpha helix arrangement, **BAMP39** showed an alpha helix structure involving only three central residues whereas **BAMP49** exhibited a fully alpha helix arrangement. Differently, **BAMP37** and **BAMP45** presented a random linear form. Structural modelling studies confirmed that the predicted tertiary structure of the peptides was maintained in the tetra-branched forms.





Antibacterial activity

Minimum Inhibitory Concentration (µM)

Name	BAMP2	BAMP37	BAMP39	BAMP45	BAMP49
Sequence	$(TLLKKVLKAAAK)_4K_2K$	$(KKLLGKNWKLM)_4K_2K$	$(LKKVLKAAAK)_4K_2K$	$(INLKKLAKL)_4K_2K$	$(GRFKRFRKKL)_4K_2K$
Origin	Dermaseptin	Mastoparan	Dermaseptin	Mastoparan	Cathelicidin
***E. coli* TG1**	1.5	3	1.5	0,7	6
***E. coli* LC711 (colR*)**	3	>25	6	6	>25
***P. aeruginosa* ATCC 27853**	6	3	6	3	6
***P. aeruginosa* FI-25 (bla_{VIM-1}*)**	12	NT	NT	NT	NT
***P. aeruginosa* FI-29 (bla_{GES-5}*)**	12	NT	NT	NT	NT
***K. pneumoniae* ATCC 43816**	6	25	12,5	12	25
***K. pneumoniae* (colR*)**	6	>25	>25	6	>25
***E. faecalis* 51299**	25	>25	>25	>25	> 25
***E. faecium* FI-48 (van_A*)**	6	NT	NT	NT	NT
***S. aureus* USA 300**	>25	>25	>25	>25	>25

** blaVIM-1, VIM-type -lactamase; bla GES-5, GES-5 type -lactamase; colR, colistin resistance; vanA, vancomycin resistance; NT: not tested.*

All the BAMPs were active against planktonic forms of Gram-negative strains of *E. coli*, *P. aeruginosa* and *K. pneumoniae*. Notably, BAMP2, BAM39 and BAMP45 were also active against strains carrying the gene for resistance to colistin, a peptide antibiotic used in clinical practice. The most promising peptide proved to be **BAMP2** also demonstrating activity against strains of *P. aeruginosa* resistant to β-lactamases.

Activity against *E. faecalis* and *S. aureus* Gram-positive strains was poor for all peptides.

Anti-biofilm activity

	E.coli TG1				*K. pneumoniae* ATCC 43816			
µM	**BAMP2**	**BAMP37**	**BAMP39**	**BAMP49**	**BAMP2**	**BAMP37**	**BAMP39**	**BAMP49**
MIC	1,5	3	1,5	6	6	25	12,5	>25
BPC	1,5	3	6	25	50	50	>50	50
MBIC	3	25	50	50	50	50	50	50

BPC: lowest concentration of peptide that results in 80% lower biofilm formation compared to untreated controls.

MBIC: lowest concentration of peptide that results in 80% reduction in preformed biofilm compared to untreated controls.

AMPs have amphipathic surfactant structure that facilitates penetration of the biofilm matrix down to the outer bacterial membrane favouring biofilm disruption and bacterial eradication *(Srinivasan, Ramanathan et al., 2021)*. Therefore, the antibiofilm activity of BAMPs was evaluated.

BAMP2 proved the best against *E. coli* biofilm, inhibiting its formation and disrupting existing layers at the MIC and double the MIC, respectively.

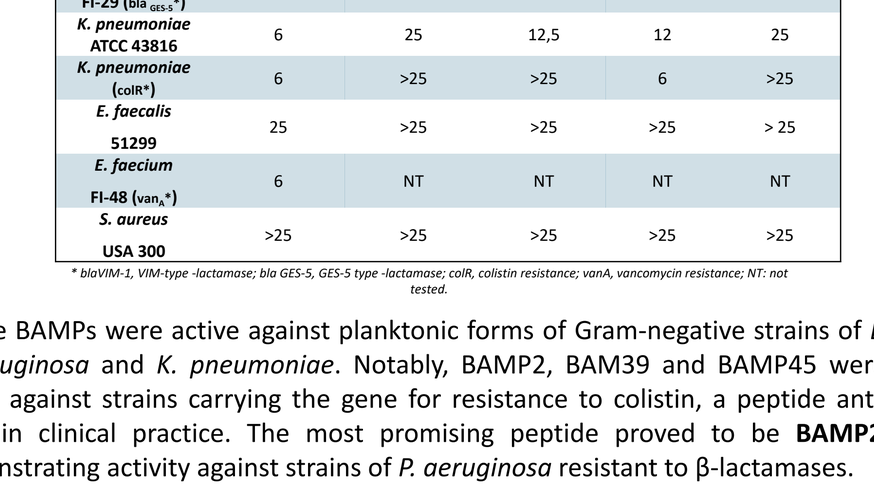
BAMP37, **BAMP39** and **BAMP49** were more efficient at inhibiting biofilm formation, and less at disrupting existing *E. coli* biofilm.

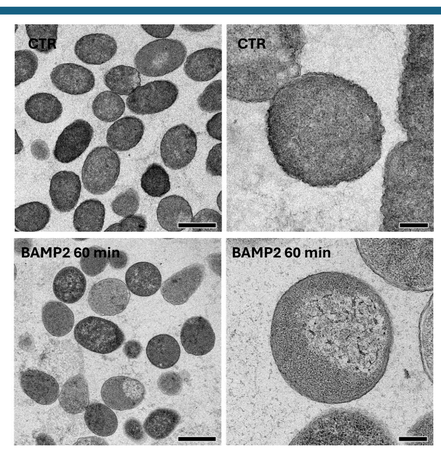
K. pneumoniae biofilm was 80% disrupted by higher concentrations, i.e. 50 µM, by all peptides.

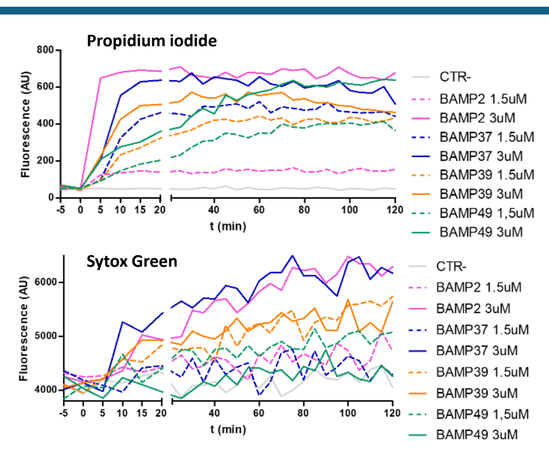
Mechanism of action

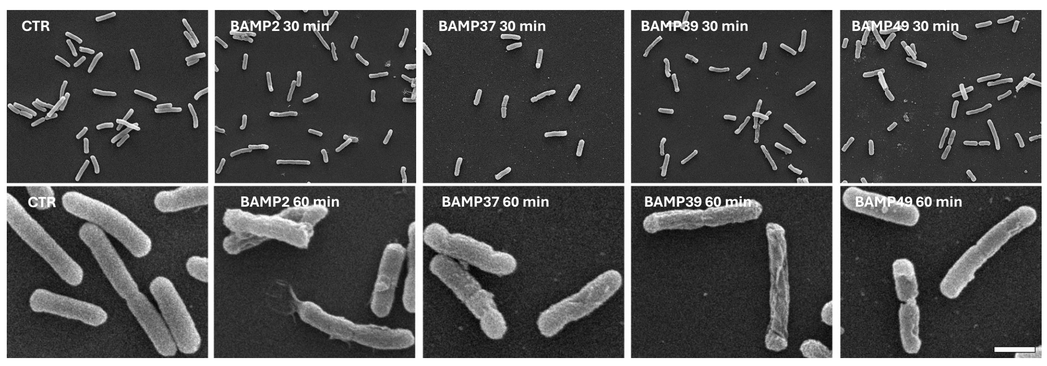
The mechanism of action of **BAMPs** was evaluated in *E. coli* TG1 with electron microscopy experiments (TEM and SEM) revealing that the peptides had a **detergent-like effect**: they disrupted the bacterial membrane causing the wrinkling of the surface, the enlargement of the cells and an overall loss of surface smoothness leading to rapid permeabilization of the membrane and bacterial death.

The loss of membrane integrity was also confirmed by the internalization of non-permeable fluorophores within 10 minutes after adding the peptides, demonstrating rapid **dysregulation of homeostasis.**









Conclusion

Among all the tested BAMPs, **BAMP2** showed to be the most effective antibacterial agent, probably thanks to its extensive amphipathic alpha helix conformation. The promising antibacterial and antibiofilm activity of these BAMPs could be exploited for the development of new therapeutic molecules to fight mild infections such as dental ones, often associated with the presence of multi-resistant biofilms. Thus, using AMPs instead of antibiotics in oral cavity infections may be beneficial in maintaining the effectiveness of standard antibiotics in more severe infections.