Investigating the antibiofilm activity of the established antimicrobial peptide indolicidin and derivatives.

Benjamin Sinclair[1,2], Supervisor: Dr. Heidi Huttunen-Hennelly[1], Co-Supervisor: Dr. Eric Bottos[2]

[1]Thompson Rivers University Department of Physical Sciences (Chemistry)

[2]Thompspn Rivers University Department of Biological Sciences

Background

- Antimicrobial resistant bacteria are a growing concern for public health.[1]
- Bacteria commonly form communities called biofilms by building a protective extracellular matrix.[2]
- Biofilm structure contributes to the antibiotic resistance of the bacteria.[2]
- Antimicrobial peptides (AMPs) are characterized by effective, non-specific action and are promising antibiofilm agents.[1,3]
- Indolicidin is an established AMP derived from bovine neutrophils.[1,3]
- It has a high tryptophan content (5 of its 13 amino acids), a +4 net charge, and an amidated C-terminus.

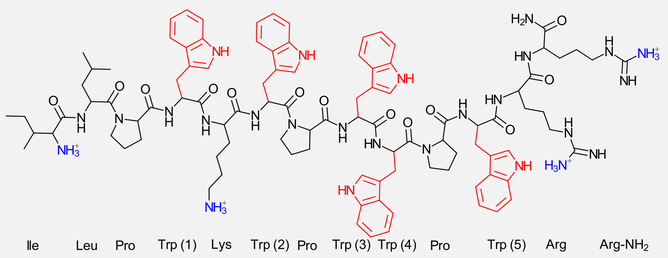


Figure 1. The structure and amino acid sequence of Indolicidin.

- High hydrophobicity contributes to hemolytic activity.
- Replacement of various Trp residues with alanine reduces hydrophobicity and decreases hemolysis while maintaining antimicrobial capability.[1,3]

Table 1. Sequences and antimicrobial activity of Indolicidin and four derivatives. Antimicrobial activity was evaluated in previous research by minimum inhibitory concentration (MIC) assays on planktonic cultures.[1,3]

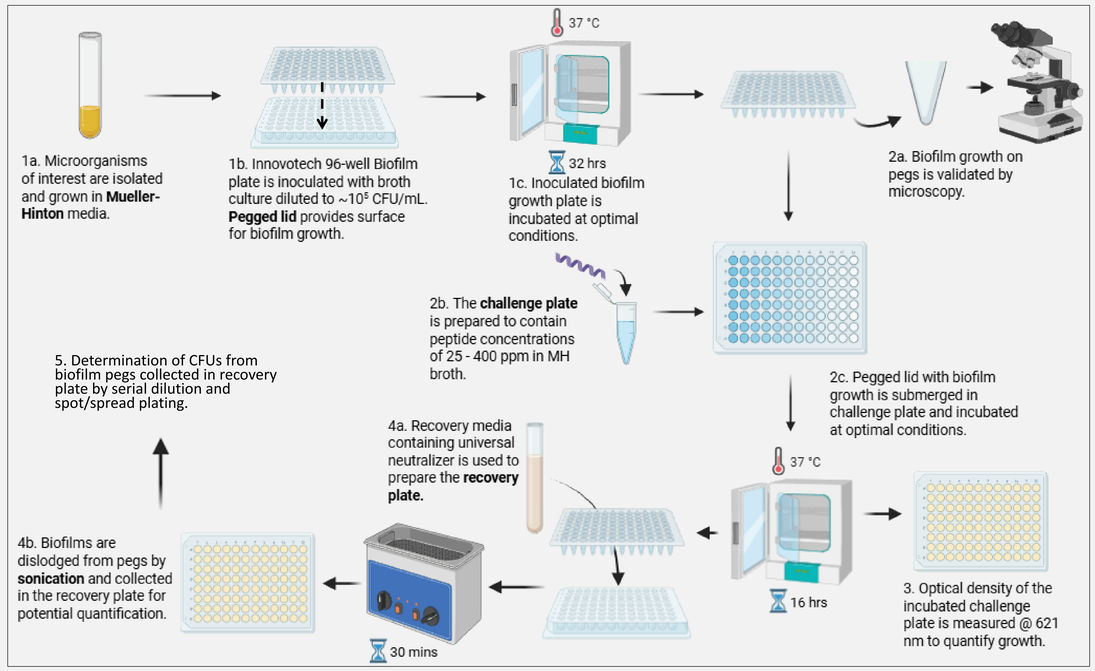
Peptide	Sequence	Antimicrobial Activity	Hemolytic Activity
Indolicidin	ILPWKWPWWPWRR-NH_2	High	High
Δ2,3	ILPWKAPAWPWRR-NH_2	Moderate	Minimal
Δ2,5	ILPWKAPWWPARR-NH_2	Moderate	Minimal
Δ1,4	ILPAKWPWAPWRR-NH_2	Moderate	Minimal
Δ4,5	ILPWKWPWAPARR-NH_2	High	Minimal

Objective

The objective of this research is to investigate the anti-biofilm capability of the established antimicrobial peptide Indolicidin and some derivatives with reduced hydrophobicity.

Methods

Minimum Biofilm Eradication Assay



Results

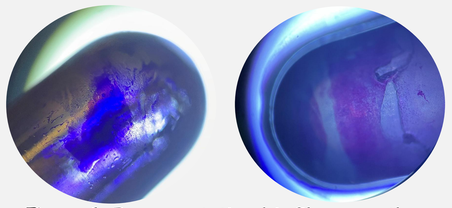


Figure 1. Representative biofilm growth images of MRSA (left, Gram +) and *E. coli* (right, Gram -) before peptide treatment.

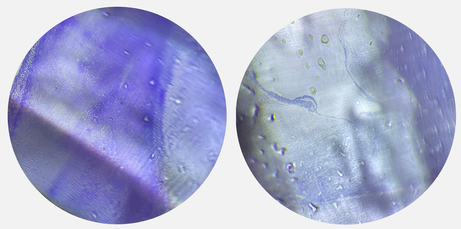


Figure 2. *Candida albicans* biofilm growth (stained with crystal violet) before (left) and after (right) treatment with 400 ppm Δ2,5.

Table 2. Minimum concentration which resulted in 50% decreased OD_{621} of three microorganism's biofilm growth media compared to control. A dash indicates the peptide did not show a 50% decrease at the highest concentration tested (400 ppm).

Peptide	*Escherichia coli*	Methicillin resistant *Staphylococcus aureus*	*Candida albicans*
Indolicidin	100 ppm	50 ppm	50 ppm
Δ2,3	400 ppm	400 ppm	400 ppm
Δ2,5	-	-	400 ppm
Δ1,4	-	-	-
Δ4,5	-	-	-

Table 3. Decrease in OD_{621} of biofilm growth media compared to control of the highest concentration peptide treatment well (400 ppm). A value of 100% would indicate no decrease in growth and a value of 0% would indicate a sterile well.

Peptide	*Escherichia coli*	Methicillin resistant *Staphylococcus aureus*	*Candida albicans*
Indolicidin	9.4%	17%	4.4%
Δ2,3	41%	36%	39%
Δ2,5	60%	54%	48%
Δ1,4	87%	83%	58%
Δ4,5	76%	67%	54%

Measurement of the OD_{621} of the challenge plate wells before and after peptide treatment can determine if the peptide prevents biofilm derived cell growth in the media (see Tables 2 & 3, Figure 3) but does not measure activity against cells in the biofilm structure itself.

Results cont.

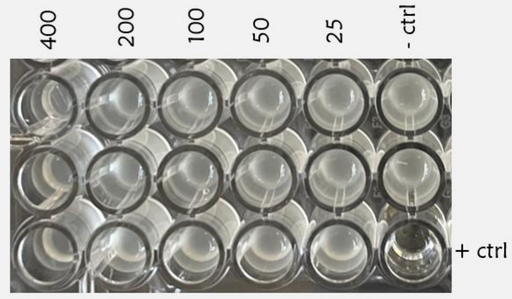


Figure 3. Challenge plate wells containing 25-400 ppm Δ2,3 after 16h incubation with *Escherichia coli* biofilms on peg lid. Turbidity of wells suggests biofilm cells were able to grow in the broth even with 400 ppm peptide treatment.

Table 4. Activity of Indolicidin against biofilms of three microorganisms reported as post-treatment colony forming units (CFUs). Biofilm cells were recovered from the pegs into recovery media by sonication, followed by estimation of CFUs by serial dilution and spot plating. A greater than sign (>) indicates colonies were too concentrated to quantify at the dilutions used.

	Growth Control estimated CFUs	200 ppm Indolicidin estimated CFUs	100 ppm Indolicidin estimated CFUs
E. coli	$>4 \times 10^7$	10^5	10^6
MRSA	$>4 \times 10^7$	No Data	5×10^4
C. albicans	$>4 \times 10^7$	10^5	$>10^6$

- Indolicidin derivatives Δ2,5, Δ1,4, and Δ4,5 did not show significant antibiofilm activity.
- The Δ2,3 derivative showed some activity and Indolicidin showed moderate activity (see Tables 2-4).

Discussion

- Indolicidin and its derivatives expectedly exhibit less activity against biofilm cultures compared to planktonic cultures used in traditional MIC assays.[1,3]
- Only indolicidin itself exhibited promising antibiofilm activity, suggesting the reduced hydrophobicity of alanine is detrimental to antibiofilm activity, but was necessary originally to reduce hemolysis.
- The protective extracellular matrix of biofilms likely contributes to lower AMP activity.

Future Work

- Investigation of the viability of biofilm cells after peptide treatment could be done.
- DNA binding assays of Indolicidin and its derivatives would give insights into mode of action and perhaps identify critical residues or motifs.

References: 1. Podorieszach, A. P., & Huttunen-Hennelly, H. E. K. (2010). The effects of tryptophan and hydrophobicity on the structure and bioactivity of novel indolicidin derivatives with promising pharmaceutical potential. Organic & Biomolecular Chemistry, 8(7), 1679–1687. doi: 10.1039/B921248E; 2. Shree, P., et al. (2023). Biofilms: Understanding the structure and contribution towards bacterial resistance in antibiotics. Medicine in Microecology, 16, 100084. doi: 10.1016/j.medmic.2023.100084; 3. Mercer-Brunelle, A. (2024). DETERMINING ANTIMICROBIAL SUSCEPTIBILITY AND HEMOLYTIC ACTIVITY OF INDOLICIDIN DERIVATIVES. Thompson Rivers University.