Medical Microbiology Seminar

Date: Dec 17, 2025 Time: 10:00-11:00 AM

Antimicrobial peptides (AMPs) from black soldier fly larvae: cecropin and DLP4 as broad-spectrum against antibiotic-resistant bacteria

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Co-Advisor: Asst. Prof. Dr. Jutarop Phetcharaburan , Prof. Dr. Yupa Hanboonsong

Introduction

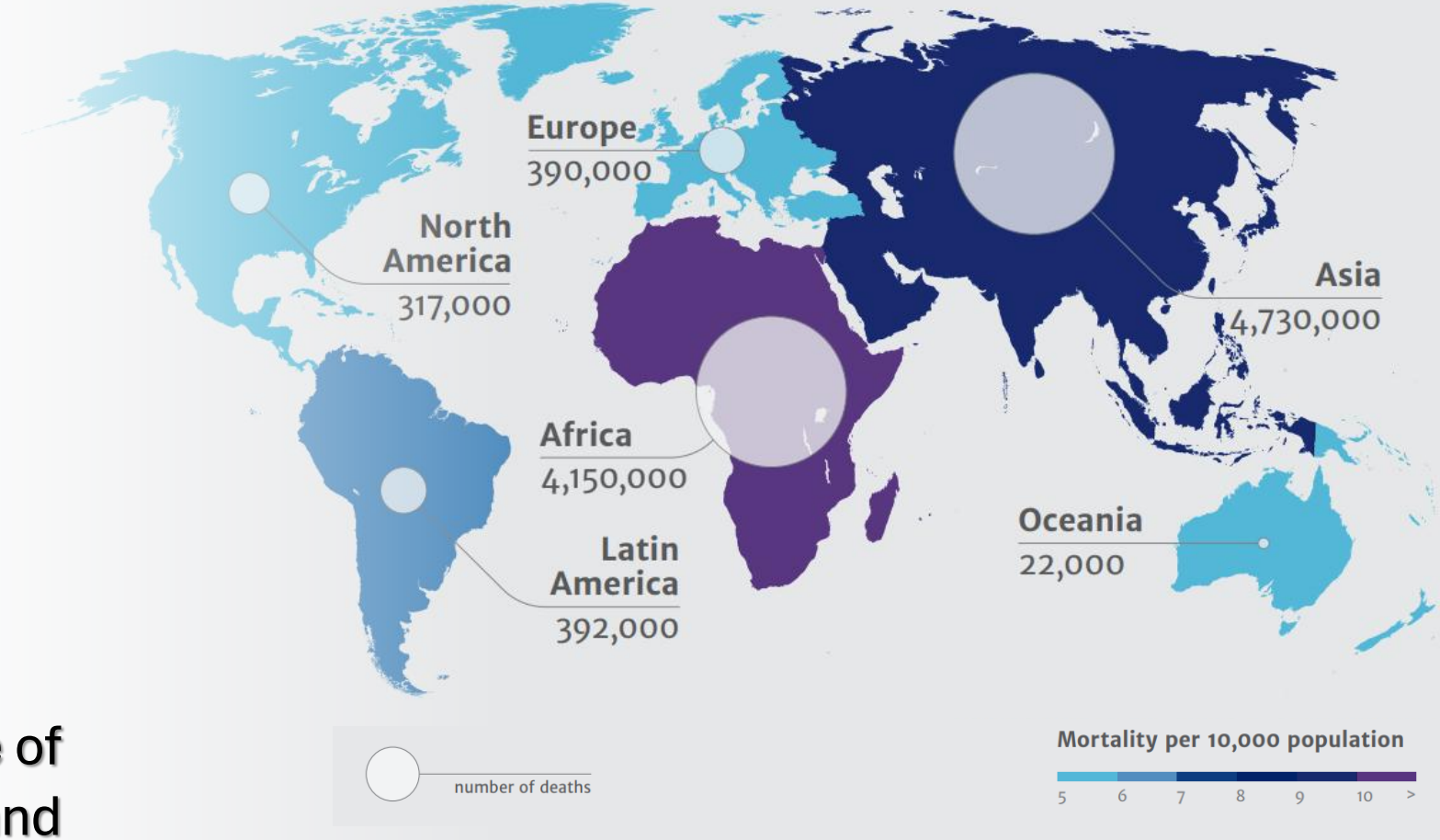
World Health Organization

Antimicrobial resistance 1.27 million global deaths in 2019

Cause by

Misuse, overuse, and long-term use of antibiotics in humans, **animals**, and plants.

https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance



(Review on Antimicrobial Resistance, 2014)

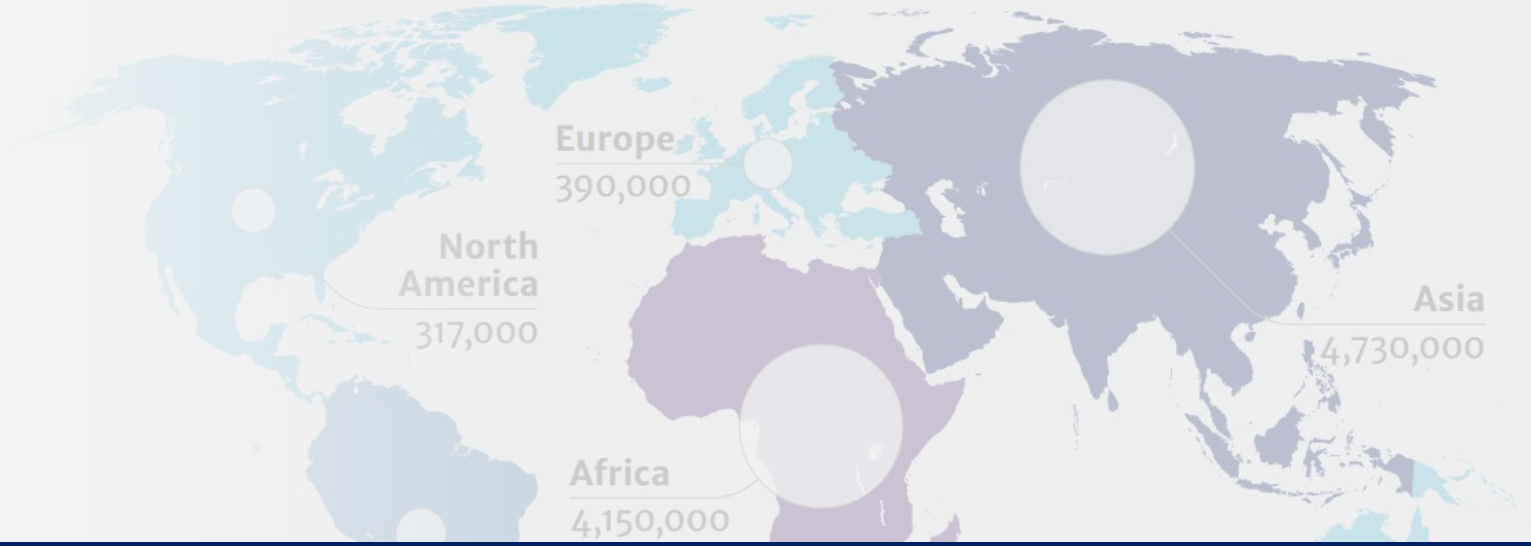
According to the review on antimicrobial resistance (2014)

The increasing AMR rate, **150 million people could die prematurely by 2050**

Overall, AMR may cause **10 million deaths per year worldwide**

Introduction

World Health Organization



Antimicrobial resistance
1.27 million global deaths

Consequently, One new strategy
Antimicrobial peptides (AMPs) are being explored as alternatives to
Traditional antibiotics are used to combat the rising threat of antimicrobial resistance.

antibiotics in humans, animals, and plants.

https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

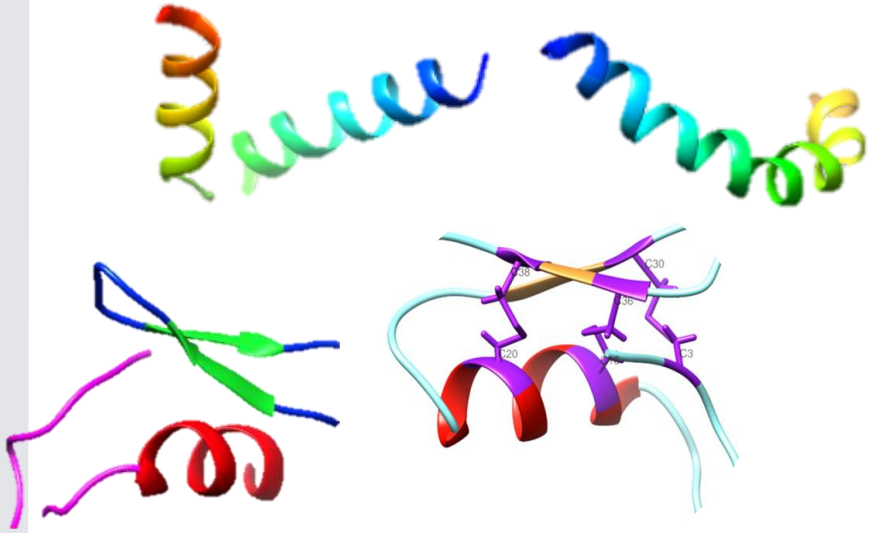
(Review on Antimicrobial Resistance, 2014)

According to the review on antimicrobial resistance (2014)

The increasing AMR rate, **150 million people could die prematurely by 2050**
Overall, AMR may cause **10 million deaths per year worldwide**

Antimicrobial peptide

(AMPs)



Structure and information of AMP: Manniello et al., (2021)

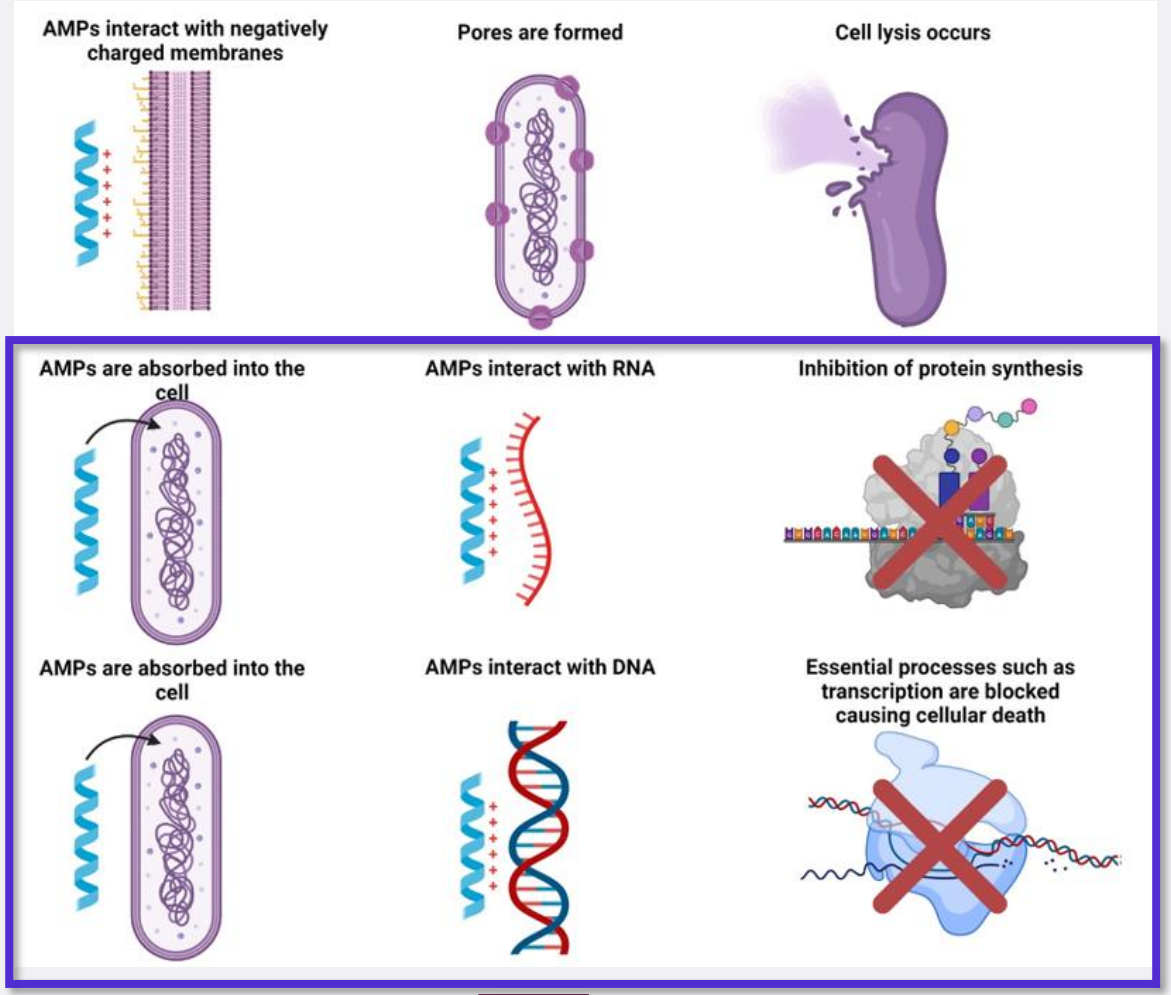
Small molecules: 10–100 amino acid residues

- **Mostly, cationic (positive charge),**
- **hydrophobic, and hydrophilic**
- Permeative components of the innate immune system
- Rapid action and show activity against bacteria, viruses, and fungi

AMPs action mechanism

Picture of the mechanism from Lima et al.,(2022)

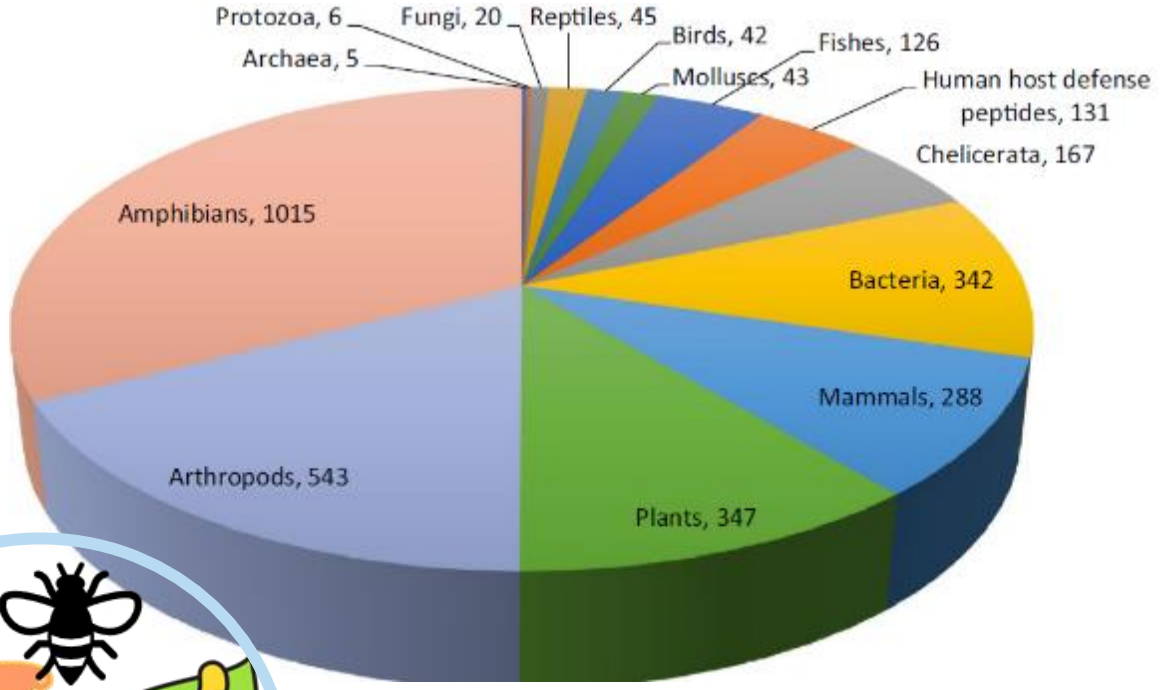
1 **Membrane Targeting Mechanism**



Targeting Interactions with DNA, RNA, and proteins 2

Antimicrobial peptide

Diversity of AMPs found in various organisms

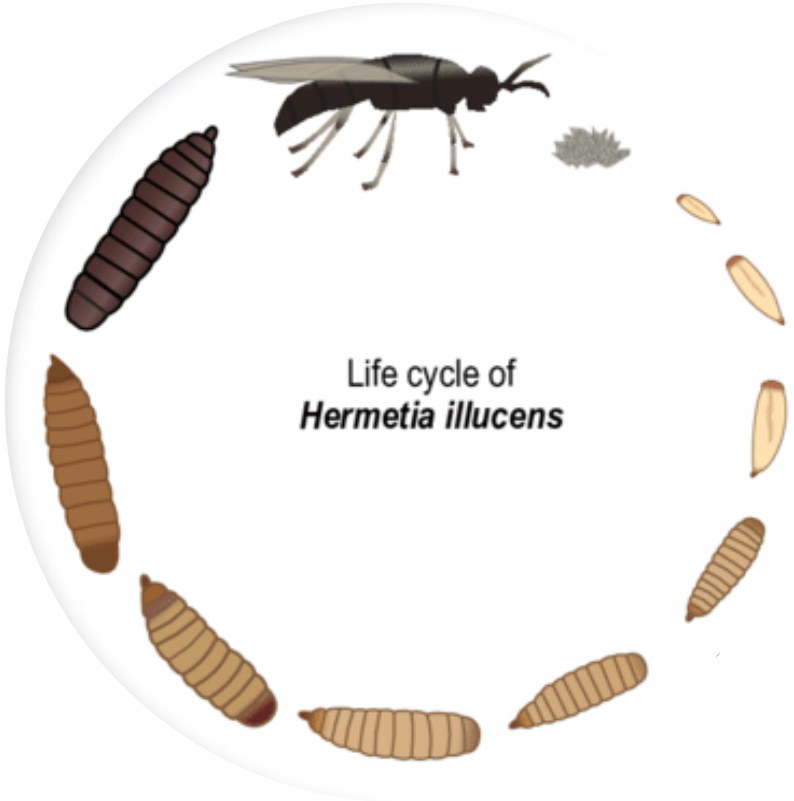


http:// aps. unmc. edu/ AP. 2019

Nayab et al., 2022 and Pimchan et al., 2024

Insects are one of the most famous sources of AMP

324 insect-derived AMPs



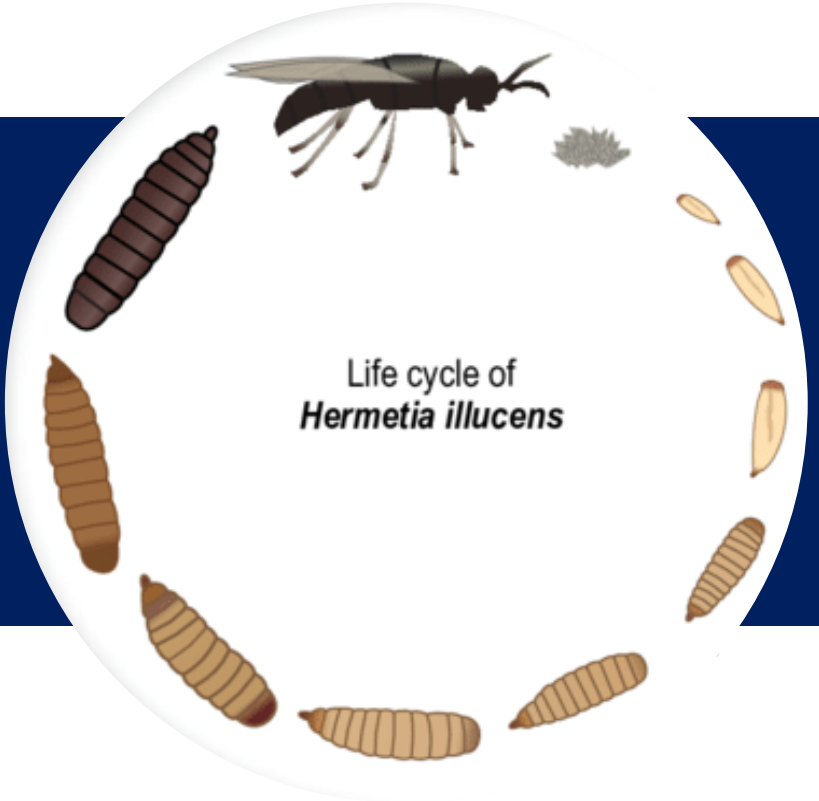
(*Hermetia illucens*)

Black soldier fly

Antimicrobial peptide

Diversity of AMPs found in various organisms

One of the most appealing insects for the AMP production



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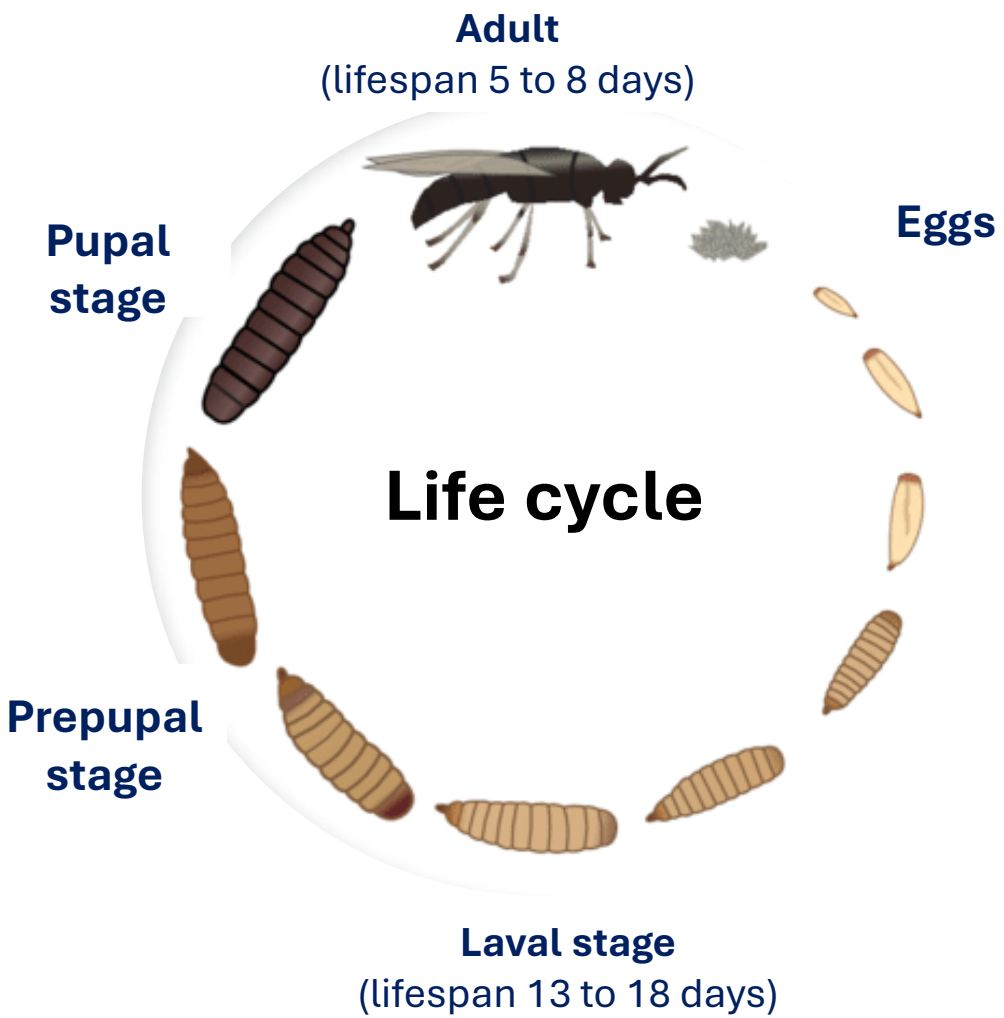
Insects are one of the most famous sources of AMP
324 insect-derived AMPs

(*Hermetia illucens*)
Black soldier fly

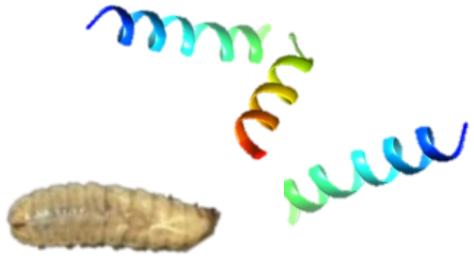
Black soldier fly; BSF

(*Hermetia illucens*)



https://www.researchgate.net/figure/The-life-cycle-of-the-black-soldier-fly-Hermetia-illucens-modified-from-De-Smet-et_fig1_349570282

AMPs derived from Black Soldier Fly larvae (BSF)



- **Defensin:**

Defensin-like peptides (DLP1-4), Hidefensin-1, Hill-BB (C6571, C16634, C46948 and C7985)

- **Cecropins:**

Cecropin 1, Cecropin-like peptides (CLP1-3)

- **Attacins:**

HI-attacin

- **Sarcotoxin:**

Sarcotoxin 1, 2a, 2b and 3

Pimchan et al., (2024)

Seminar papers

1st Paper

Developmental and Comparative Immunology 152 (2024) 105111

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/devcompimm

Molecular characterization and antimicrobial activity of cecropin family in *Hermetia illucens*

Jian Peng [a,b,c,1], Lu Li [b,e,1], Yan Wan [c,1], Yifan Yang [c], Xiaoqin An [c], Kexin Yuan [c], Zhilang Qiu [c], Yinhui Jiang [c], Guo Guo [a], Feng Shen [b,e,*], Guiyou Liang [a,d,**]

Peng et al. 2024
Impact Factor: 2.4 (2024)

2nd Paper

Current Research in Microbial Sciences 9 (2025) 100469

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

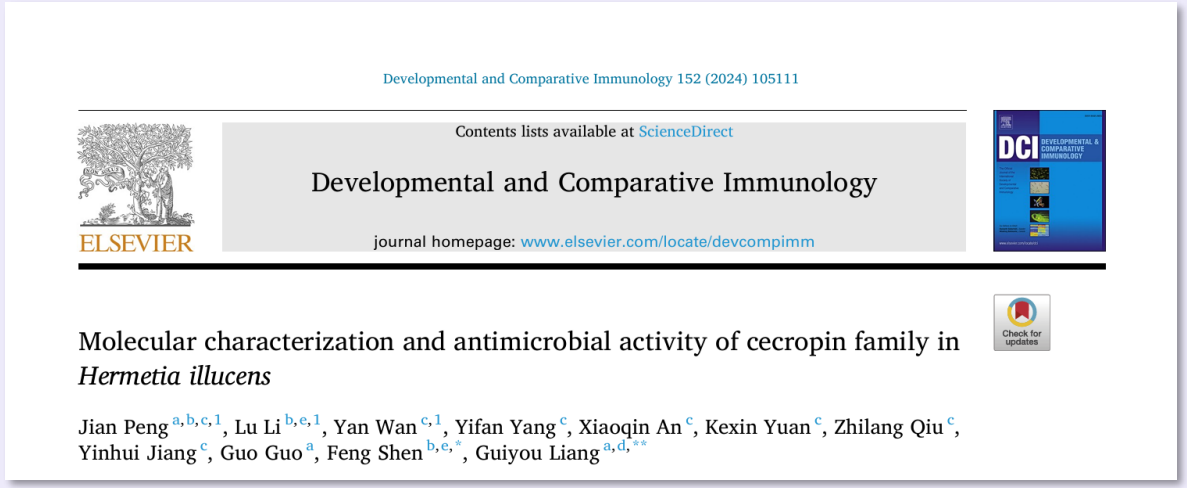
journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

The Black Soldier Fly *Hermetia illucens* Larva Presents an Antimicrobial Activity in Response to *Clostridioides difficile* Exposure

Aviel Melchior [a], Maya Azrad [b], Boris Fichtman [a], Avi Peretz [a,b,*]

Melchiora et al. 2025
Impact Factor: 5.8 (2024)

1st Paper



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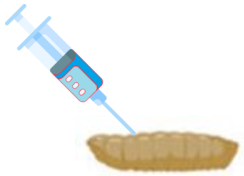
- To study the **quantity of cecropins and the genome structure** of *H. illucens*.
- To investigate the **antibacterial spectrum of the cecropin family.**
- To evaluate the **antibacterial activity of selected cecropins against *E. coli*.**

Overview

Genomic location and gene structure analysis of cecropin in *H. illucens*

Using TBtools

Transcriptome analysis of *H. illucens* after microbial stimulation



Assessment of AMPs :

- The minimum inhibitory concentration against microbial
- Effects of salt, trypsin, and serum on the antibacterial activity
- Cytotoxicity assay
- Kinetics of sterilization
- SEM and membrane integrity affect

Genomic location and gene structure analysis of cecropin

The Genomic Location

- TBtools · The GTF/GFF function

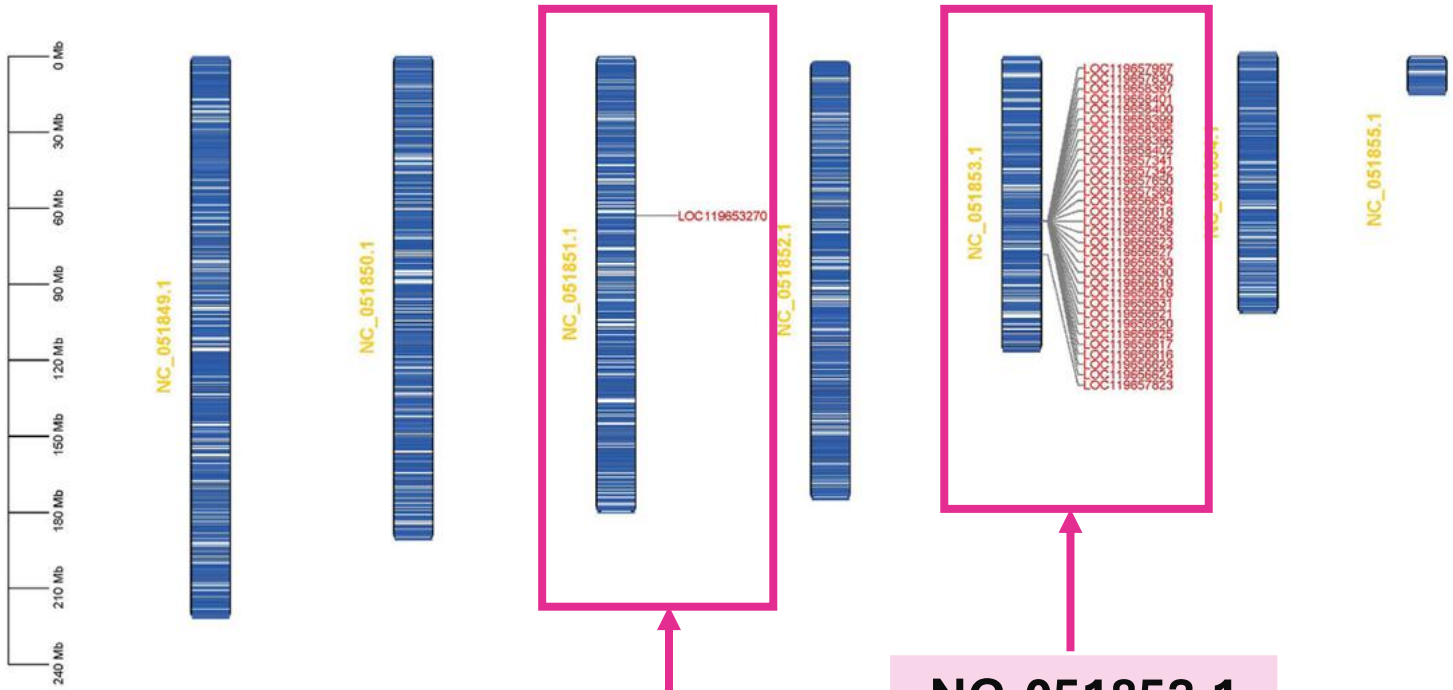
Gene structure analysis

- The One Step Build an ML Tree function

The molecular phylogenetic tree

- Neighbour-Joining method in MEGA7 (GenBank database)

Chromosomal location of 33 cecropins in *H. illucens*



NC-051851.1

1 cecropin

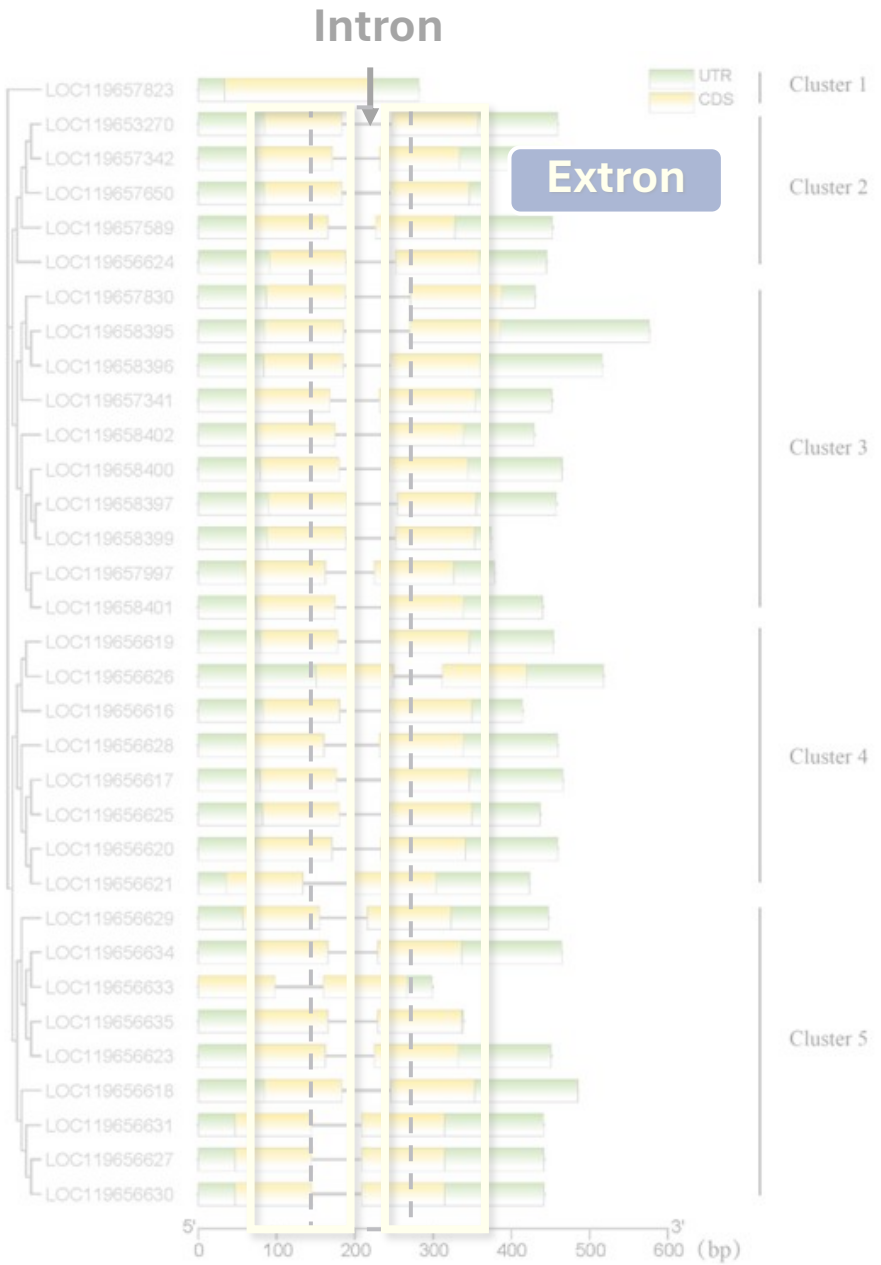
LOC119653270

NC-051853.1

32 cecropins

1. Analysis of the genome structure of the cecropin family *(cont.)*

Gene structure of cecropin family genes in *H. illucens*



5 clusters
two exons and one intron

One Step Build a ML Tree of TBtools

Methods

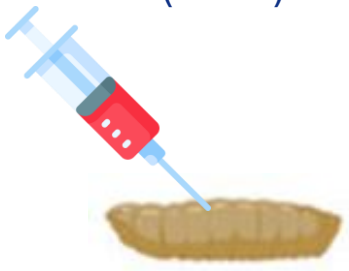
Different transcriptomes in *H. illucens* changed with microorganisms

RNA extraction and RNA sequencing

Test group

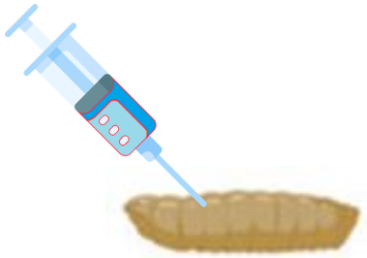
C. albicans : *S. aureus* : *E. coli*

(1:1:1)



Control group

PBS



24 h

The total RNA extracted: Trizol

The Illumina platform, Hisat2 Tools Soft mapping with the Reference Genome

Identification and annotation of differentially expressed genes

Differentially Expressed Genes (DEGs) Analysis

- DESeq2
- Selection Criteria: FDR < 0.05
- $\log_2$(fold change) ≥ 1

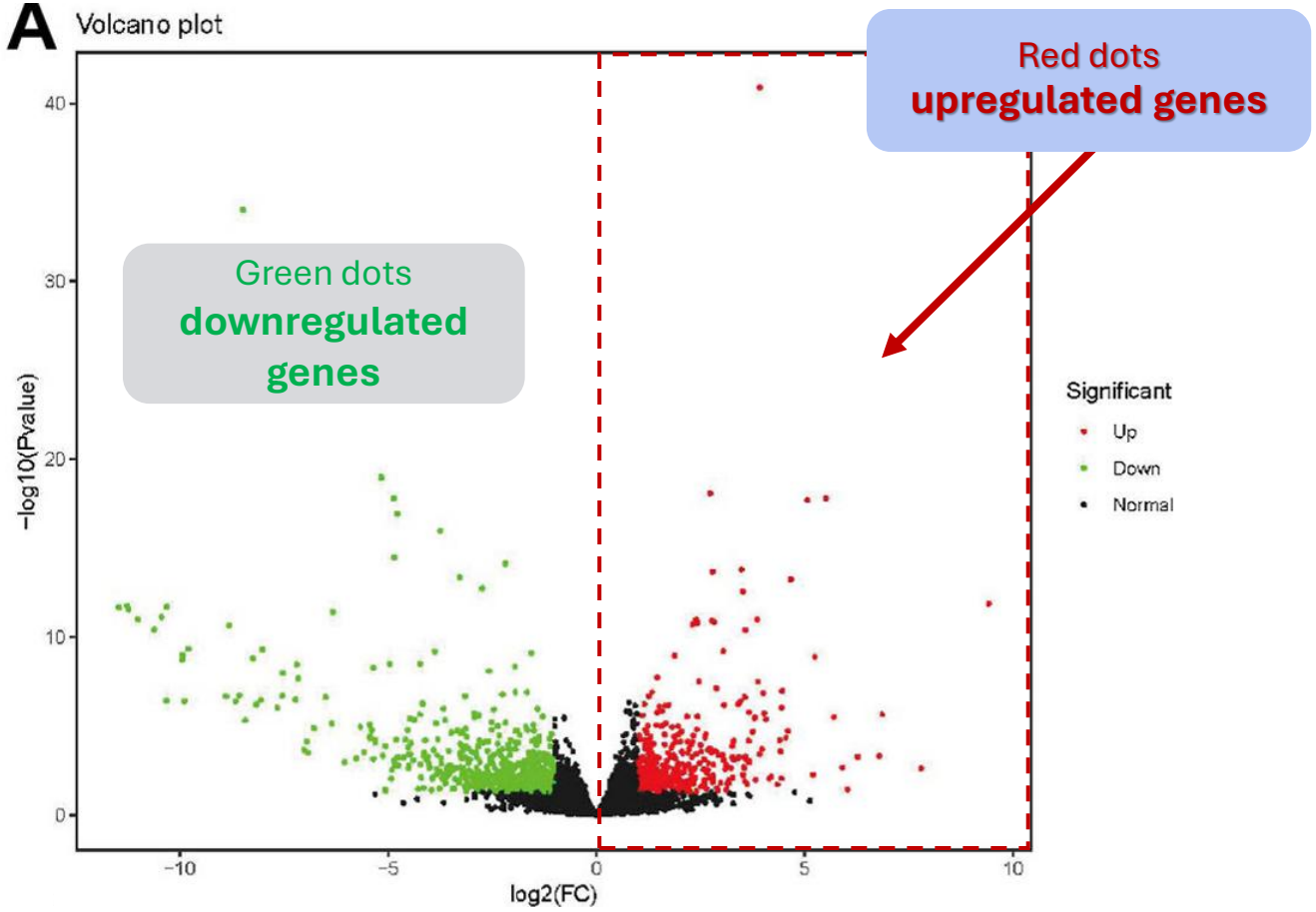
The expression of related genes was verified by

qPCR

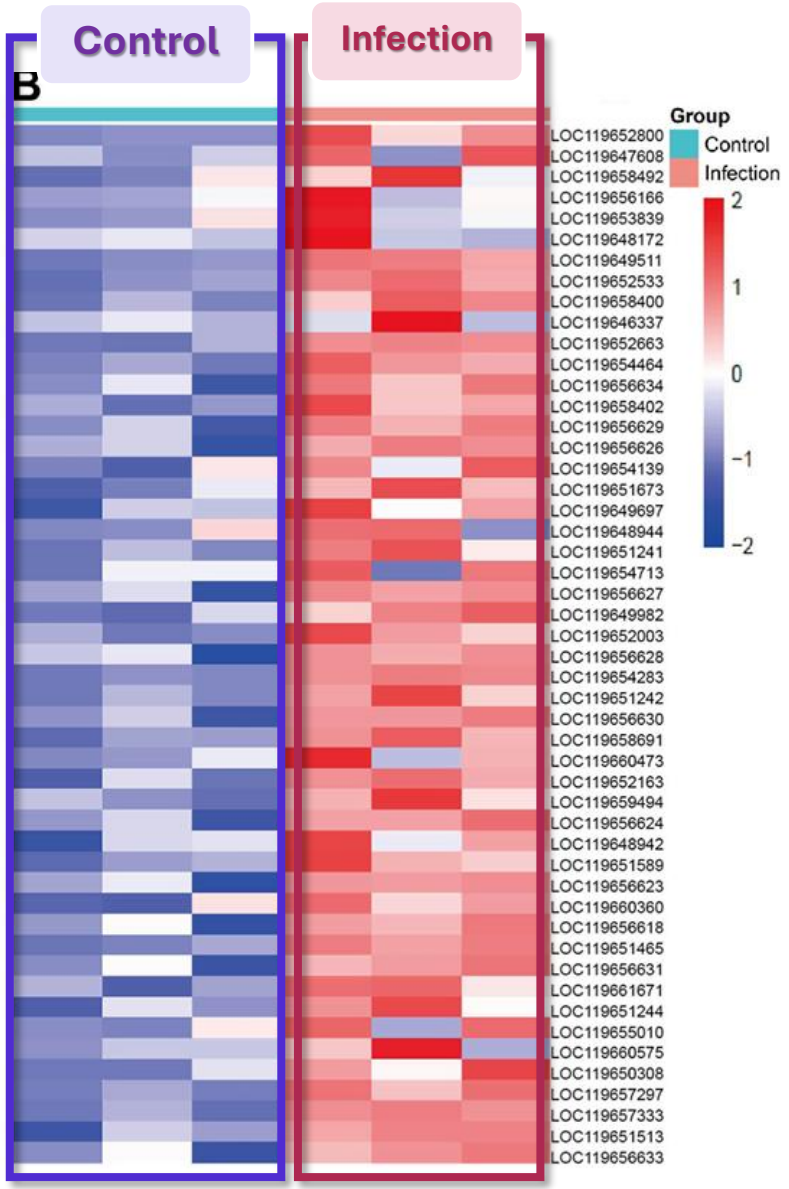
Results

Transcriptome analysis of *H. illucens* after microbial stimulation

Volcano Plot of Differentially Expressed Genes After Microbial Infection



Top 50 Upregulated Genes (Heatmap)



Results

Transcriptome analysis of *H. illucens* after microbial stimulation *(cont.)*

Table 3: Differential analysis of cecropin family genes

	Gene ID	Peptides	Description	log2 Fold_change	Corrected *p*-value
1	LOC119653270	H13	cecropin-like peptide 2	0.27	7.74×10^{-1}
2	LOC119656616	H14	RecName: Full = Cecropin-like peptide 1; Short = CLP1; Flags: Precursor	3.27	5.86×10^{-4}
3	LOC119656617	H5	cecropin-like peptide 2	2.66	4.64×10^{-3}
4	LOC119656618	H6	cecropin-like peptide 3	3.59	7.38×10^{-4}
5	LOC119656619	H5	cecropin-like peptide 2	3.11	
6	LOC119656620	H1	cecropin-like peptide 3	3.3	
7	LOC119656621	H5	cecropin-like peptide 2	3.0	
8	LOC119656623	H6	cecropin-like peptide 3	3.6	
9	LOC119656624	H8	cecropin-like peptide 3	3.6	
10	LOC119656625	H5	cecropin-like peptide 2	3.1	2.71×10^{-3}
11	LOC119656626	H8	cecropin-like peptide 3	4.46	9.56×10^{-7}
12	LOC119656627	H6	cecropin-like peptide 3	4.07	4.30×10^{-6}
13	LOC119656628	H8	cecropin-like peptide 3	3.98	2.14×10^{-4}
14	LOC119656629		cecropin-like peptide 3	4.47	1.11×10^{-7}
15	LOC119656630	H6	cecropin-like peptide 3	3.89	3.30×10^{-8}
16	LOC119656631	H6	cecropin-like peptide 3	3.58	5.27×10^{-4}
17			peptide 3		10^{-}
18	LOC119656634	H4	cecropin-like peptide 3	4.61	2.00×10^{-5}
19	LOC119656635	H6	cecropin-like peptide 3	3.41	6.74×10^{-4}
20	LOC119657341	H7	cecropin-like peptide 3	-	-
21	LOC119657342	H12	cecropin-like peptide 2	-	-
22	LOC119657589	H10	cecropin-like peptide 2	2.05	2.07×10^{-2}
23	LOC119657650	H15	cecropin-like peptide 3	2.47	3.25×10^{-8}
24	LOC119657823		cecropin-A2-like	-	-
		7830	H11	cecropin-like peptide 3	1.12	2.78×10^{-1}
		57997	H2	cecropin-like peptide 3	3.27	1.36×10^{-4}
		58395	H3	cecropin-like peptide 3	1.88	8.26×10^{-2}
		58396		cecropin-like peptide 3	-	-
		58397	H2	cecropin-like peptide 3	2.31	7.98×10^{-5}
30	LOC119658399	H2	cecropin-like peptide 3	3.3	7.29×10^{-5}
31	LOC119658400	H2	cecropin-like peptide 3	5.25	1.33×10^{-9}
32	LOC119658401	H9	cecropin-like peptide 3	2.99	1.65×10^{-2}
33	LOC119658402	H9	cecropin-like peptide 3	4.56	4.65×10^{-5}

Note:"-"indicates that it is not detected in the transcriptome.

33 cecropin genes

15 peptide group: H1 –H15

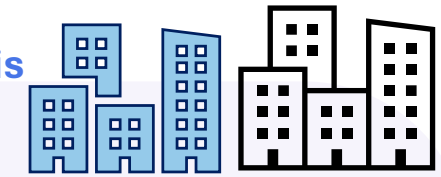
33 genes with log2FC > 0

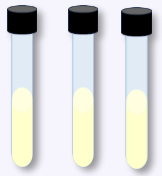
upregulated expression

Methods

1. Determination of the minimum inhibitory concentration (MIC) of cecropin

The AMPs H1-H15 were synthesized by Gil Biochemical Co., Ltd., Shanghai,
using solid-phase chemical synthesis

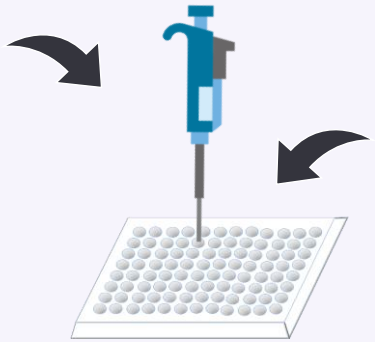




logarithmic growth stage,
1×10^6 CFU/mL

The peptides AMP

128 μg/ml
to 2 μg/ml



Gram-negative
- *A. baumannii* (ATCC 17978)
- *E. coli* (ATCC 25922)
- *K. pneumoniae* (ATCC 13883)
- *P. aeruginosa*
- *V. parahemolyticus*

Gram-positive
- *S. aureus* (ATCC 25923)
- *B. subtilis* (ATCC 29212)
- *S. epidermidis* (ATCC 35984)
- *E. faecalis* (ATCC 29212)

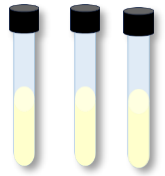
Fungus
- *C. albicans*

serially diluted

incubated
37°C, 18-20 h.

According to the Clinical Laboratory Standards Institute (CLSI)

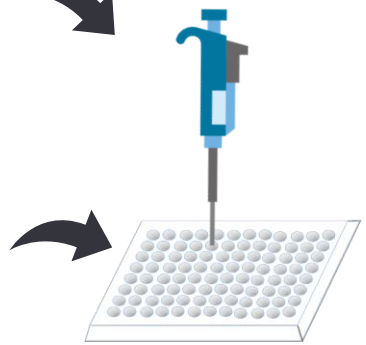
2. Effects of salt, trypsin, and serum on the antibacterial activity of AMPs



bacterial suspension
1×10^6 CFU/mL

The peptides AMP

128 μg/ml
to 2 μg/ml



Different salts
- NaCl (150 mM)/
- KCl (4.5 mM)/
- $MgCl_2$ (1 mM)/
- $CaCl_2$ (2 mM)/
- trypsin (1 mg/mL)/
- 10% fetal bovine serum

incubated 37°C, 20 h.

Results

Antibacterial effect of the cecropin family

Table 1: Antibacterial activities of AMPs H1-H15 (MIC, μg/mL, μM)

Table 1

Antibacterial activities of antimicrobial peptides H1-H15 in *H. illucens* (MIC, μg/mL, μM).

	Strains	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15
Gram-negative bacteria	*E. coli* (ATCC25922)	4(0.8)	4(0.8)	4(0.8)	8(1.6)	16(3.2)	4(0.8)	4(0.7)	>128 (25.6)	8(1.6)	8(1.7)	8(1.5)	8(1.7)	16(3.0)	16(3.2)	16(3.4)
	K. pneumoniae (ATCC700603)	32(6.4)	32(6.6)	16(3.1)	32(6.5)	16(3.2)	16(3.2)	16(2.9)	>128 (25.6)	64(13.0)	16(3.3)	16(3.1)	32(6.9)	16(3.3)	32(6.5)	16(3.4)
	A.baumannii (ATCC19606)	32(6.4)	32(6.6)	32(6.2)	32(6.5)	64(12.9)	>128 (25.6)	16(2.9)	>128 (25.6)	32(6.5)	16(3.3)	64(12.4)	32(6.9)	32(6.1)	32(6.5)	32(6.8)
	P. aeruginosa (CMCC10104)	>128 (25.6)	>128 (26.2)	>128 (25)	>128 (26)	>128 (25.8)	>128 (25.6)	>128 (25.6)	>128 (25.6)	>128 (25.9)	>128 (26.7)	>128 (24.7)	>128 (27.4)	32(6.1)	64(12.9)	64(13.5)
	V. parahaemolyticus (ATCC17802)	8(1.6)	4(0.8)	8(1.6)	16(3.2)	8(1.6)	8(1.6)	16(2.9)	>128 (25.6)	8(1.6)	8(1.7)	16(3.1)	16(3.4)	16(3.0)	16(3.2)	8(1.7)
Gram-positive bacteria	*E. faecalis* (ATCC29212)	>128 (25.6)	>128 (26.2)	>128 (25)	>128 (26)	>128 (25.8)	>128 (25.6)	>128 (25.6)	>128 (25.6)	>128 (25.9)	>128 (26.7)	>128 (24.7)	>128 (27.4)	>128 (24.2)	>128 (25.9)	>128 (27.1)
	B. subtilis (BNCC109047)	16(3.2)	32(6.6)	8(1.6)	8(1.6)	16(3.2)	8(1.6)	4(0.7)	>128 (25.6)	8(1.6)	8(1.7)	8(1.5)	16(3.4)	8(1.5)	8(1.6)	8(1.7)
	S. epidermidis (ATCC35984)	>128 (25.6)	>128 (26.2)	>128 (25)	>128 (26)	>128 (25.8)	>128 (25.6)	>128 (25.6)	>128 (25.6)	>128 (25.9)	>128 (26.7)	>128 (24.7)	>128 (27.4)	>128 (24.2)	>128 (25.9)	>128 (27.1)
	S. aureus (ATCC6538)	>128 (25.6)	>128 (26.2)	>128 (25)	>128 (26)	>128 (25.8)	>128 (25.6)	>128 (25.6)	>128 (25.6)	>128 (25.9)	>128 (26.7)	>128 (24.7)	>128 (27.4)	>128 (24.2)	>128 (25.9)	>128 (27.1)
Fungus	*C. albicans* (ATCC10231)	>128 (25.6)	>128 (26.2)	>128 (25)	>128 (26)	>128 (25.8)	>128 (25.6)	>128 (25.6)	>128 (25.6)	>128 (25.9)	>128 (26.7)	>128 (24.7)	>128 (27.4)	>128 (24.2)	>128 (25.9)	>128 (27.1)

Antibacterial Activity

Strong activity against Gram-negative bacteria

Weak activity against Gram-positive bacteria and *C. albicans*

H1, H2, and H3 showed the strongest antibacterial activity against *E. coli*

Antibacterial effect of the cecropin family *(cont.)*

Table 2: Effects of salt ions, trypsin and serum on antimicrobial activities of AMPs (MIC, μg/mL)

AMPs	PBS	NaCl	$CaCl_2$	KCl	Serum	Trypsin
H1	4(0.8)	4(0.8)	8(1.6)	4(0.8)	4(0.8)	>128(25.6)
H2	4(0.8)	4(0.8)	8(1.6)	4(0.8)	4(0.8)	>128(26.2)
H3	4(0.8)	4(0.8)	8(1.6)	4(0.8)	4(0.8)	>128(25)

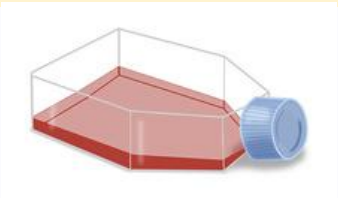
Note: The experiment was performed in triplicate and repeated three times.

- Ca^{2+} and Trypsin reduce the activity of H1–H3
- K^+, Na^+, and serum have no significant effect
- No effect of temperature 37°C for 24 h

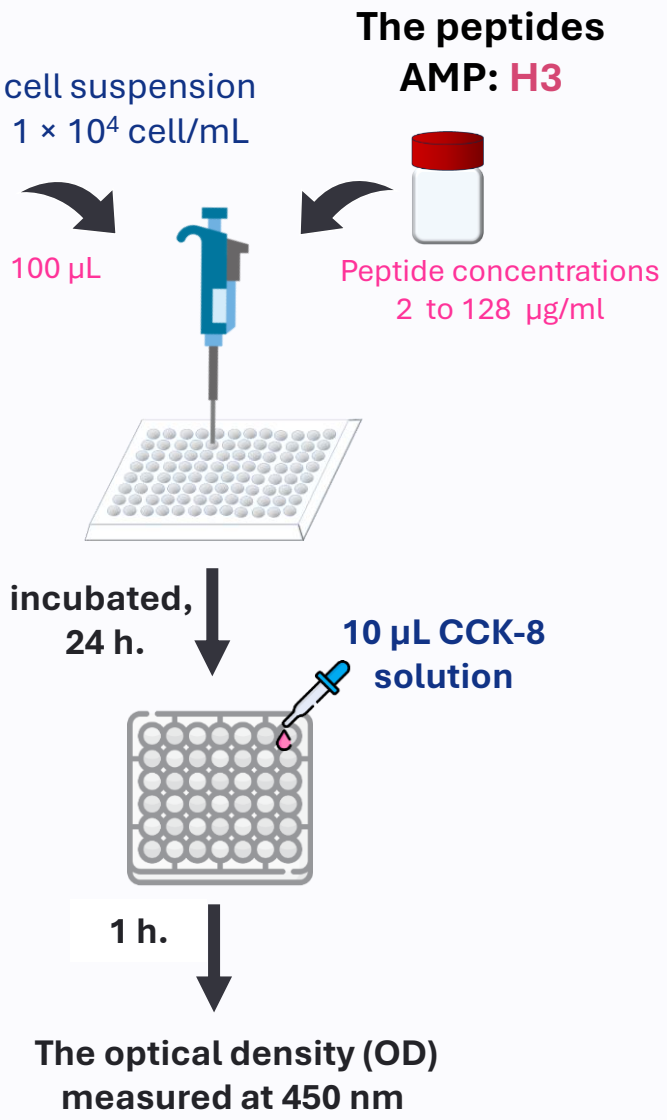
Methods

3. Cytotoxicity assay

Human normal hepatocytes LO2

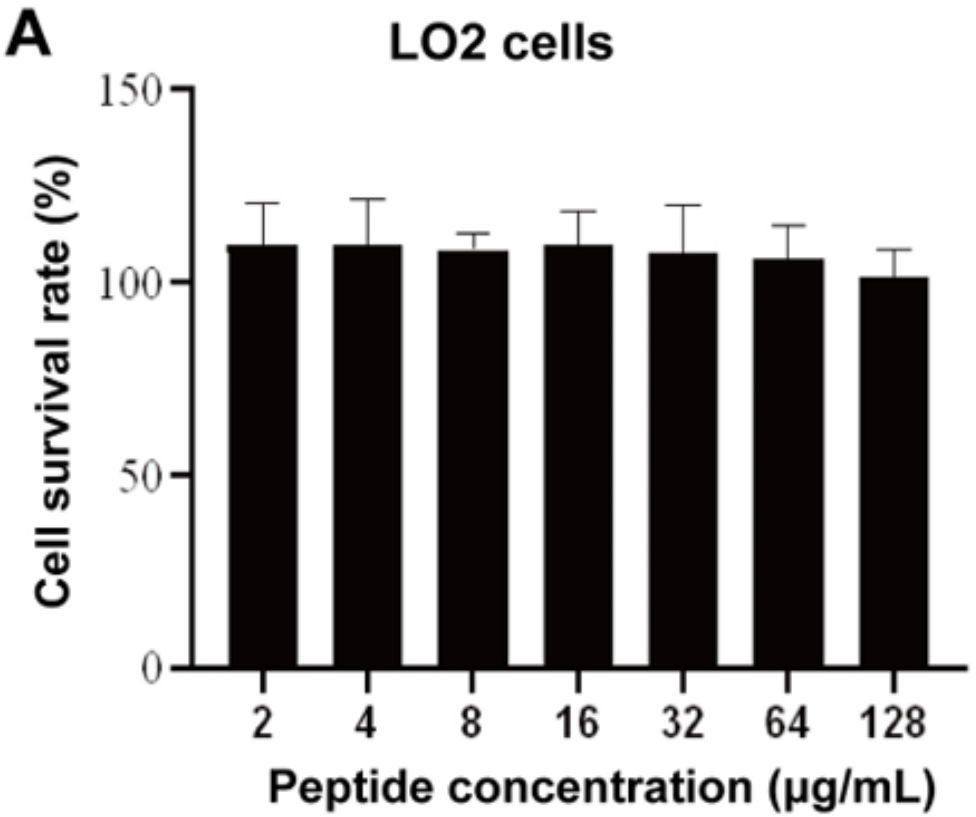


DMEM medium
+ 10% FBS and 1% penicillin-streptomycin



Results

Fig. 1: Cytotoxicity of H3 in human liver cells

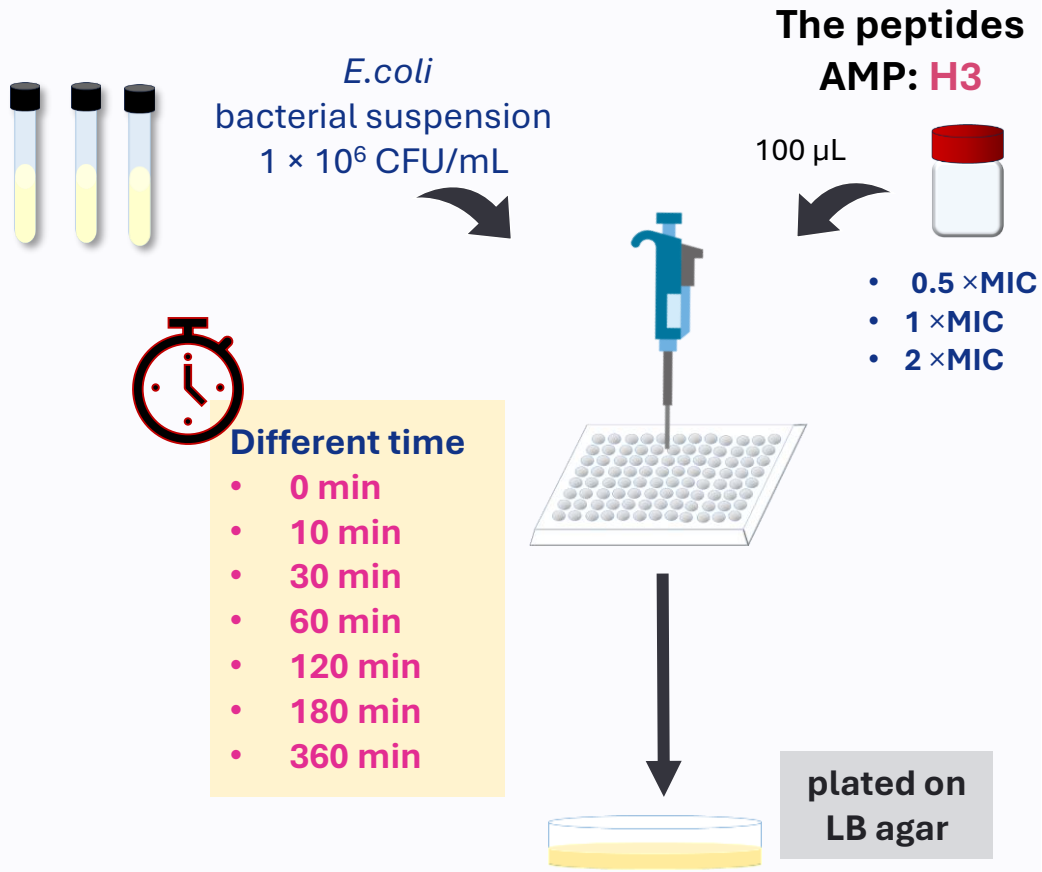


Effectiveness and Safety of H3

- No cytotoxicity observed up to 128 µg/mL
- H3 is safe for human cells

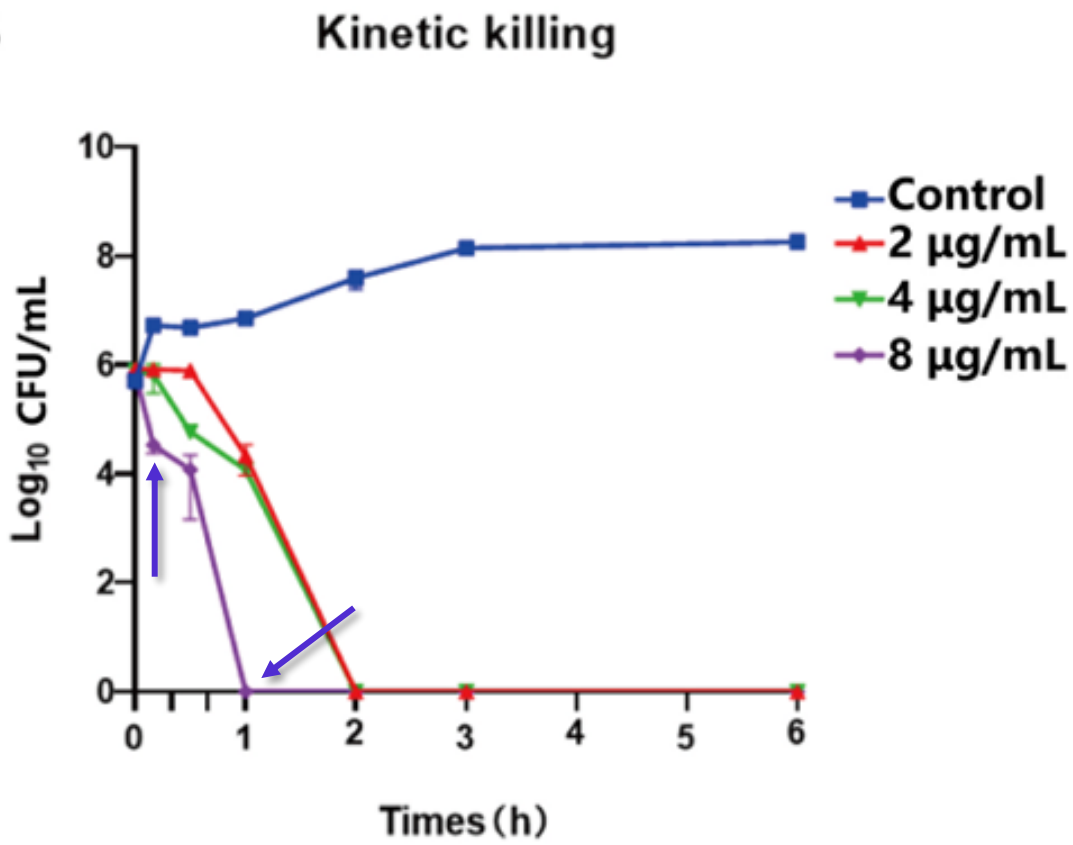
Methods

4. Kinetics of sterilization



Results

Fig.2: Time bactericidal curve of different concentrations of H3 against *E. coli*



H3 shows rapid bactericidal activity

- 2×MIC (8 μg/mL): reduces *E.coli* within 20 min
- Kills *E. coli* completely within 1 h

Methods

5. Scanning electron microscope

Control: PBS **Test:** H3 (2xMIC)

bacterial suspension
1×10^6 CFU/mL

incubated 37°C, 1 h.

centrifuged
2000 rpm, 10 min.

collected precipitate

fixed with
2.5%
glutaraldehyde

Dehydrated

gradient
tert-butanol/ethanol
mixture

dryer and
spraying gold

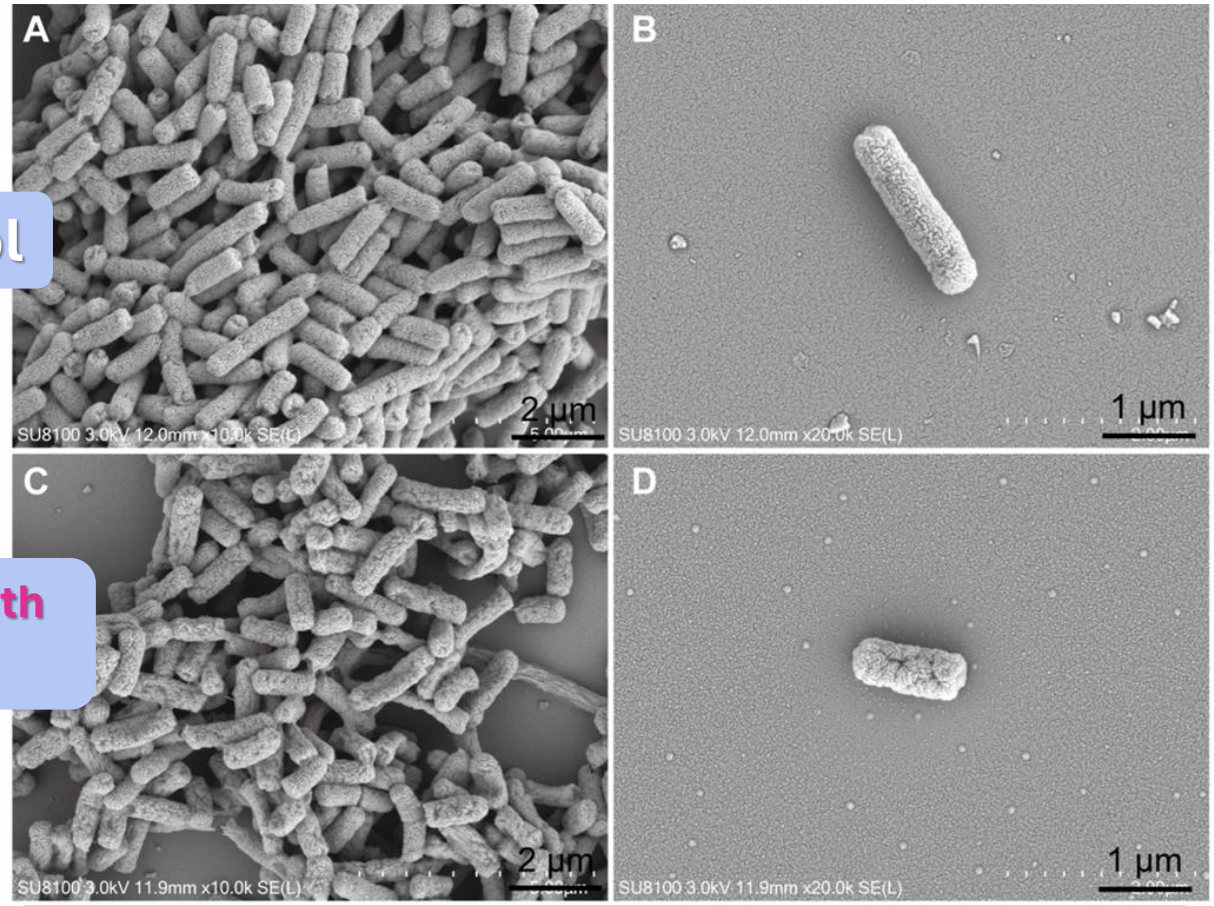
scanning electron microscope

Results

Fig. 3: Scanning electron microscope image of *E. coli* treated with H3

Control

Treat with H3



H3 shows the mechanism of the membrane

Membrane shrinkage and rupture
→ formation of pores/channels

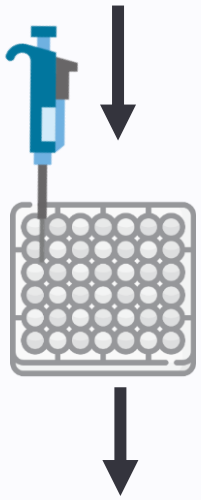
Methods

6. The permeability of the outer membrane and the inner membrane

bacterial suspension
1×10^8 CFU/mL

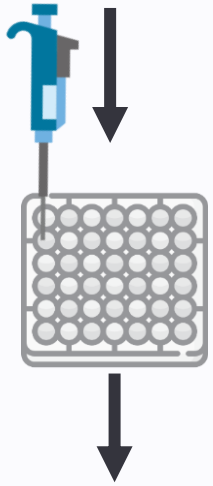
outer membrane: NPN
(n-phenylnaphthylamine)

inner membrane: PI
(Prodium Iodide)



10 μM, 30 sec

+ Peptide H3
4, 8, 16, 32, 64 μg/mL

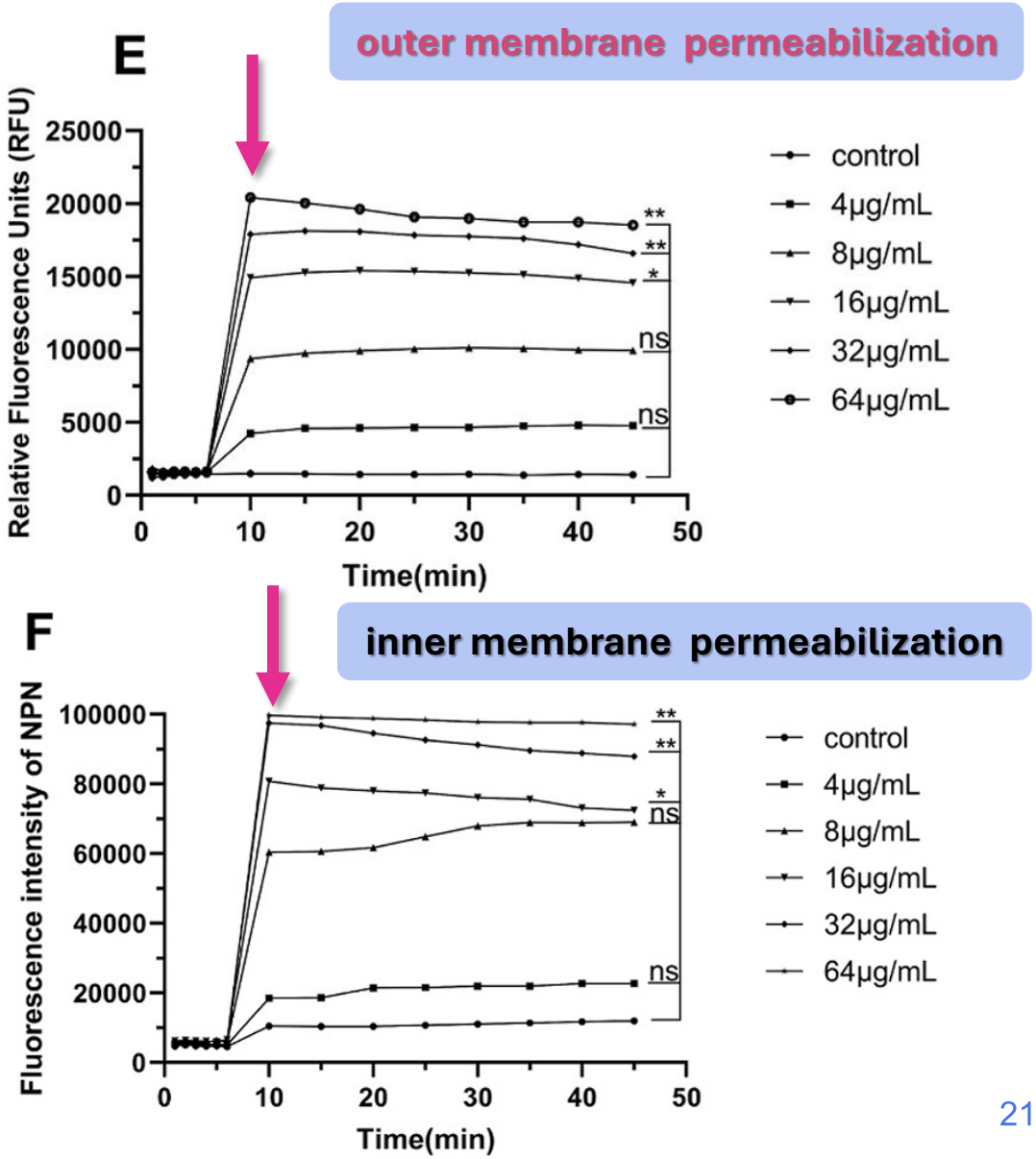


Excitation: 315 nm
Emission: 400 nm

Excitation: 535 nm
Emission: 610 nm

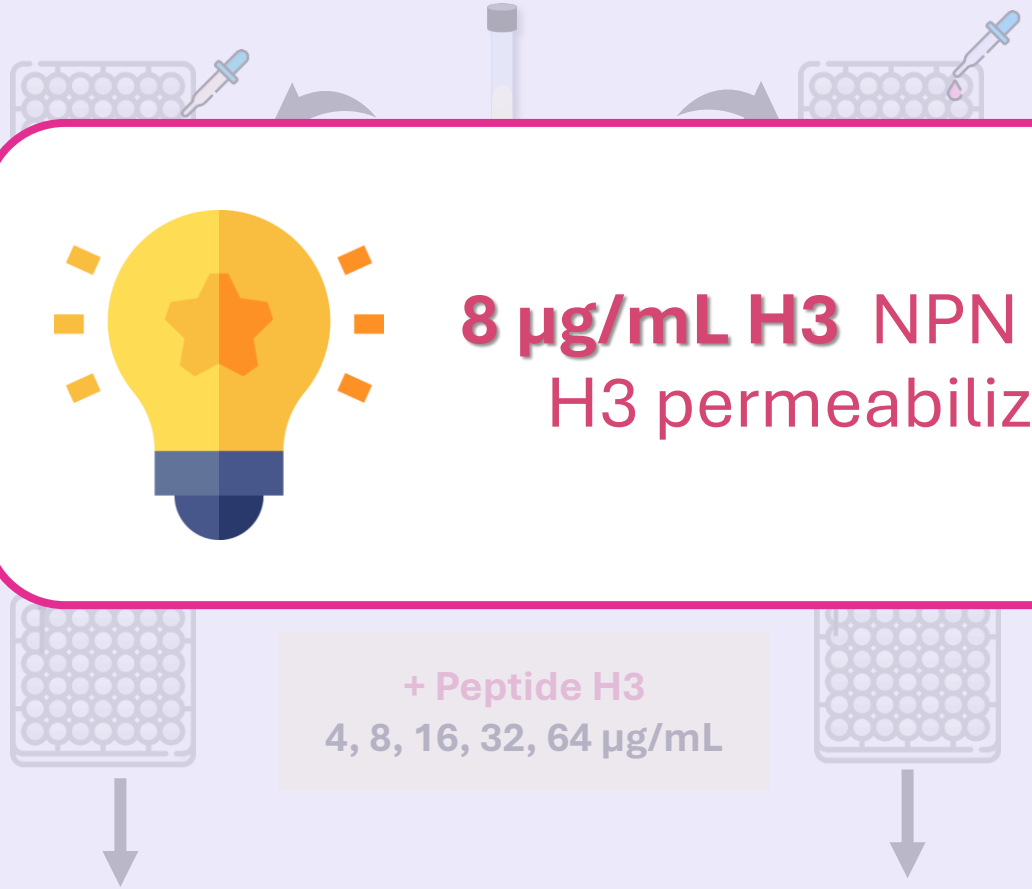
Results

Fig. 4: *E. coli* membrane integrity affected by H3



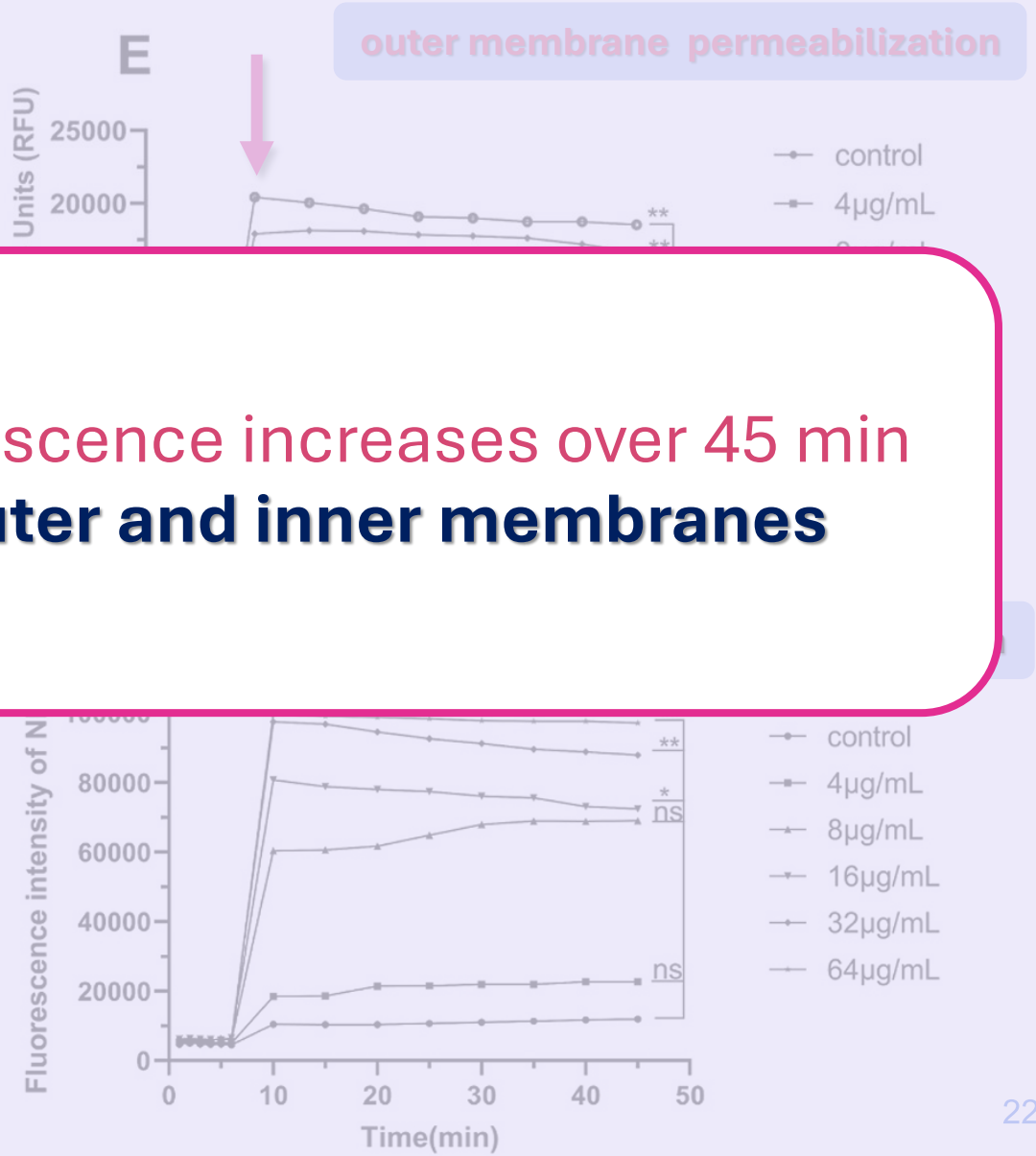
Methods

6. The permeability of the outer membrane and the inner membrane



Results

Fig. 4: *E. coli* membrane integrity affected by H3

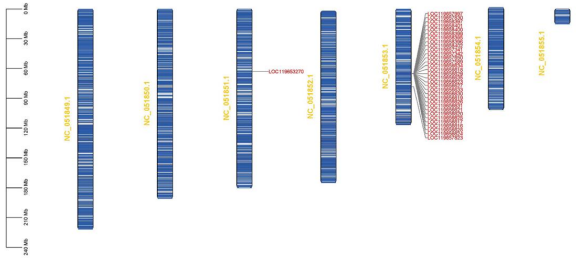


8 μg/mL H3 NPN & PI fluorescence increases over 45 min
H3 permeabilizes both **outer and inner membranes**

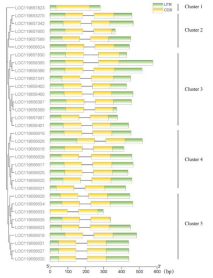
Conclusions

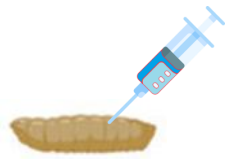
1st Paper

Genomic location and 33 cecropins in *H. illucens*



Transcriptome analysis after **microbial stimulation**





15 peptide group: H1 –H15

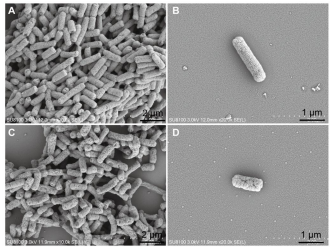
Antibacterial effect of the cecropin family

H1, H2, and H3 showed the strongest antibacterial activity against *E. coli*

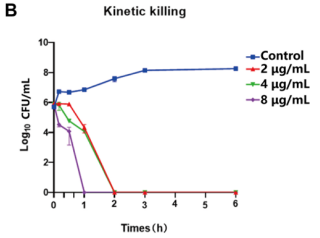
Effects of salt ions, trypsin and serum on antimicrobial activities

H3 showed the strongest

H3 cytotoxic effect
low cytotoxicity



H3 killing kinetics.
within 1 h



H3 shows a membrane-disruption mechanism

The genome analysis revealed 33 cecropin

Cecropin H3 showed the effect of environmental factors and strong activity against *E.coli*

H3 exhibited low toxicity and rapid killing kinetics.

Moreover, H3 shows a membrane-disruption mechanism

2nd Paper

Current Research in Microbial Sciences 9 (2025) 100469

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

The Black Soldier Fly *Hermetia illucens* Larva Presents an Antimicrobial Activity in Response to *Clostridioides difficile* Exposure

Aviel Melchior [a], Maya Azrad [b], Boris Fichtman [a], Avi Peretz [a,b,*]

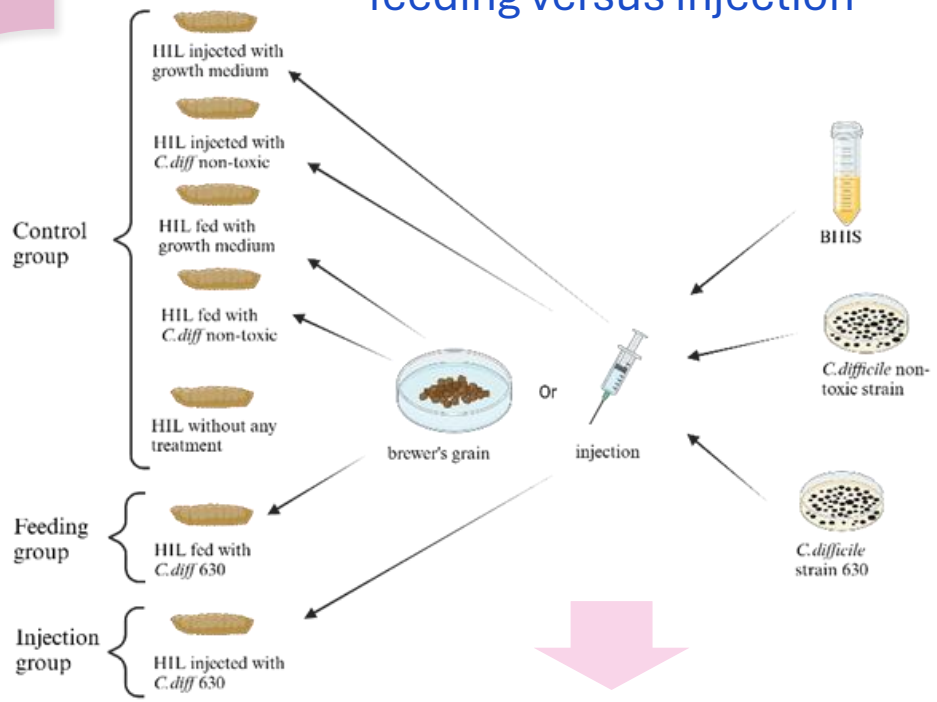
[a] The Faculty of Medicine in the Galilee, Bar Ilan University, Safed 1311502, Israel
[b] Clinical Microbiology Laboratory, Tzafon Medical Center, Poriya, Tiberias 152800, affiliated with Azrieli Faculty of Medicine, Bar Ilan University, Safed 1311502, Israel, Israel

- To investigate whether BSFL hemolymph has an **antimicrobial effect against *C. difficile***
- To determine the effect of *C. difficile* exposure mode **(feeding with infected food versus injection)** on BSFL antimicrobial activity.

Overview

BSFL exposure to *C. difficile*

feeding versus injection



Hemolymph extraction

Assessment:

- Hemolymph antimicrobial activity testing
- AMPs gene expression
- Cell viability levels measurement
- Morphological alterations in the bacterial membrane

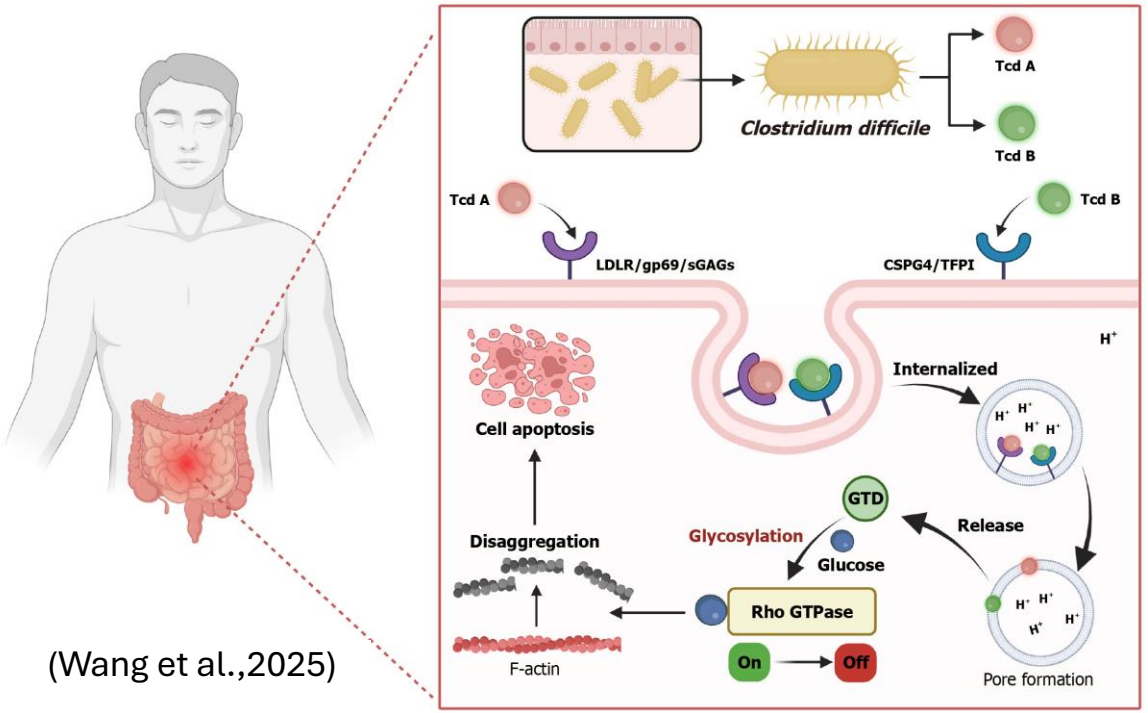
2nd Paper

Clostridioides difficile
C. difficile

- Gram-positive
- Obligate anaerobic
- **Toxin**: Toxin A and Toxin B
- Vegetative form or in highly resistant spore form

https://microbiologyinpictures.com/bacteria-micrographs/gram-stain/gram-positive/clostridium-difficile-gram-stain.html

The mechanism of the C. difficile infection



(Wang et al.,2025)

the key virulence factors

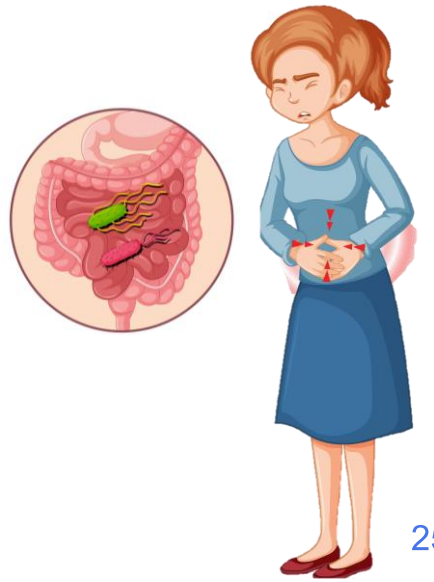
Toxin A (TcdA)
Toxin B (TcdB)
(These toxins are glucosyl transferases)

targeting Rho proteins

disruption of
F-actin and the formation of microtubule protrusions.

Clinical manifestations

- mild diarrhea to severe
- pseudomembranous colitis
- toxic megacolon
- colonic perforation



(Pourliotopoulou, Karampatakis, & Kachrimanidou, 2024)

Materials and methods

Insect and bacterial growth

BSF larvae

Fourth instar larvae

28 ± 2 ◦ C

brewer's grains medium

C. difficile

***C. difficile BAA-1382* strain 630**
(a toxin-producer strain)

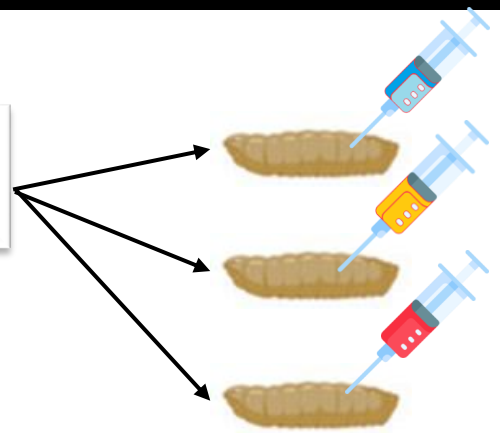
C. difficile BAA-1801
(a non-toxin-producer strain)

+ Control group without treatment

BSFL exposure to *C. difficile* treatments

Injection group

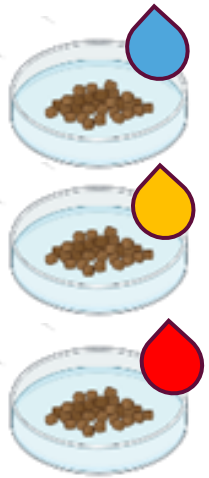
10 µL



1. BHIS medium
2. *C. difficile BAA-1801* (non-toxin strain)
3. *C. difficile BAA-1382* strain 630 (toxin strain)

Feeding group

brewer's grains medium



1. BHIS medium
2. *C. difficile BAA-1801* (non-toxin strain)
3. *C. difficile BAA-1382* strain 630 (toxin strain)

24 h

Hemolymph extraction

Methods

1. Hemolymph antimicrobial activity testing

Clinical isolates from the Tzafon Medical Center, Poriya, Israel

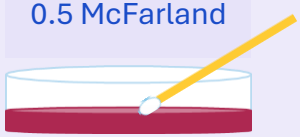
C. difficile vancomycin-resistant isolates (n=6)

C. difficile metronidazole-resistant isolates (n=6)

C. difficile 6 vancomycin- and metronidazole-resistant isolates (n=6)

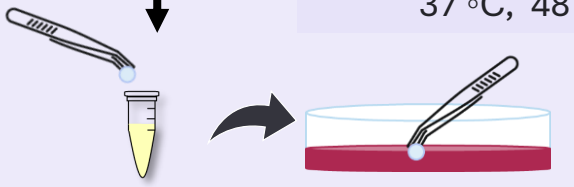
Disk diffusion method

0.5 McFarland



Brucella blood agar

37 ◦C, 48 h.



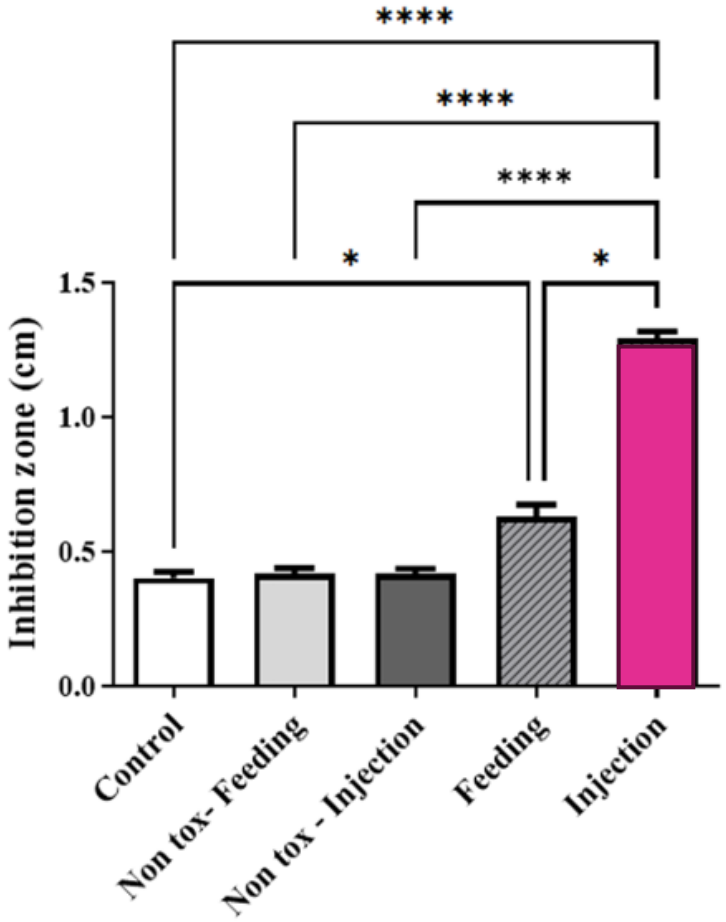
Hemolymph from immunized

1. **Non-toxic Injection**
2. **Toxic Injection (injection)**
3. **Non-toxic Feeding**
4. **Toxic Feeding (feeding)**
5. Control

Results

Hemolymph inhibition assay

Fig.5: Hemolymph from immunized BSFL had an inhibitory effect against *C. difficile* isolates



The toxic injection treatment showed the **largest inhibition zone** among all treatments

Results

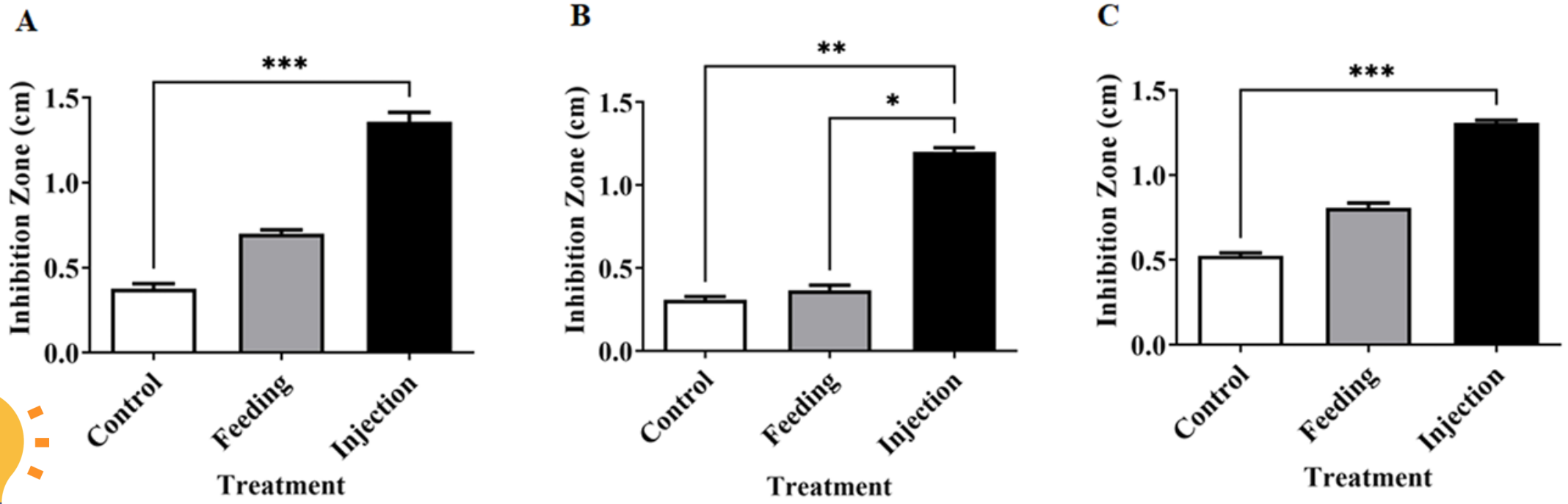
Hemolymph inhibition assay *(cont.)*

Fig.6: The effect of hemolymph from injection and feeding methods against various antibiotic-resistant *C. difficile* isolates

(A) vancomycin-resistant *C. difficile* isolates

(B) metronidazole-resistant *C. difficile* isolates

(C) vancomycin- and metronidazole-resistant *C. difficile* isolates



Hemolymph from toxin-injected BSFL showed significantly higher inhibition against drug-resistant *C. difficile*

Feeding treatment did not significantly affect the inhibition zone compared to the Control group.

Methods

2. AMPs gene expression

Treatments

collected
Fat body

1. Feeding with *C. difficile*
2. Injection with *C. difficile*
3. Injection of BHIS (Control 1)

or without any treatment (Control 2)

RNA extracted from the fat body

TRIzol Kit

theqPCRBIO cDNA Synthesis Kit

cDNA synthesized

real-time RT PCR

The expression levels of five AMP genes

Targeted genes

1.DLP4
2. DLP3
3. attacin B-like
4. cecropin-like
5. stomoxyn

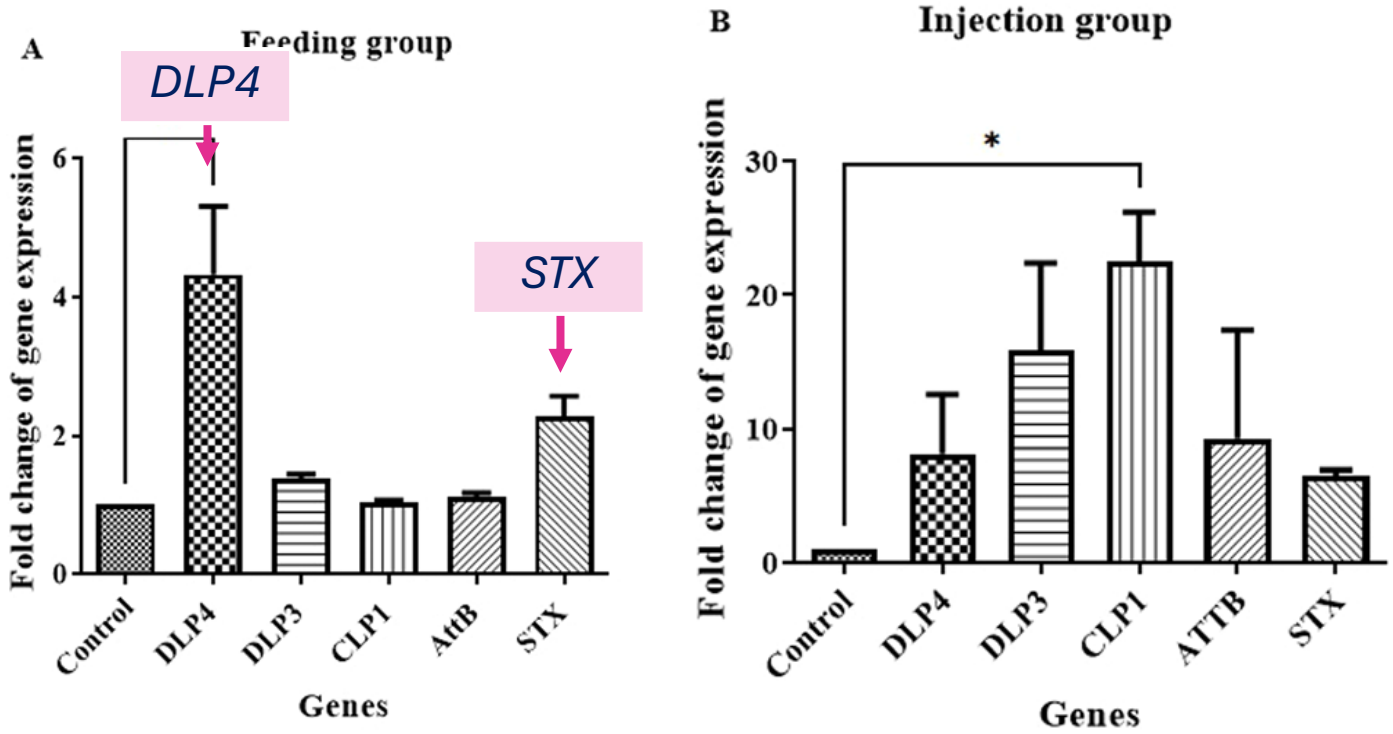
calculated

Gene expression levels
(the 2-ΔΔCT method and the fold-change)

* BSFactin gene: housekeeping gene

Results

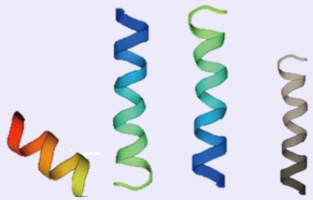
Fig.7: AMP gene expression levels were altered following *C. difficile* exposure



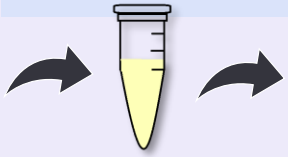
All five AMP genes showed a high increase

Methods

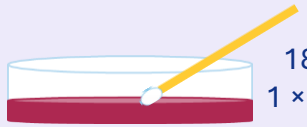
3. Evaluation of the inhibitory effects of five synthesized AMPs



Dissolved AMPs
(2 mg/ml)



1 **Disk diffusion method**



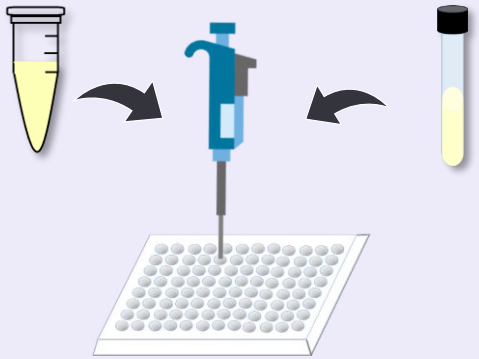
18 *C. difficile*
1×10^5 CFU/mL

Five synthesized AMPs

1. DLP4
2. DLP3
3. attacin B-like
4. cecropin-like
5. stomoxyn

purchased from
GL Biochem, China

2 **The MIC of DLP4**

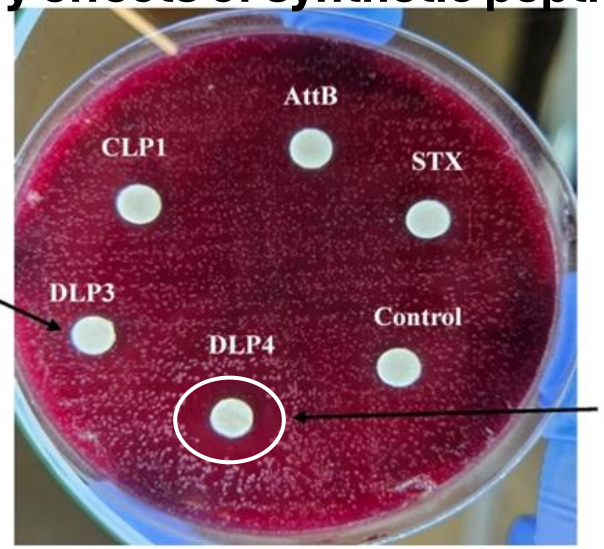


8 – 2000 µg/ml

incubated 37°C, 48 h., anaerobe

Results

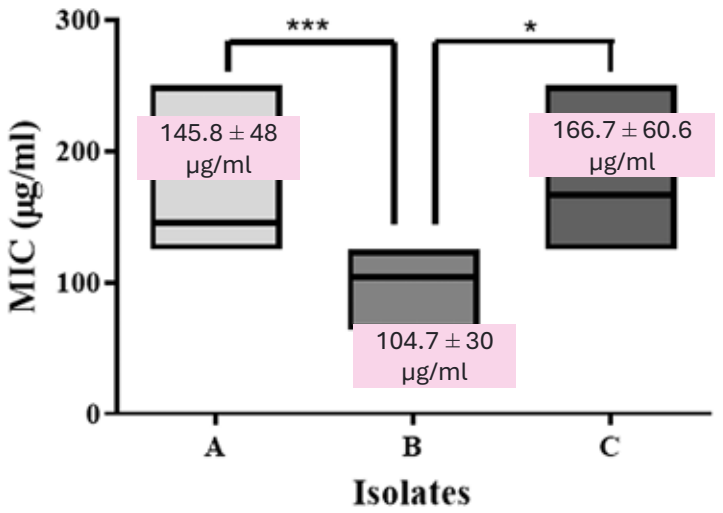
Fig.8: Inhibitory effects of synthetic peptides on *C. difficile* growth



No inhibition- there are colonies near the disk.

Inhibition- there are no colonies near the disk.

Fig.9: DLP4 minimal inhibitory concentration (MIC)



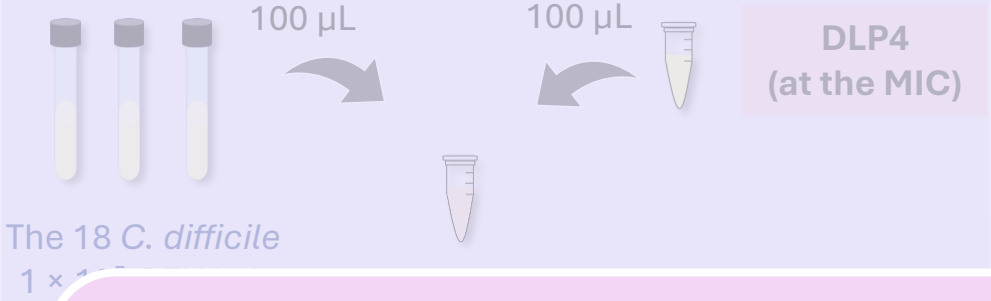
(A) Vancomycin + Metronidazole-resistant

(B) Vancomycin - resistant

(C) Metronidazole - resistant

Methods

4. Difficile colony-forming units counting



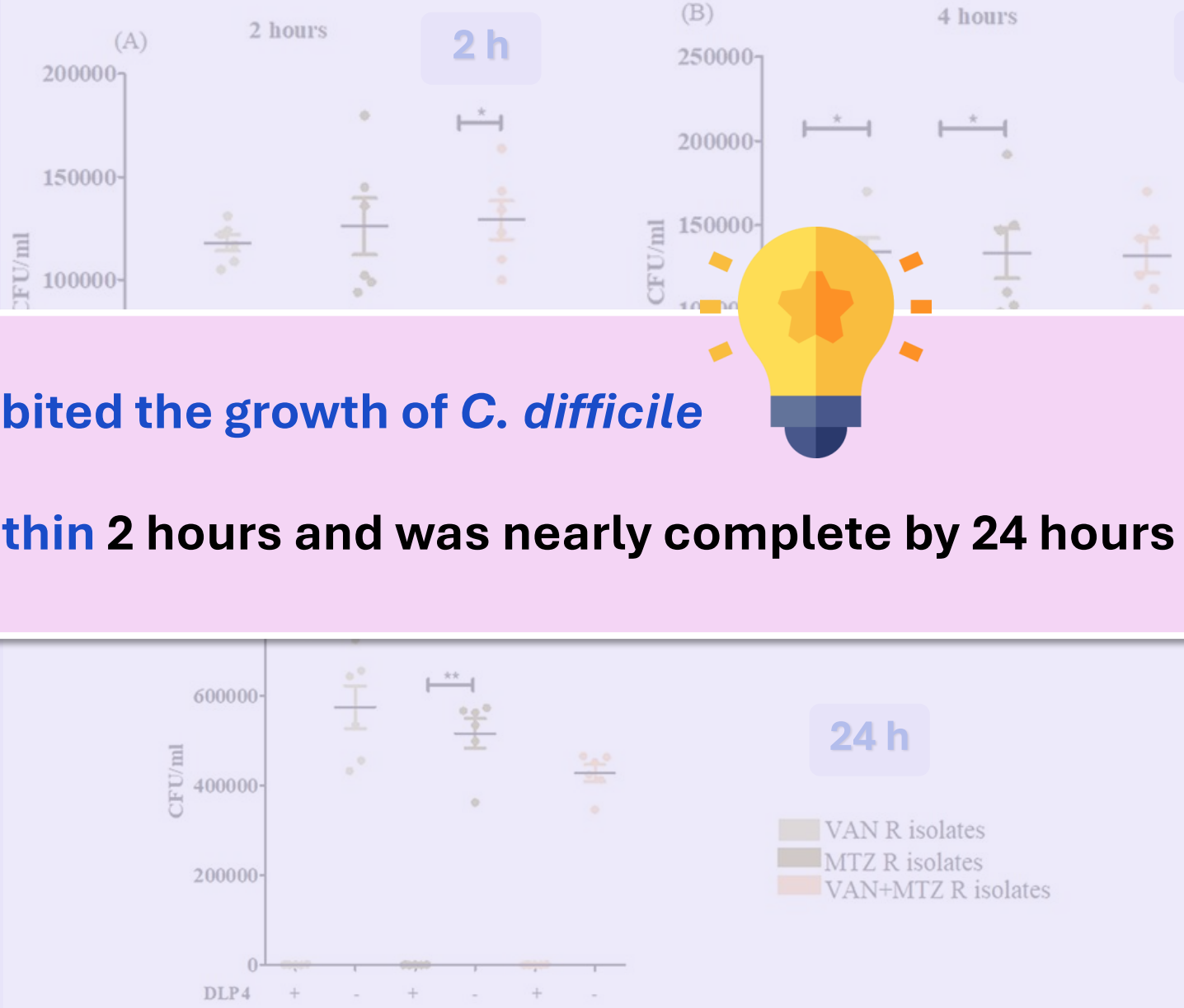
selective Chromagar medium
(ChromID™ C. difficile (CDIF)

48 h.

counted
Colony-forming units (CFU)

Results

Fig.10: The effect of DLP4 on *C. difficile* growth



DLP4 inhibited the growth of *C. difficile*

Inhibition occurred rapidly within 2 hours and was nearly complete by 24 hours

Methods

5. DLP4 cytotoxic effect on caco-2 cells

Human colon adenocarcinoma cell line (Caco-2, HBT37)

DLP4 (16–500 µg/ml)

cell suspension 3×10^4 cell/mL

DMEM medium + 10% FBS and 1% penicillin-streptomycin

incubated, 2, and 24 h.

50 µL XTT solution

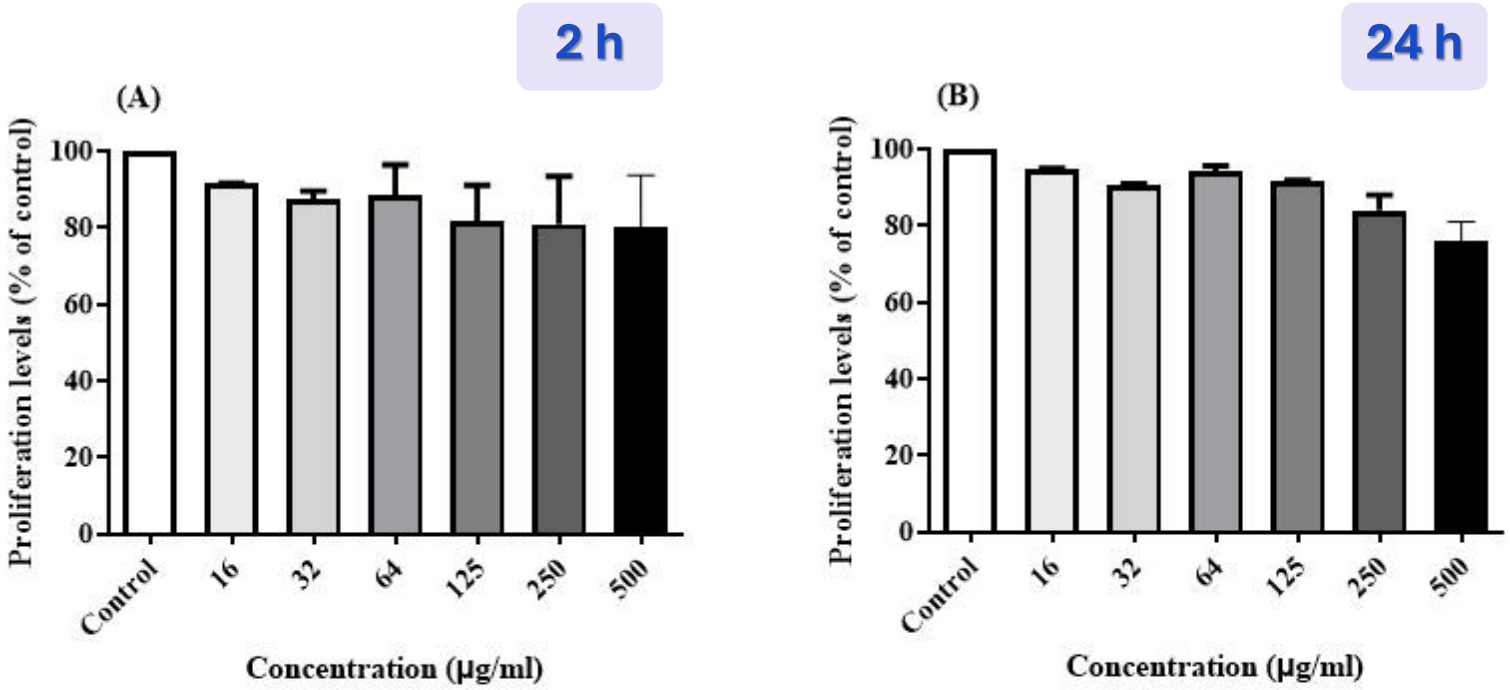
2 h.

The optical density (OD) measured at 630 nm, 450 nm

Results

Fig.11: DLP4 cytotoxic effect on Caco-2 cells

Effect of different DLP4 concentrations on Caco-2 cells



DLP4 shows very low cytotoxicity toward Caco-2 cells, even at the highest concentration (500 µg/ml)

Methods

5. DLP4-Induced bacterial membrane damage

Control: DDW

Test: DLP4 (125µg/ml)

C. difficile 630
0.5 McFarland

incubated 37°C, 1, 2 h.

Transfer
. 24-well plate.

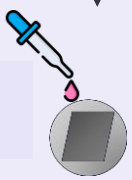
Fixation
Carnovsky's fixative

wash

post-fixed with
1 % osmium tetroxide

Dehydrated
gradient
ethanol series

Critical point drying



Coating
iridium

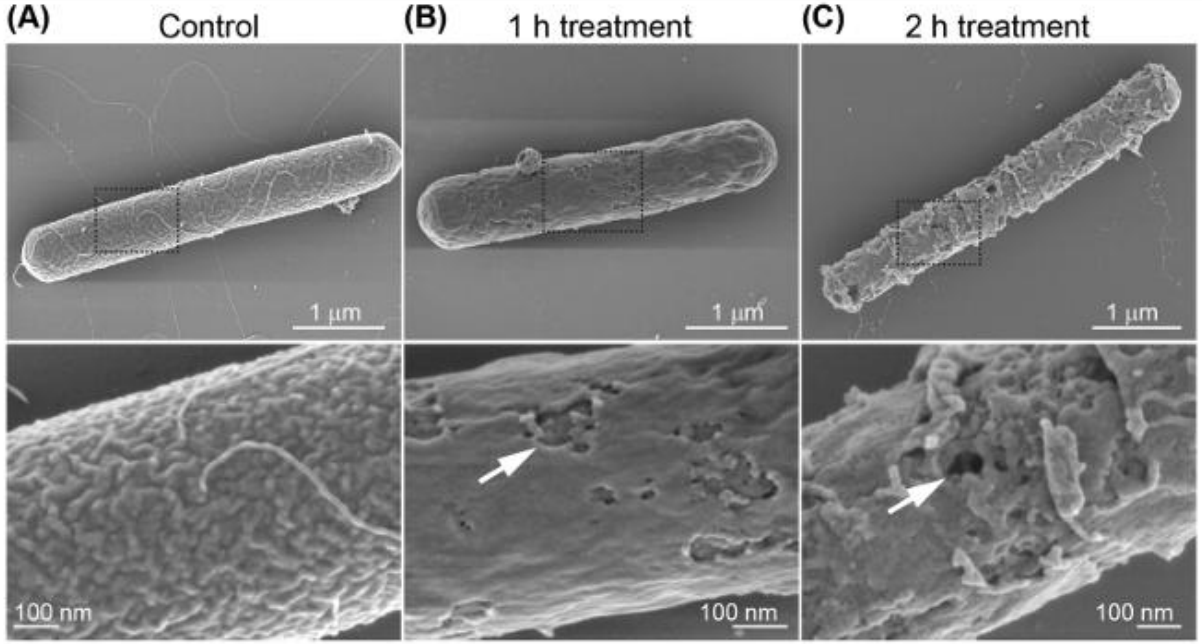
mounted
on aluminum stubs

scanning electron microscope

Results

Fig.12: Morphological alterations in *C. difficile* bacteria following DLP4 exposure

DLP4 damages the cell wall and outer layer of *C. difficile*.
occurs rapidly, with pores appearing within 1 h.



Morphological changes suggest that the **DLP4 mechanism involves cell wall disruption**

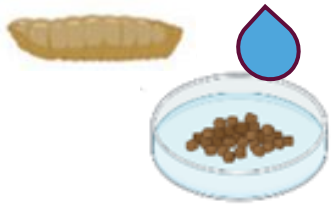
Conclusions

2nd Paper

BSFL exposure to *C. difficile*

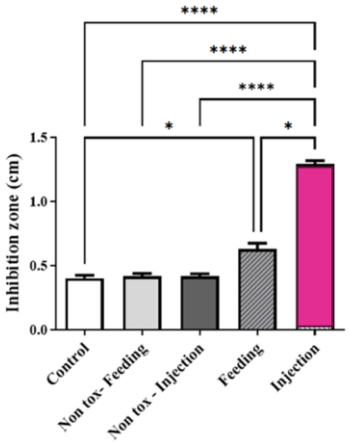
Injection

Feeding



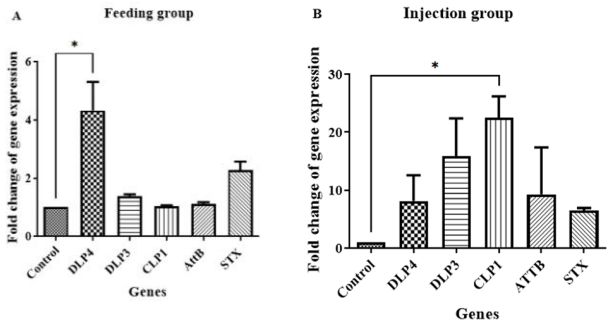
feeding versus injection

Hemolymph inhibition assay



The injection mode **largest inhibition zone**

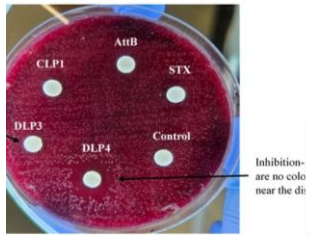
AMP gene expression



Five synthesized AMPs

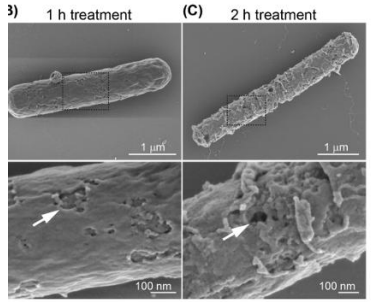
1. DLP4
2. DLP3
3. attacin B-like
4. cecropin-like
5. stomoxyn

DLP4



DLP4 cytotoxic effect

low cytotoxicity



DLP4 involves cell wall disruption
Observation using SEM

DLP4 was the most potent AMP

DLP4 exhibited rapid-onset bactericidal activity within 1 hour and showed no cytotoxicity in human epithelial cells
DLP4 antimicrobial properties against antibiotic-resistant *C. difficile* isolates

Criticisms

1st Paper

Advantages

- Strong data on the screening of Cecropin family AMPs and the analysis of cecropin gene structures derived from BSF larvae
- Strong data indicate that a cecropin AMP (H3) exhibits strong activity against *E. coli*

Disadvantages

All methods assess the antibacterial activity of synthetic AMPs, not of extracts directly derived from BSF

2nd Paper

- Strong data on defensin-like peptide activity against *C. difficile*
- Initial observations provide strong information that AMPs extracted directly from the hemolymph of BSF larvae exhibit activity against *C. difficile*

This study focuses on five synthetic AMPs specifically selected for *C. difficile*

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Introduction part

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Thank you for your kind attention