SYNERGISTIC ANTIMICROBIAL INTERACTION OF HONEY AND BACTERIOPHAGE IN *ESCHERICHIA COLI* BIOFILMS

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Introduction

Wound colonization by biofilms-forming bacteria is one of the main obstacles to the treatment of chronic wounds, causing a number of biological and financial problems. Biofilms are structured communities of bacterial cells enclosed in a self-produced polymeric matrix, adhered to inert or living surfaces, blocking antibiotics and patient's immune cells from reaching bacteria. Bacterio(phages), viruses infecting exclusively bacteria, and honey are being considered as valuable alternatives to treat a variety of infections (Table 1). Phages are harmless to mammalian cells, specific for target bacteria therefore not affecting commensal microflora, have the ability to self-replicate as long as the host is present, and are effective against antibiotic resistant bacteria. Honey is a complex substance with broad spectrum antimicrobial activity, essentially attributed to the high sugar content, low pH, the presence of hydrogen peroxide and methylglyoxal that reacts with important biological molecules (RNA, DNA and proteins). Honey had yet the potential to promote tissue regeneration, cicatrization, decrease inflammation, improving wound healing. In this work the combination of those two antimicrobial agents was considered in the control *E. coli* biofilms.

Table 1 – Advantages of honey and bacteriophages

	Honey	Bacteriophage
Antimicrobial effect	✓	✓
Low cost of production	✓	✓
Efficient against antibiotic resistant bacteria	✓	✓
Ability to degrade the biofilm matrix	✓	✓
Promote regeneration, cicatrization, decrease inflammation	✓	✗
Ability to induce insensitive mutants	✗	✓
Self-replication in the site of infection	✗	✓
Specificity	✗	✓
Dangerous to mammalian cell	✗	✗

Goal of the study

Evaluation of the combinatorial effect of **Portuguese honey** and **phages** in controlling *E. coli* biofilms

Methods

Biofilm Formation

Escherichia Coli
24h and 48h,
37 °C, 120 rpm

Biofilm Treatment

During 6, 12 and 24 h

- Phage EC3a
- Honey PF2
- Phage EC3a +
- Honey PF2

Treatment Efficacy

CFU Counts
SEM
Flow Cytometry

Results

24 h *E. coli* biofilms

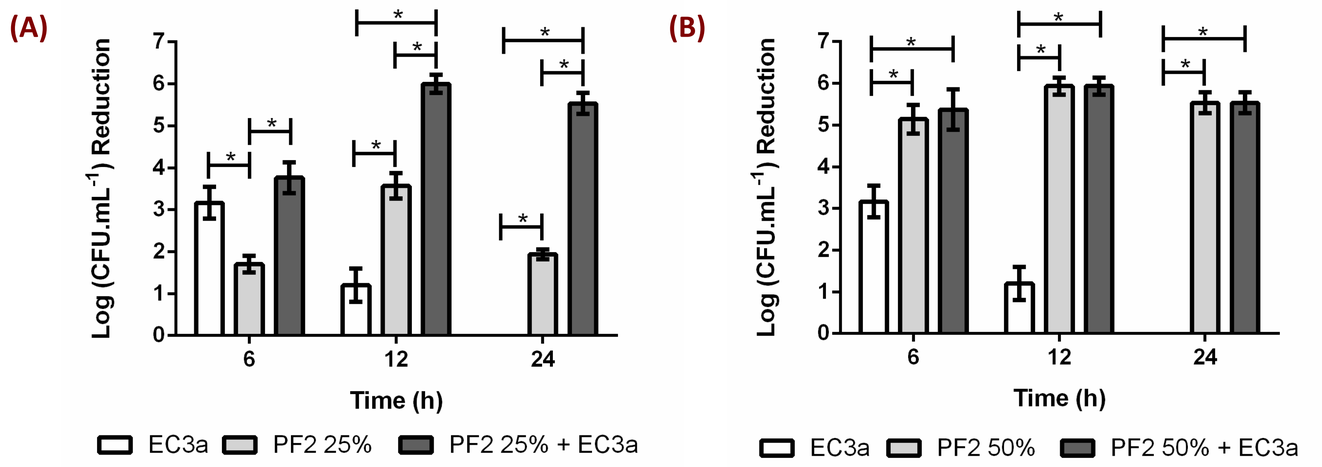

Fig. 1. Antibiofilm effect of EC3a, PF2 and EC3a+PF2 on 24 h-old *E. coli* biofilms. (A) 25% (w/v) PF2; (B) 50% (w/v) PF2. *Sig. differences P< 0.05.

48 h *E. coli* biofilms

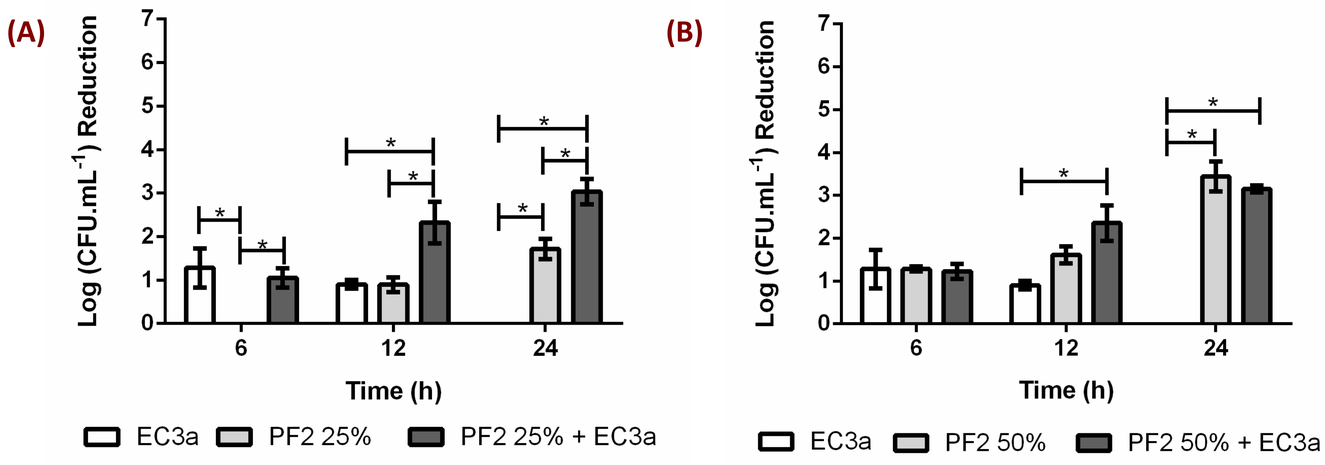

Fig. 2. Antibiofilm effect of EC3a, PF2 and EC3a+PF2 on 48 h-old *E. coli* biofilms. (A) 25% (w/v) PF2; (B) 50% (w/v) PF2. *Sig. differences P< 0.05.

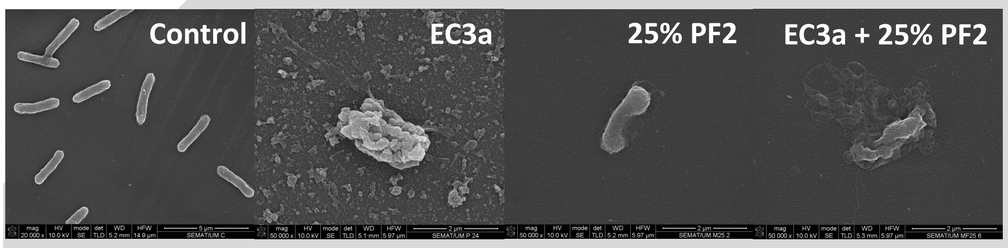

Fig. 3. SEM micrographs showing the effect of EC3a, 25% (w/v) PF2 and EC3a+PF2 on 24 h-old *E. coli* biofilms.

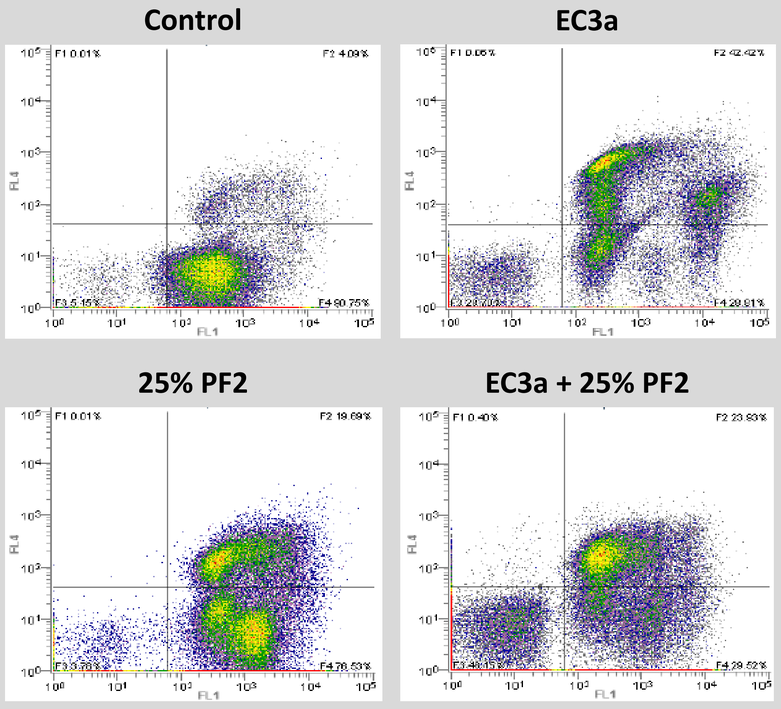

Fig. 4. Cell viability analysis after 12 h application of single and combined treatments to 24 h-old *E. coli* biofilms, using Flow Cytometry. Representative dot plot FL1 (xx axis) vs FL4 channel (yy axis) showing *E. coli* cells stained with SYTO BC (250 nM) and PI (20 µg/mL).

Main conclusions

- The Portuguese honey PF2 50% (w/v) showed great antibiofilm effect with **6-log cell reduction** in 24 h-old biofilms.
- **A synergistic effect** was observed with PF2 25% (w/v) and EC3a in 24 and 48 h-old biofilms.
- 48 h-old biofilms were **more difficult to combat** than 24 h-old biofilms.
- This is a promising strategy for biofilm control that will be further tested with other pathogens and using *ex vivo* models.

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