Synergistic antimicrobial interaction between honey and phage against *Escherichia coli* biofilms

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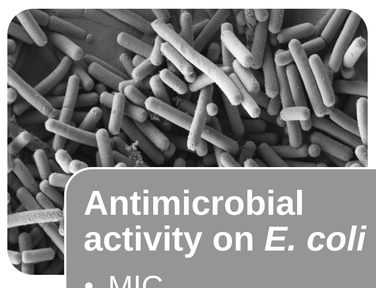
Introduction

Chronic wounds that take months, years or may even never heal present a major biological and financial problem on both individual patients and the broader health system. Chronic wounds afford a hostile environment of damaged tissues that allow bacterial proliferation and further wound colonization. Wound colonization by bacterial biofilms is one of the main obstacles of chronic wounds healing. Biofilms are structured communities of bacterial cells enclosed in a self-produced polymeric matrix and adhered to an inert or living surface. *Escherichia coli* is among the most common colonizers of infected wounds and it is a prolific biofilm former. Living in biofilm communities, cells are protected, become more difficult to control and eradicate, and less susceptible to antibiotic therapy. Due to the vast increase of antibiotic resistant bacteria, there is a renewed interest in pre-antibiotic therapies. Years before the discovery of modern antibiotics, bacteriophages (phages) that are bacterial viruses, and bee hive products such as honey were extensively used for their antimicrobial properties. Phages, are the natural bacterial enemies and have proven efficacy towards antibiotic-resistant bacteria, have self-replicating nature, do not interfere with the commensal flora and many studies acknowledge that phages can destroy, to varying extent, mono and mixed biofilm populations. Honey, on the other hand, has a broad spectrum antibacterial activity against bacteria and its high viscosity provides a protective barrier against infections being suitable for skin care, promoting the wound healing, tissue regeneration and anti-inflammatory process. This work presents insights into the proceedings triggering *E. coli* biofilm control with phage, two Portuguese (PT) honeys and their combination, achieved through standard antimicrobial activity assays, zeta potential and flow cytometry studies and further visual insights sought by SEM and TEM microscopy.

Methods

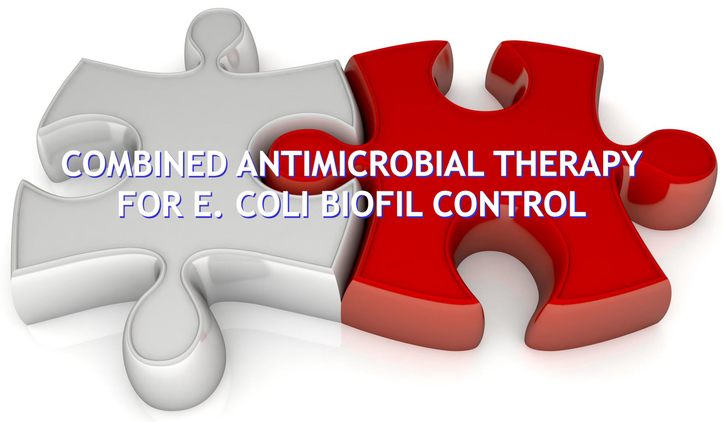
Escherichia phage vB_EcoS_EC3a
- TEM
- Genome

2 PT honeys
- Physicochemical (pH, color, protein, pollen analysis, conductivity)
- MGO & HMF



Antimicrobial activity on *E. coli*
- MIC
- Biofilm control
- SEM & TEM
- Flow cytometry

Study Aim



Results

BACTERIOPHAGE EC3a CHARACTERISTICS

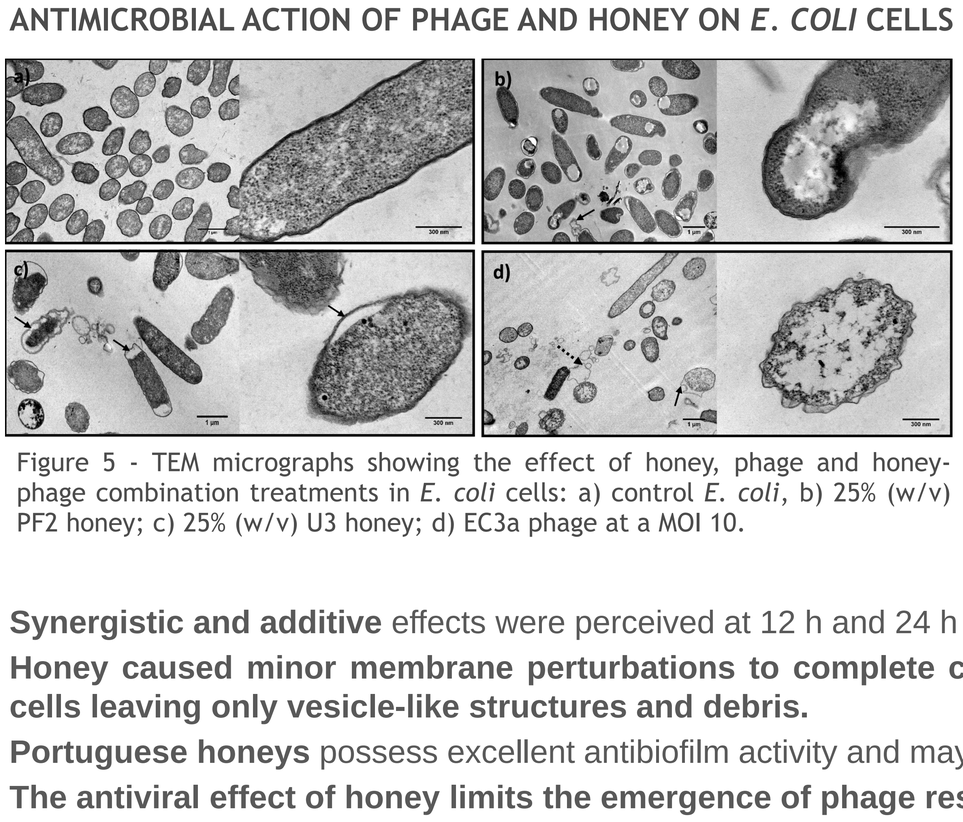


Figure 1 - Characteristics of EC3a a) plaque morphology, b) virion particle, and c) circular view of phage vB_EcoS_CEB-EC3a genome and tblastx comparison with phages M12, RTP and T1, respectively.

ANTIMICROBIAL & ANTIVIRAL EFFICACY ASSESSMENT

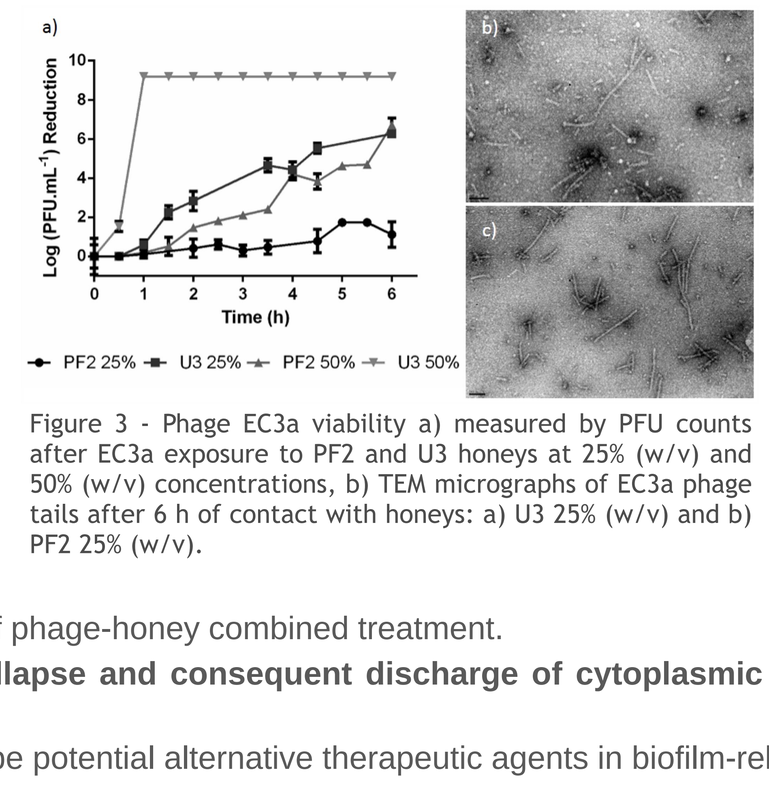


Figure 2 - Antibiofilm effect of phage EC3a, honey and of the phage-honey combination on 24 h-old *E. coli* biofilms. a) PF2 honey at 25% (w/v), b) PF2 honey at 50% (w/v), c) U3 honey at 25% (w/v), d) U3 honey at 50% (w/v).

ANTIMICROBIAL ACTION OF PHAGE AND HONEY ON *E. COLI* CELLS

Figure 5 - TEM micrographs showing the effect of honey, phage and honey-phage combination treatments in *E. coli* cells: a) control *E. coli*, b) 25% (w/v) PF2 honey; c) 25% (w/v) U3 honey; d) EC3a phage at a MOI 10.

Figure 3 - Phage EC3a viability a) measured by PFU counts after EC3a exposure to PF2 and U3 honeys at 25% (w/v) and 50% (w/v) concentrations, b) TEM micrographs of EC3a phage tails after 6 h of contact with honeys: a) U3 25% (w/v) and b) PF2 25% (w/v).

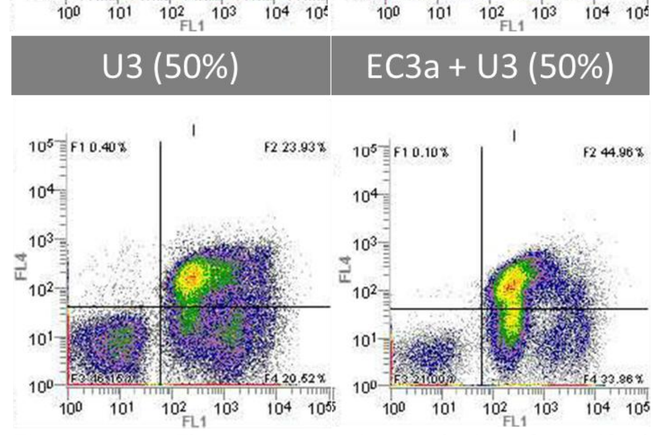


Figure 4 - Flow cytometric analysis after 12 h application of single and combined treatments to 24 h-old *E. coli* biofims. Representative dot plot FL1 (xx axis) vs FL4 channel (yy axis) showing *E. coli* cells stained with SYTO BC (250 nM) and PI (20 µg/mL).

Conclusions

Synergistic and additive effects were perceived at 12 h and 24 h of phage-honey combined treatment.

Honey caused minor membrane perturbations to complete collapse and consequent discharge of cytoplasmic content, and phage completely destroyed cells leaving only vesicle-like structures and debris.

Portuguese honeys possess excellent antibiofilm activity and may be potential alternative therapeutic agents in biofilm-related wound infection.

The antiviral effect of honey limits the emergence of phage resistant phenotypes

The use of diluted honey solutions is advantageous, not only due to a potential lower cost of treatment, but also might be therapeutically more desirable as a topical rinsing solution maximizing the tolerability and practicality of the delivery technique.

The pioneering combined delivery of phage and honey is thus a promising antimicrobial alternative towards *E. coli*.

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