SYNERGISTIC ANTIMICROBIAL INTERACTION OF HONEY AND BACTERIOPHAGE IN ESCHERICHIA COLI BIOFILMS

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 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. Bacteriophages (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, cicatrisation, decrease inflammation, improving wound healing. In this work the combination of those two antimicrobial agents was considered in the control E. coli biofilms.

Methods

Biofilm Formation

Escherichia Coli

24 h and 48 h, 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 observation
• TEM observation
• Flow Cytometry

Results

Main conclusions

• The Portuguese honey PF2 50% (w/v) showed great antibiotic 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.

Table 1 – Advantages of honey and bacteriophages

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<tr>
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<th>Honey</th>
<th>Bacteriophage</th>
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<tbody>
<tr>
<td>Antimicrobial effect</td>
<td>✓</td>
<td>✓</td>
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<td>Low cost of production</td>
<td>✓</td>
<td>✓</td>
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<td>Efficient against antibiotic resistant bacteria</td>
<td>✓</td>
<td>✓</td>
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<td>Ability to degrade the biofilm matrix</td>
<td>✓</td>
<td>✓</td>
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<td>Promote regenerance, cicatrisation, decrease inflammation</td>
<td>✓</td>
<td>✓</td>
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<td>Ability to induce non-pathogenic mutants</td>
<td>✓</td>
<td>✓</td>
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<td>Self-replication in the site of infection</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Specificity</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Dangerous to mammalian cell</td>
<td>✓</td>
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Goal of the study

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

Phage viability

Fig. 1. EC3a viability after 8 h of contact with honey, measured by CFU counts after EC3a exposure to PF2 at 25% (w/v) and 50% (w/v) concentrations.

Fig. 2. Viability of phages in honey: them makes honey (B. Portuguesa). Detection antimicrobial solutions (1). The viability of phages in honey is measured by CFU counts. The viability of phages in honey is measured by CFU counts.

Fig. 3. SEM micrographs showing the effect of EC3a, 25% (w/v) PF2 and EC3a+PF2 after 12 h treatment on 24 h-old E. coli biofilms.

Fig. 4. TEM micrographs showing the effect of EC3a, 25% (w/v) PF2 and EC3a+PF2 after 12 h treatment on 24 h-old E. coli cells.

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