

Potential of bacteriophage K as an antimicrobial strategy against biofilms of *Staphylococcus aureus* in the food industry

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The potential of phage K in the removal and prevention of *Staphylococcus aureus* biofilms on stainless steel surfaces was examined. The host range of the phage was tested on 18 strains from the food industry. Two out of six sensitive strains were selected for study of biofilms.

Immature 6-h-old biofilms were challenged with a wide range of multiplicities of infection (MOI, 0.01-500) for 18 h at 25°C. A noticeable effect on cell number was appreciated at MOIs ≥ 10 , but no effect was detected at lower MOIs. Subsequently, 24 h-old biofilms (with a higher cell density and a more dense extracellular matrix) were challenged too at MOIs between 0.01-3.0 for 24 h at 25°C. Again, a significant effect was only found at MOIs > 1 .

The effectiveness in the prevention of biofilm formation was examined by infecting planktonic cultures of *S. aureus* with sub-lethal and lethal doses of phage K (MOIs 1·10⁻⁷-10.0). A notable effect on cell number and biofilm biomass was observed from MOIs $> 1 \cdot 10^{-5}$.

The potential of phage K as a tool for biohygienization in the food industry acting specifically against biofilms of *S. aureus* was thus shown. However, neither biofilm cells were completely removed nor biofilm formation was totally prevented, and a sub-population of live cells was left in both cases. As a result, the effectiveness of two co-adjuvant-based strategies was subsequently assessed.

Combining DNase (0,1-10 mg/mL) with phage K (MOI of 0.3-3.0) did not show an additional effect on the removal of 24 h-old-biofilms at 25°C. A 30 min pre-treatment at 37°C aimed to enhance DNase activity also had not effect. DNase did not show any effects by itself either. In contrast, combining cis-2-decenoic acid (1 - 100 nM) with phage K (MOI of 0.03-0.3) seemed to show some effects on biofilms.