

Staphylococcus epidermidis biofilms are resistant to phage infection due to the protective effect of the biofilm matrix

Luís Melo^{a,*}, Fernando Oliveira^a, Sanna Sillankorva^a, Begoña Pérez-Cabezas^b, Manuel Vilanova^b, Joana Azeredo^b, Nuno Cerca^b

^a CEB-Centre of Biological Engineering, University of Minho, Braga, Portugal

^b ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal

Staphylococcus epidermidis are involved in a set of different nosocomial infections, namely in indwelling medical devices, where they form infective biofilms. These complex tridimensional structures are very tolerant to antibiotics. Moreover, staphylococcal bacteriophages were shown to be inefficient against biofilms [1]. In this study the previously isolated *S. epidermidis*-specific Twortlikevirus phiIBB-SE1 (SEP1) [2] also shown very low activity against *S. epidermidis* biofilms compared to planktonic cells. Consequently, the aim of this study was to disclose the factors that impair SEP1 efficacy against biofilms. For this purpose, phage infection assays were carried out in bacterial populations with different growth states (simulating the heterogeneous population physiology within a biofilm) and bacterial cells scraped from the biofilm. The results pointed out that SEP1 was able to infect and eradicate stationary-phase cells within 8 of infection. Interestingly, the results showed that modulated biofilms with distinct metabolic activities did not alter phage efficacy. In opposition, SEP1 caused a 2-log reduction on scraped biofilm cells.

We therefore propose that the physiology of biofilm might not be hindering phage infection but some matrix component might be interfering with the outcome of biofilm control. To assess this hypothesis, biofilm matrix was first added to exponential cells together with phage, and after 24 h it was observed a reduction in phage efficiency. PNAG is the main component of most *S. epidermidis* biofilms and its secretion is induced by glucose. The possibility of an interaction between SEP1 and PNAG was assessed by varying the concentration of glucose in the culture media. Results showed that SEP1 infected more efficiently cells grown in the absence of glucose. PNAG is described as an evasion mechanism of *S. epidermidis* cells to antibiotics and to the immune system [3], consequently, our results suggest that PNAG can work as a decoy, allowing the bacterium to evade from phage infection.

Keywords: bacteriophage, Twortlikevirus, *S. epidermidis*, staphylococci, biofilms

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