Genomic characterization of the *Staphylococcus epidermidis*-specific bacteriophage SEP1 and evaluation of its lytic activity against bacterial under different metabolic states

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Staphylococcus epidermidis is one of the most frequent causative agents of nosocomial infections, predominantly in patients with indwelling medical devices. This microrganism may form biofilms which are microbial structures very tolerant to the host immune defenses and to antibiotherapy. Therefore, studies are needed in order to develop effective methods for biofilm control. Currently, bacteriophages (phages) are seen as an important tool to combat pathogenic organisms. These bacteria-specific viruses are generally very efficient antibacterial agents and possess many advantages over antibiotics. The present study concerns the search for virulent phages with broad host range for *S. epidermidis* biofilm therapy.

Using wastewater treatment plant raw effluents, a novel phage was isolated and characterized. This virus was named phiIBB-SEP1 and TEM micrographs suggested that it belonged to the *Twortlikevirus* genus. Phage phiIBB-SEP1 is able to infect 41 *S. epidermidis* clinical isolates used in this study, and contrarily to other polyvalent viruses of the *Twortlikevirus* genus, phiIBB-SEP1 is highly specific for *S. epidermidis* strains. The genome of this phage was fully sequenced and presents the typical structure of a member of the *Twortlikevirus*. However, when compared to other staphylococcal members of this genus, it showed DNA sequence identities no greater than 58.2%, suggesting that phiIBB-SEP1 is a new species within this subfamily.

Efficacy studies results showed that phage phiIBB-SEP1 is able to cause a 6 Log CFU per ml reduction of the cell titre in less than 2h for some of the clinical strains in exponential phase; and, in less than 4h for stationary phase cells (using a multiplicity of infection of 1). This phage has also the capacity of reducing, by up to 2 Log CFU per ml, 24h scraped biofilm cells, and in some strains it was observed 50% cell reduction. Besides CFU counting, this cell reduction was confirmed by flow cytometry counting. Additionally, live/death flow cytometry staining allowed the observation that this phage kills biofilms bacteria in different metabolic states.

These are promising results, since phage phiIBB-SEP1 presents a broad host strain range and the ability to control *S. epidermidis* bacteria in different metabolic states.

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