Characterization of a new twortlikevirus infecting *Staphylococcus epidermidis* that exhibits activity against biofilm and stationary bacterial populations

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Staphylococcus epidermidis is a major causative agent of nosocomial infections, mainly associated with the use of indwelling devices, on which this bacterium forms structures known as biofilms. Due to biofilms high tolerance to antibiotics, virulent bacteriophages were previously tested as novel therapeutic agents. However, several staphylococcal bacteriophages were shown to be inefficient against biofilms.

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 SEP1 is able to cause a 6 Log CFU per ml reduction of the cell titer 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. Besides CFU counting, this cell reduction was confirmed by flow cytometry counting. Additionally, live/dead flow cytometry staining allowed the observation that this phage kills biofilms bacteria in different physiological states including dormant cells. These are promising results, since the rare feature presented by this phage of infecting cells with reduced metabolic activity allied with its high broad host strain range suggest its use for therapy purposes.