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Isolation and optimization of the production of *Staphylococcus epidermidis* bacteriophage from environmental samples

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Staphylococcus epidermidis is now among the most important nosocomial pathogenic agents owing its virulence to the adhesion and biofilm-forming abilities on medical surfaces, such as catheters. Biofilm control by antibiotics is often innefective and new strategies of biofilm control are being sought. One promising strategy is the use of bacteria-specific virus, known as bacteriophages, to control infections by pathogenic bacteria. Bacteriophages, also know as phages, have been suggested to be one of the most abundant biological agents on the planet. Phages are currently suggested as possible alternatives to antibiotics for the treatment of bacterial diseases in humans to minimize the pathogen loads in medical devices. The goal of this study was to isolate new phages with potential bactericidal activity against *S. epidermidis* clinical isolates. Bacteriophages were isolated from an effluent from Waste Water Treatment Plants or from Hospital efluents, using a set of 40 bacterial strains as background. Five phages were isolated but when determining the phage titer the achieved concentration was around 10^5 pfu/ml and this titer was reduced 1 fold in two week's time. In order to increase the concentration of bacteriophages, since the obtained concentration was not sufficient to use in biofilm assays, severall optimization steps were performed, using previous described isolation protocols, namelly: using different concentrations of $CaCl_2$, using different concentrations of top agar, using different buffers, and using different phage filtration systems. For the optimization protocols we selected the bacteriophage with the higher titters and found that an optimized protocol was achieved by using Tris Buffer, Top Agar at 0.4%, and purification with $CsCl_2$ gradient (q = 1.3, 1.5, and 1.7) with ultracentrifugation at 100,000g for 1 h at 4^0C. This phage titter was determined to be around 10^8-10^9 pfu/ml. The optimized produced phage was then characterized by determining the lytic spectrum. The phage was able to lyse 13 strains, and of these strains 10 had the biofilms genes present. Finally, the 10 selected strains were tested for biofilm formation, using the microtiter assay, and it was confirmed that they formed biofilms in TSB supplemented with 1% glucose. For future work; we need to determine if we have lytic or temperate phages with DNA sequence analyses and to test the phage against to the Biofilm formation of relevant bacterial strains.

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