Antibiofilm activity of a natural bacteriophage against multi-drug resistant *Pseudomonas aeruginosa*

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Introduction

A biofilm is communities of surface-linked microbial cells surrounded by extracellular exopolysaccharides. Microbial species are considered to be mostly found in the form of biofilms in environment, and few percent of microbial biomass is really found in the planktonic form (Kostakioti, Hadjifrangiskou, and Hultgren 2013). *Pseudomonas aeruginosa* is an opportunistic pathogen which is considered as one of the important causes of hospital-acquired infections such as pneumonia, wound infections, urogenital and intra-abdominal sepsis (Page and Heim 2009). Phages are hopeful alternatives for antibiotics and they can eliminate the biofilms and pathogens effectively (Ghasemian, Bavand, and Moradpour 2017). The objectives of this work are assessing the characterization and anti-biofilm activity of a naturally isolated phage on *P. aeruginosa*.

Methods

Enviormental water resources and sewage samples were screened to find affective bacteriophages against *P.aeruginosa*. The collected samples were subjected to enrichment in TSB medium using methods of Kropinski (Clokie and Kropinski 2009).

For determination of the phage morphology, a drop of isolated phage suspension was mounted on the Formvar-coated copper grid, the prepared thin film subjected to negative staining as per the standard procedures. Grids were observed under a transmission electron microscope (TEM, Philips Bio Twin, CM100, Netherlands), at an accelerating voltage of 75KV.

Phage genome was extracted by the standard method of the phenol-chloroform extraction. The purified nucleic acid was treated with DNase I and RNase A. For restriction analysis; the enzymes were added to the extracted genome and treated nucleic acids evaluated using 1% agarose gel. The proteins of the phage capsid were examined using SDS-PAGE according to the Laemmli method (Laemmli 1970). Purified phages were precipitated by ice-cold acetone. The samples were then electrophoresed on 10% SDS-PAGE slab gel.

Determination of the bacterial host range of the isolated phage

The double-layer agar test was used to determine the ability of the phage to infect other bacterial strains. Bacterial cells were added to 3 mL of liquefied agar and poured on solid agar to prepare double-layer agar plates. A volume of 10 µL of the phage was applied on each bacterial lawn. After overnight incubation at 37 ºC, the formation of spots (zone of inhibition) was recorded as a positive result indicating the susceptibility of examined bacteria to the lytic phage. Control (negative) samples were done with buffer instead of the phage.

Results

Isolation & molecular characterisation

The isolated phage has a species-specific host range for *P. aeruginosa*. Morphological analysis by transmission electron microscopy revealed the isolated phage is an icosahedral particle with ~50 nm diameter (figure 1).

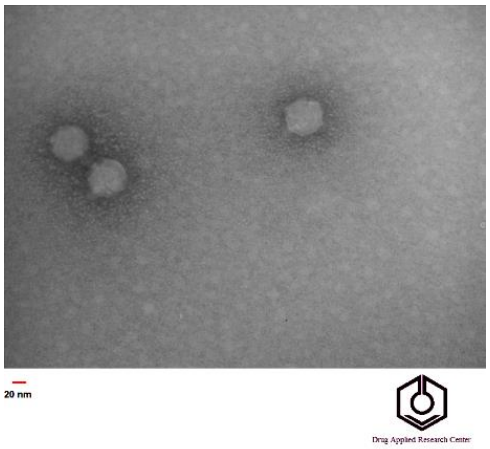

Figure 1. Electron micrograph of phage particles under a transmission electron microscope. Scale bar represents 20 nm.

Based on transmission electron microscopy (TEM), the isolated phage is a icosahedral particle with ~50 nm diameter (figure 1). Genome purification of the phage and subsequent treatment with RNase, DNase, and restriction analyses revealed that it contains a dsDNA genome. SDS-PAGE (10%) analysis of the phage proteins resulted in a band pattern shown in figure 2. The major band of the phage proteins is ~35 kDa. Other dominant protein bands on SDS-PAGE are seen at approximately 60 and 90 kDa, which may be attributed receptor binding proteins of the phage.

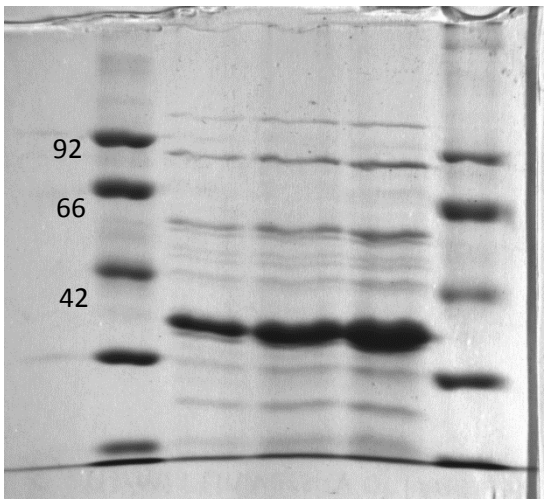

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Figure 2. Proteome of the phage (a). Lane first and last, size marker, lane 2, 3 and 4 acetone precipitated phage protein with different concentration.

Antibacterial activity of the isolated phage

The phage exhibited 86.82 and 86.86% biofilm inhibition at PFU of 10 and 100, respectively. Furthermore, the phage is effective on some antibiotic resistant clinically-isolated *P. aeruginosa*.

Discussion

In a screening set-up, spot testing and plaque assay were used to identify suitable bacterial virus for potential application in phage therapy of *P. aeruginosa*. The results of this study shows that the natural phages are effective agents against biofilms. Due to increasing resistant pathogens to antibiotics, alternative agents such as phages are promising tools for treating bacterial infections.

References

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