Separation of Bacteriophage Tails to Develop Novel Recognition Elements

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Abstract

As the world's population grows, the use of antibiotics became an indispensable medical treatment and prevention for bacterial infections. However, the constant, broad and sometimes inconsiderate use of antibiotics resulted in antibiotic resistance bacteria which represent a global health concern. The lack of early diagnosis and pathogen identification intensifies the cause of this resistance. Biosensors allow a fast, reliable and ease-to-use medical diagnosis with the support of recognition elements. In this context, bacteriophages represent promising recognition elements for identification of antibiotic resistant bacteria due to their exceptional characteristics. The current phage-based biosensors are not effective since bacteriophages infect and induce lysis of the host bacteria in their natural state. The aim of this Master´s Thesis was to enhance and develop advance bacteriophage recognition elements for a fast and reliable pathogen identification by the separation of bacteriophage´s heads from their tails, thus eliminating the infection capacity, but retaining their recognition ability. The overall process consisted in *E. coli* specific T4 bacteriophage production by infection of bacterial cultures, preparation and amplification of a T4 phage stock and testing mechanical (water bath and probe sonication) and non-mechanical (osmotic shock) disruption methods for the separation of phage tails. The 25 W probe ultrasonication demonstrated to be the most promising method to achieve the proposed purpose.

Keywords: Bacteriophage, Antibiotic Resistant Bacteria, Biosensors, Advanced Recognition Elements, Bacteriophage Separation.

1. Introduction

The emergence of pharmaceutical antibiotics in the mid-20th century, along with the better understanding of disease and sanitation, revolutionized healthcare and drastically improved both quality of life and life expectancy. Antibiotics uncovered a new era in medicine by rapidly becoming an indispensable medical tool [1]. However, the broad and often inconsiderate use of antibiotics in human and veterinary medicine, and also in agriculture, resulted in the widespread antibiotic resistance in a variety of microbiota in several ecological compartments. Because of this, antibiotic-resistant bacteria are a major and increasing global health concern. This increase of resistance is mostly due the lack of early diagnosis, which play an important role in successful identification of the infectious pathogen and its treatment.

Nowadays, the use of biosensor technology in the field of medical diagnostics has allowed a cost-effective, fast, reliable, and easy-to-use sensing platform. Biosensors are analytical devices that utilize recognition

elements such as enzymes, nucleic acids and antibodies for the detection of a specific target [2]. Because of obstacles like false positives, isolation and purification steps that may be time-consuming and expensive, recent evolutions in biotechnology have created the possibility to develop novel affinity-based recognition elements that overcome the limitations encounter. Bacteriophages are novel innovative recognition elements due to their exceptional characteristics, such as their high affinity and specificity for their targets, fast and cheap production, their stability and ease to be modified [2,3,4,5,6,7].

As naturally-occurring bacterial parasites, bacteriophages are viruses with the capability to infect and kill specific bacteria. The reproduction of viral bacteriophage particles occurs only within the host cell where their machinery is available to replicate phage´s own genome, making these viruses ultimately dependent on the bacterial host for survival [1]. Unlike the wide range of bacteria targeted by antibiotics, bacteriophages are highly specific for the target bacterial host and do not affect other beneficial microbes [8,9,10]. A renewed interest in bacteriophage potential emerged in order to control especially multidrug-resistant bacteria [3]. A high number of different bacteriophages, each displaying a different protein on their surface, can be used as a target specific recognition element of a biosensor, distinctively the ones with high affinity and specificity for a specie of resistant bacteria [11].

Current phage-based biosensors for research and medical diagnosis are not completely efficient for target identification once phages, in their native structure, are capable to induce the host bacterial cell lysis upon attachment and infection. This limits bacteria identification and tracking in a biosensor device.

This Master´s Thesis has the objective of generating advanced recognition elements for the identification of antibiotic resistant bacteria by fragmentation of bacteriophage´s heads from their tails, thus eliminating the infection capacity. By isolating the tails which are the main apparatus of recognition and attachment to the target bacteria, phages would maintain their ability to identify the target without lysing it. The process should be effective and suitable to be applied in a biosensor platform in order to improve the field of medical diagnosis.

To this end, a *E. coli* host strain and a specific T4 bacteriophage were used. The first stage of this experimental study consisted in the rehydration and preparation of the biological material for all experiments, specially a working cell bank and the determination of the *E. coli* host strain growth data. A phage stock was prepared as well by inoculating a *E. coli* culture with a phage stock solution. For this, the volume of medium used, a MOI of 0.1 and the exact time of infection were applied to obtain the highest yield of bacteriophage production.

The second stage of the experimental plan comprised in the test of mechanical (sonication by water bath and probe sonication) and non-mechanical (osmotic shock) methods to promote the disaggregation of phage tails from their heads. In each disruption process the phages were analyzed by their titre through a double-layer plaque assay and by their protein content through SDS-PAGE and Bradford assay. In the most promising method, phages were visualized by TEM (Transmission Electron Microscopy).

2. Materials and Methods

The experimental process developed in this Master´s Thesis for the separation of an *E. coli* specific bacteriophage tails towards the production of advanced recognition elements can be described by the flowchart presented in **Figure 1**.

2.1. Cell Culture

E. coli dried bacterial content was supplied in an ampoule by the German Institute DSMZ – German Collection of Microorganisms and Cell Cultures with the designation *E. coli* DSM 613 strain B,B (Luria).

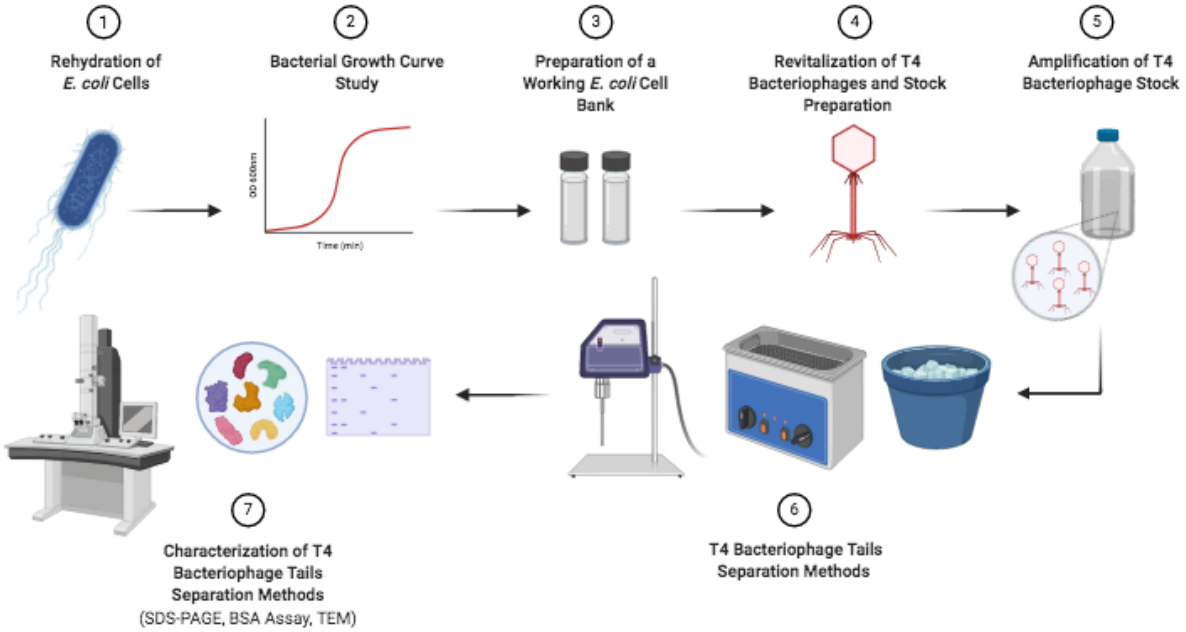

Figure 1 – Graphic representation of the general experimental process developed in this Master´s Thesis for the generation of advanced recognition elements by the separation of bacteriophages´tails. Image created in BioRender.com – accessed 5th November 2020.

The ampoule glass where the dried *E. coli* bacterial cells were kept, was carefully broken and the inner vial was taken out. 0.5 mL of tryptic soy broth (TSB) medium was added to the dried pellet inside of the vial and let to rehydrate up to 30 minutes. The content was gently mixed with an inoculation loop to homogenize the suspension. About 100μL of suspension was plated in an agar plate and incubated overnight at 37°C. The rest of suspension fraction was transferred into a sterilized eppendorf®, mixed with 50% glycerol and stored at -80°C. To determine the *E. coli* growth data, a fresh culture of bacterial cells was grown in TSB medium at 250 rpm and 37°C overnight. A new inoculum was prepared in shake flasks with 500 mL TSB medium and agitated at 250 rpm at 37°C. The culture growth was followed for a period of 4h and every period of 15-30 minutes two samples were taken. The first sample was used to measure the OD at 600nm for determine the different growth phases of the bacterial cells, and the second sample was used to determine the number of viable cells by plating 100 μL of the appropriate dilutions of each sample. After incubation of the plates overnight at 37°C, the colonies of *E. coli* were counted.

2.2. Bacteriophage Stock

Specific *E. coli* bacteriophages dried content was supplied in an ampoule by the German Institute DSMZ – German Collection of Microorganisms and Cell Cultures with the designation Phage T4 DSM 4505. For the revitalization of the dried T4 bacteriophages, an agar plate was prepared and the *E. coli* host was plated using the top agar layer method containing 4 mL of top agar, 100 μL of fresh grown host culture and 40 μL $MgCl_2$ 5 mM. The ampoule containing the phages was carefully broken and the inner vial was taken out. Inside the vial there was a filter paper containing the dried phage suspension, which was placed in the middle of the host plate. 0.1 mL of TSB medium was added on the surface of the filter paper and the plate was incubated at 37 °C overnight. The next day it was possible to visualize a clear zone around the filter paper which was where the lysis occurred. For preparing a phage suspension, 2-5 mL of 1x SM buffer (10x SM buffer is composed of 1 M NaCl; 0.5 M Tris-HCl

pH 7.4; 100 mM $MgSO_4 \bullet 7H_2O$; 0.3 g of gelatin and milliQ H_2O) was added to the plate and left at room temperature while slowly rotating on a shaker for at least 4h. Afterwards the phage suspension was harvested and centrifuged (Eppendorf® 5810R Centrifuge) at 8000 g at 4°C for 10 minutes. The supernatant was collected and filtered in a 0.45 μm Nalgene™ Rapid-Flow™ sterile disposable Bottle Top Filter PES membrane to remove the remaining bacteria. The revitalized phage suspension was stored at 4°C.

In order to have sufficient quantities of bacteriophage stock for the experiments it was necessary to perform both solid and liquid medium amplifications.

2.3. Bacteriophage Separation Methods

2.3.1. Osmotic Shock

For the process of osmotic shock, sodium acetate 5 M was used for the dissociation of phage tails. The experiment was always carried on ice where 500 μL of phage stock were mixed to 500 μL of sodium acetate for different periods of exposure (2, 5, 10, 15, 20, 30 minutes). After each time of subjection 1:100 volumes of milliQ H_2O were added. A sample of 1 mL was collected for each time and stored at 4°C. Afterwards the suspensions were centrifuged (Eppendorf® 5810R Centrifuge) at 3500 g for 30 minutes at 4°C. Both pellets and supernatants were collected and stored at 4°C for further testing. Pellets were ressuspended in 5 mL of 1x SM buffer. The process of osmotic shock was adapted from the literature as described by Herriott *et al.* and Liu *et al.* [12,13]. These authors were trying to obtain ghost phages by osmotic shock and actually observed that the technique caused the separation of heads from tails.

2.3.2. Water Bath Ultrasonication

A VWR Ultrasonicator bath operating at 45 kHz and 80 W was used to perform the disruption test on phages during a period of 40 minutes. Samples of 500 μL were collected at 1, 5, 10, 15, 20, 25, 30 and 40 minutes of exposure to the sonicator and stored at 4°C for further experiments. The water bath was always carried at 10°C.

2.3.3. Probe Ultrasonic Homogenization

A Bandelin Sonopuls Ultrasonic Homogenizer HD 3200 operating at 20 kHz was used to carry out the disruption test on phages using a MS 72 probe. Sonication was performed at 25 and 50 W for 10 cycles of 4 minutes of exposure and 1 minute of pause. Samples of 1 mL were collected at 5, 10, 15, 20, 30 and 40 minutes of sonication and stored at 4°C for further experiments. Both processes were adapted from the literature described by Anderson *et al.* and Pinto *et al* [14,15]

2.4. Bacteriophage Characterization

2.4.1. SDS-PAGE and Protein Quantification

The protein content of phages was determined by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The SDS-PAGE analysis was done on polyacrylamide gels with 12% T (total monomer concentration), 3.3% C (weight percentage of crosslinker) for the resolving layer and 4% T, 3.3% C for the stacking layer. 20μL of phage samples were added to 25 μL of 2x Laemmli Sample buffer (Bio-Rad) and denaturated by addition of 5 μL of dithiothreitol (DTT, 1 M, Sigma). The samples were then heated in a water bath at 100°C for 10 minutes. The phage samples and the Bio-Rad Precision Plus Protein™ Standards Dual Color Marker were then loaded into the gel. Electrophoresis was conducted at 90 V, with the gels submerged in 1x running buffer (10x running buffer composed of 250 mM Tris-HCl, 1% SDS, 1.92 M glycine, pH 8.3). Staining was performed with Coomassie Brilliant Blue and afterwards with Silver Nitrate. Silver Staining protocol included a fixation step with 30% ethanol + 10% acetic acid for at least 2h, a wash step with 30% ethanol for 10 minutes, a second wash step with milliQ H_2O for 2x10 minutes, a sensibilization step with 0.02% of sodium thiosulfate for 10 minutes, a third wash step with milliQ H_2O 3x30 seconds, a staining step with 0.15% silver nitrate for 30 minutes, a development

step with 3% sodium carbonate + 0.05% formaldehyde, and a stop step with 5% acetic acid for 15 minutes. Images of the gels were obtained with a GS-800 Calibrated Densitometer (Bio-Rad). Osmotic shock phage samples were purified and concentrated with Amicon® Ultra-4 100 kDa Centrifugal Filter Units (Merck), before sample preparation and lead into the gel, in order to remove the sodium acetate, which could interfere with the running of the gel.

2.4.2. Bradford Assay

The protein quantification present in the phage samples submitted to the disruption methods was determined by the Bradford protein assay (Pierce Coomassie Bradford Protein Assay Kit, Thermo Fisher Scientific™) and compared with the protein content in the stock phage solution. A set of protein standards was prepared by diluting the contents of one Bovine Serum Albumin (BSA) standard ampoule (2 mg/mL) five times into clean eppendorfs®, in Tris-HCl 25 mM pH 9 buffer. It was pipetted 50 μL of each standard, control and testing samples into a 96 microwell plate well. Duplicates were made. 200 μL of Coomassie solution was added to each well and the plate was mixed on a plate shaker for 30 seconds and then incubated for 10 minutes at room temperature. Absorbance was read at 595 nm with a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific™).

2.5. Bacteriophage Morphology Analysis

The morphology of *E.* coli T4 bacteriophages submitted to fragmentation processes were analyzed with TEM at the MicroLab facilities in Instituto Superior Técnico.

For the analysis of T4 bacteriophages submitted to fragmentation methods, the staining process with uranyless was performed as described by *Electron Microscopy Sciences* [16]. The grid was deposited on a sample drop of these phage samples and then a droplet of uranyless solution was added on top and let to absorb for 1 minute. The excess of dye was removed with a tissue paper. The grid was then deposited on the uranyless solution for 1 minute and let to dry for another 5 minutes. Afterwards the grid was placed in the TEM device to visualize the samples.

3. Results and Discussion

3.1. *E. coli* Growth Data

E. coli is a robust, gram-negative and high versatile bacterium that is characterized by its ease of maintenance and rapid growth in laboratory conditions. These qualities make *E. coli* one of the model organisms in the field of microbiology studies. The proliferation of a bacterial population and its rate of growth are limited by its genetics and environmental condition such as temperature, acidity (pH), oxygen levels, water, micro and macro nutrients and toxins. In a batch system, viable bacterial cells cultivated in TSB medium and incubated at a constant optimal temperature of 37°C, with agitation of 250 rpm and pH 7, are capable of reproducing quickly and their dynamics of population proliferation can be measured periodically by increasing the number of cells as a function of the incubation time [17,18,19]. This relation makes it possible to obtain a growth curve of *E. coli* cells as present in **Figure 2**.

When the microbial growth of the culture is carried out in batch, the culture goes through 4 distinct phases. Initially, when bacteria are inoculated, despite being metabolically active, cell division does not occur immediately since they need a period of adaptation to the medium where they are inserted. This adaptation period is called the latency phase in which the culture tends to synthesize enzymes and co-enzymes for its physiological activities in order to benefit from environmental changes [18]. Due to this, the latency phase occurs in the pre-inoculum and for that reason, this state does not appear in the determined *E. coli* growth curve. When bacteria are fully adapted to the environment and all of the nutrients necessary for their

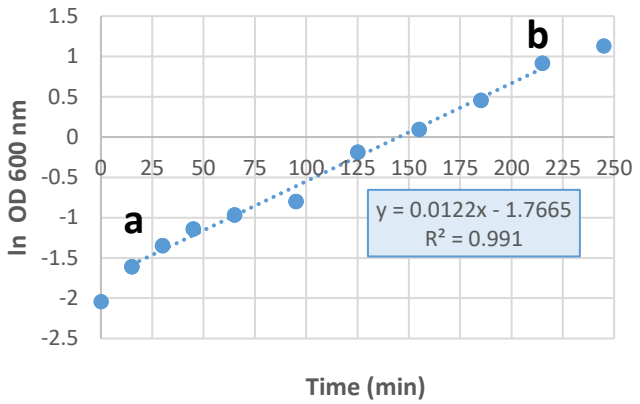

Figure 2 – *E. coli* growth curve. Identification of growth phases: (a) exponential phase between 0 and 220 minutes; (b) deceleration phase at 220 minutes.

growth are available, cells start to multiply continuously and at a constant rate, being this state denominated the exponential phase. The exponential phase can be characterized in the growth curve by the letter (a) between 0 and 220 minutes. After the depletion of the limiting nutrient or accumulation of toxic products by bacteria, cells enter a deceleration phase which can be characterized in the growth curve by the letter (b) at 220 minutes. Further, cells enter in the stationary phase, where the proliferation rate becomes zero [17,18].

By taking a sample of bacteria from the culture at every 15-30 minutes, it was possible to determine the number of viable cells through the plating the dilutions of those samples. This information in addition to the determination of the different phases of *E. coli* proliferation through the measuring of the OD, is crucial to understand the bacteria growth data, specially the exponential phase since its when the bacteria receptors are more exposed and available for the bacteriophage recognition and attachment. The counted colonies of *E. coli* at each time of bacterial growth allowed to determine that there were 1.52×10^7 CFU/mL of bacteria in the exponential phase, characterized by an OD between 0.20 and 0.26.

3.2. T4 Bacteriophage Stock Amplification

After the knowledge on bacteria content it was necessary to know the phage content. A titration assay showed that the revitalized phage suspension had 4.32×10^7 PFUs/mL which is low taking into account that a typical phage lysate contains approximately 10^8 to 10^{11} PFUs/mL. Due to this, it was necessary to perform both solid and liquid medium amplifications in order to have sufficient quantities of bacteriophage stock for all of the experiments. The solid medium amplification allowed to obtain a titre of 2.38×10^9 PFUs/mL. Although the phage content was already satisfactory to proceed on the experiments, it was not yet the desire titre. In this case, a liquid medium amplification was performed and a final stock phage suspension was obtained with a titre of 2.86×10^{10} PFUs/mL.

3.3. Bacteriophage Separation Methods

During their infection process, bacteriophages attach to the receptors in the surface of the specific bacterial cell and inject their genetic material inside them in order to create new progeny. This action leads to the rupture of the bacterial cell and release of the phage progeny. Although its a fast and efficient way to amplify and produce new phages, it compromises the recognition of target cells when used in biosensors. Due to this, the generation of phages with only their tails (recognition and attachment portion) and lack of their heads (transmission of genetic material portion) could allow the identification of bacterial targets without them lyse. For this purpose, three different approaches, mechanical (osmotic shock) and non-mechanical (water bath and probe ultrasonications) were accomplished to achieve the generation of phages as a new recognition element.

The fastest way to understand the effect of the fragmentation processes was to analyze the infection capacity of the submitted phages. All of the assays demonstrated a decrease in the ability to maintain infectiveness by one to two orders of magnitude in comparison to the initial phage content that was used in the beginning of each procedure, in exception for the water bath ultrasonication which was not effective. Despite these results, the decline of the infection capacity could be due to: i) burst of phage capsids; ii) generation

of openings in the capsid of the phages, which may have lead to the escape of the genetic material; and iii) actual separation of phage tails from their heads. Nevertheless, PFU counting plaques does not differentiate between undamaged intact phages and damaged “leaky” phages which are technically whole, however may have openings in the capsid which may or not allow the escape of the DNA, as so they can still infect the bacterial host as long as they own enough genetic material for that process [21]. Furthermore, the plaque counting methods is not an exact method due to possible dilution and plaquing associated errors.

To further evaluate if the phage fragmentation by the disruption methods, an assay based on measuring protein concentration was performed. For each Bradford assay, a calibration curve was plotted by varying the initial concentration of BSA (data not shown). The Bradford assay showed a protein concentration of 62 - 64 μg/mL in the initial phage stock. In the osmotic shock samples, zero or very low protein contents was detected when compared to the protein concentration on the phage stock (data not shown). This result could be explained by the high dilutions (100x) that were made to perform the osmotic shock itself. The analyzed protein samples from the water bath ultrasonication and 25 W probe ultrasonication presented high values of protein concentration (57 μg/mL and 58 μg/mL respectively) that almost reached the content of the phage stock. Although reported in literature by Guerlava *et al.* and Spiden *et al*. that after mechanical or non-mechanical cell disruption techniques appears to be an increase in protein concentration [20,21], it does not necessary mean that the same results should be obtained for two reasons: i) this experimental work is done with phages which are much resistant and robust than bacterial cells or yeasts, and ii) the main objective is to separate body parts of the phages, not disrupt them, so in theory, the protein content would be less than the protein content released by burst phages. Consequently, the level of protein content in the Bradford assay analysis would not be mandatorily much higher than the protein content of the stock. Additionally, the Bradford assay is only applicable to differentiate between whole undamaged phages and phages which have released their metabolites. It is not applicable to distinguish between phages which are ruptured and more extreme levels of fragmentation [20,21,23,24]. The 50 W probe ultrasonication presented very low values of protein concentrations which is compatible to protein degradation by the rapid increase of temperature at the tip of the probe which can compromise the biological samples [25].

Further, to determine what proteins were detectable and to acquire more information about the effect of the fragmentation processes, all of the phage samples were subjected to a denaturing SDS-PAGE. This technique allows for a quick analysis of the gels because enables to estimate the molecular weight of each phage corresponding protein band. By the construct of a standard curve that plots the log of the molecular weight from the ladder versus the migration of each ladder band, it was possible to calculate the molecular weight for all of the phage bands (data not shown). The bands were identified by their molecular weight, compared to literature and characterized as proteins which belong to the head, tail and tail fibres [22,23] (data not shown).

3.4. Bacteriophage Morphology Analysis

The *E. coli* T4 bacteriophages submitted to a 25 W probe ultrasonication were visualized by TEM since it was the fragmentation process with the most promising and reliable results.

Intact T4 bacteriophages were reported by Miller *et al.* to be composed of a long contractile tail and an icosahedral head. These characteristics indicate that these phages belong to the T-even bacteriophages from the *Myoviridae* family [23]. **Figure 3** presents images of a sample of T4 bacteriophages submitted to a 25W probe ultrasonication at 40 000x magnification.

The submitted T4 phages appear to be with their tails separated from their respective capsids and their baseplate and tail fibers are also visible, which indicated that the recognition portion may be intact and functionalized.

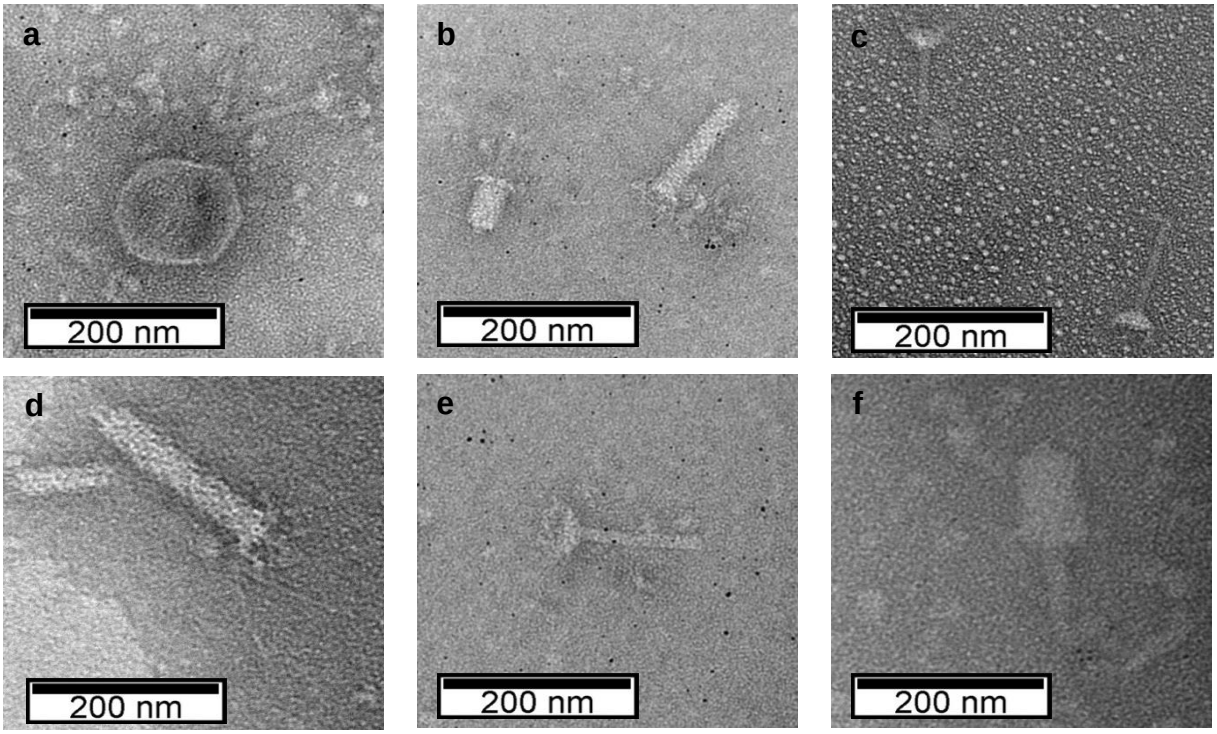

Figure 3 – TEM images of specific *E. coli* T4 bacteriophages submitted to a 25 W probe ultrasonication stained with uranyless. Images obtained at 40 000x magnification at a 200 nm scale in IST MicroLab. (a) phage capsid separated of their tail apparatus with 15 minutes of probe ultrasonication; (b) contracted and non-contracted tail apparatus separated from phage capsid with 15 minutes of probe ultrasonication; (c) tail apparatus with visible baseplates separated from phage capsid with 15 minutes of probe ultrasonication; (d) tail apparatus with visible tail fibers separated from phage capsid with 15 minutes of probe ultrasonication; (e) tail apparatus with visible baseplates separated from phage capsid with 20 minutes of probe ultrasonication; (f) tail apparatus with visible baseplates separated from phage capsid with 20 minutes of probe ultrasonication.

These observations may be an evidence that the 25W probe ultrasonication was effective for the generation of bacteriophages comprising only the recognition apparatus suitable to be applied in a biosensor platform in order to improve the field of medical diagnosis. Even with no images visualized of the intact T4 bacteriophage, the morphology of the capsid and tails analyzed appear to be in accordance to Miller *et al.* [23].

4. Conclusion

This Master´s Thesis aimed to enhance and develop advance bacteriophage recognition elements for a fast and reliable pathogen identification by the separation of bacteriophage´s heads from their tails, thus eliminating the infection capacity, but retaining their recognition ability. In the initial experimental work, concentration and diafiltration procedures in the amplification of the phage stock, appeared to be important for phage stability since only small losses in phage titre were detected when storing the stock for three months. When the lysate was stored in the culture media after centrifugation, without concentration and diafiltration processes, a phage precipitation showed up, leading to a large loss in the titre.

The *E. coli* host strain growth study provided an understanding of the behavior of these bacterial cells, and also important information such as OD_{600nm} (to know when the exponential phase occurs) and CFUs for the infection assays. The exponential phase was crucial to determine because it's in that interval when bacteria receptors are more exposed and available for the bacteriophage recognition, attachment and infection.

After having a functional cell bank and phage stock, the second stage of this study consisted in the attempt to separate the tails of the phages from their heads to prevent the infection and subsequent lysis of the host cells, which compromises the recognition of target cells when used in biosensors.

Taking into account all of the four dissociation methods, the one that showed more promising results was the 25 W probe ultrasonication, phage samples were analysed in TEM. TEM images showed a successful fragmentation of the phage tails from their capsids during 15 and 20 minutes of ultrasonication. The other three disruption methods were not analysed by TEM, however it would be a way to take more detailed conclusions on the fragmentation of the bacteriophages.

For future work, supplementary disruption assays could be performed by changing or adding some of parameters such as temperature or power in probe ultrasonication or different salt concentrations for the osmotic shock. dDNA quantification assay such as PicoGreen® could also be strategy to measure the concentration of nucleic acids after the fragmentation processes once a high concentration of DNA would indicate the release of genetic material in consequence of the detachment of the phage heads from the tails. Furthermore, enzymatic digestion could also be performed as a disruption technique to separate phage heads in the specific aminoacids. Additionally, a two-phase separation assay with PEG (Polyethylene Glycol) or a column chromatography would be approaches worth to try in order to separate and purify phages tails from the heads. PEG separation would allow for the majority of the tails to rest on the upper phase since they have lower density and the heads, which have higher density due to the DNA encapsulation, to rest in the lower phase. Afterwards, a diafiltration step could also be added in order to exchange the phage media into a suitable buffer. Separation of the tails from the heads in the medium could also be achieved by chromatography which is substantially faster, more consistent, and allows for system automation when compared to the PEG two-phase system. Ion-exchange chromatography (IEC) is the most promising mode of operation that could be used once the heads appear to be more negatively charged than the tails, consequently being retained in a positive charged anion-exchange resin. Size-exclusion chromatography (SEC) is another possibility to consider for this attempt of separation in order to remove non-encapsidated phages (just with tail apparatus) that would elute at last due to their smaller size in comparison to intact phages. At last affinity chromatography could be another way to separate the tails from the heads, however in this case the tails required to be expressing a recombinant protein in order to interact with the affinity molecule present in the column. The phage heads would not interact with the column and elute first. The desire recombinant tails would only elute in the presence of a solvent of higher salt concentration.

For instant, it could be produced bacterial cells with a plasmid expressing a His-tag in the proteins of the tail fibers. Thus, during phage assembly, phages with a His-tag in their tail fibers would be present. After phage fragmentation processes, this tag could be used to capture the tails that bind to the column coated with immobilized metal ions such as nickel or copper. In the same way, a biotin-streptavidin recognition assay could be used to separate the tails from the heads. Once again bacterial cells with a plasmid expressing biotin in the tail fibers could be produced and after phage assembly, phages with biotin attach to their tail fibers would be present. After this, the capture could proceed with a column coated with streptavidin. Besides that, the bacterial cells could simply be immobilized in the column to be recognized by the tail fibers and be captured without engineer the phages. After confirmation of phage tail separation and purification, trials for the functionalization of the tails to biosensors could start in order to provide a fast reliable pathogen identification and improve the medical diagnosis field.

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