The isolation of bacteriophages from Beninese water samples against clinical *Acinetobacter baumannii* strains

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Tiivistelmä — Referat — Abstract

The objective of this thesis was to isolate and characterized phages from Beninese wastewater samples against clinical *Acinetobacter baumannii* strains for phage therapy use. *A. baumannii* is one of the most threatening nosocomial bacteria because most of the strains are resistant towards all commonly used antibiotics. One promising alternative treatment method could be phage therapy that utilizes lytic phages to dispose of specific bacteria.

In this thesis, seven phages infecting clinical *A. baumannii* strains were isolated and two of them were characterized more in detail. Phages vB_AbaA_fBenAci001 (fBen-Aci001) and vB_Aba_fBenAci002 (fBen-Aci002) were members of the *Friunavirus* genus of the *Autographiviridae* family. In addition, they were the only phages characterised from their respective species to date. The genome analysis revealed 82.2% identity between the phages. No genes indicating lysogenic lifecycle, or genes encoding bacterial toxins or antibiotic resistance were identified from either of them. Phage fBen-Aci001 were infecting 4% and fBen-Aci002 were infecting 9% of tested 23 clinical *A. baumannii* isolates. Phylogenetic tree which was constructed based on whole genome sequences was compared to the trees that were made using tailspike proteins and capsid proteins. No correlation between genome-wide tree and trees built based on single genes were seen.

In conclusion, the Beninese hospital wastewater appeared to be a good source for *A. baumannii* phages, as several phages were isolated and they were infecting clinical multidrug resistant strains isolated from Finnish patients. Phages fBen-Aci001 and fBen-Aci002 were concluded to be potential candidates to be used in the phage therapy though the narrow host range might negatively affect their usability.

Avainsanat — Nyckelord — Keywords

Acinetobacter baumannii, bacteriophage, phage, phage therapy, antibiotic resistance, nosocomial infection, *Friunavirus*, vB_AbaA_fBenAci001, vB_AbaA_fBenAci002

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Bakteriofagien eristäminen Beniniläisistä vesinäytteistä kliinisiä *Acinetobacter baumannii* kantoja vastaan

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Tiivistelmä — Referat — Abstract

Tämän tutkielman tavoitteena oli eristää ja karakterisoida faageja Beniniläisitä jätevesinäytteistä kliinisiä *Acinetobacter baumannii* -kantoja vastaan käytettäväksi faagihoidoissa. *A. baumannii* on yksi vakavimmista sairaalabakteereista, koska suurin osa kannoista on resistenttejä kaikille yleisesti käytetyille antibiooteille. Yksi lupaavista vaihtoehtoisista hoitomenetelmistä on faagihoito, joka hyödyntää lyyttisiä faageja tiettyjen bakteerien hävittämiseksi.

Tässä tutkielmassa eristettiin seitsemän faagia, jotka infektoivat kliinisiä *A. baumannii* -kantoja. Kaksi faageista karakterisoitiin tarkemmin. Faagit vB_AbaA_fBenAci001 (fBen-Aci001) ja vB_Aba_fBenAci002 (fBen-Aci002) kuuluivat *Autographiviridae* -heimoon ja *Friunavirus*-sukuun. Tähän mennessä tutkituista faageista ne olivat ainoat oman lajinsa edustajat. Genomianalyysin mukaan faagit ovat keskenään 82,2 % identtisiä. Faageista ei tunnistettu lysogeeniseen elinkiertoon viittaavia geenejä. Myöskään bakteeritoksiineja tai antibioottiresistenssiä koodaavia geenejä ei löydetty. Faagi fBen-Aci001 infektoi 4 % ja fBen-Aci002 infektoi 9 % testatuista 23 kliinisestä *A. baumannii* -kannasta. Koko genomin sekvenssin perusteella rakennettua fylogeneettistä puuta verrattiin puihin, jotka tehtiin käyttämällä häntäpiikkiproteiineja ja kapsidiproteiineja. Korrelaatiota koko genomin laajuisen puun ja yksittäisten geenien perusteella rakennettujen puiden välillä ei havaittu.

Beniniläinen jätevesi vaikutti olevan hyvä lähde *A. baumannii* -faageille, koska siitä voitiin eristää useita faageja. Lisäksi eristetyt faagit infektoivat suomalaisista potilaista eristettyjä kliinisiä monilääkeresistenttejä kantoja. Faagit fBen-Aci001 ja fBen-Aci002 olivat lupaavia ehdokkaita faagihoidoissa käytettäväksi, mutta kapea isäntäkirjo voi vaikeuttaa niiden käyttöä.

Avainsanat — Nyckelord — Keywords

Acinetobacter baumannii, bakteriofagit, faagit, faagihoito, antibioottiresistenssi, sairaalainfektio, *Friunavirus*, vB_AbaA_fBenAci001, vB_AbaA_fBenAci002

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INTRODUCTION

First antibiotic resistant bacteria were discovered in 1960's (Centers for Disease Control and Prevention, 2019b) and since then their rates have been rising rapidly all over the world due to the misuse of antibiotics. Especially gram-negative bacteria are causing problems to healthcare systems in the form of extended hospitalization periods, lower recovery rates, and higher costs (Cassini et al., 2019; Mauldin et al., 2009; Playford et al., 2007). In 2017, the World Health Organization published a global priority list of multidrug resistant (MDR) bacteria, where carbapenem-resistant *Acinetobacter baumannii* appeared in the top of the first priority group, for which the situation is critical (World Health Organization, 2017). *A. baumannii* is also a part of the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which is a group of pathogens with exceptionally high resistance towards antibiotics (Rice, 2008).

A. baumannii is a gram-negative, non-fermentative aerobic coccobacillus and a significant nosocomial pathogen worldwide (DeLong et al., 2014). It is associated with a wide spectrum of hospital-acquired infections, such as pneumonia, blood stream infections, urinary tract infections and wound infections (Playford et al., 2007). These infections are especially dangerous for immunocompromised people and patients in intensive care units.

When *A. baumannii* starts to spread in a hospital environment, it is very difficult to eliminate the bacterium as it can form biofilms on biotic and abiotic surfaces. Typically, the bacteria spread in the hands of hospital staff, in plastic medical devices as well as in rails and door handles (Centers for Disease Control and Prevention, 2019a). In addition, *A. baumannii* has the ability to tolerate detergents, ultraviolet light, and dehydration, as well as survive on various surfaces for prolonged periods of time (Peleg et al., 2008).

Besides being resilient, *A. baumannii* has numerous antibiotic resistance mechanisms and due to the misuse of antibiotics, the majority of the strains have become resistant towards all antibiotics. Besides being remarkably high, MDR rates of *A. baumannii* has been rising more rapidly than for any other gram-negative bacteria. Between 2004 and 2014, global rates of MDR *A. baumannii* has increased from 23% to 63% while MDR rate of other gram-negative bacteria included in the study (e.g. *K. pneumoniae, P. aeruginosa* and *Escherichia coli*) were below 20% during the whole study period, and the rates remained

fairly constant (Giammanco et al., 2017). There is also a great variability in MDR rates between different regions. In Europe, combined resistant rates of *Acinetobacter* spp. to three antibiotics (fluoroquinolones, aminoglycosides and carbapenems) varied from 0% to over 95% in 2019 depending on the reporting country (European Centre for Disease Prevention and Control, 2020). Against MDR *A. baumannii* strains, colistin is a last-line antibiotic that can be used to dispose it. However, it has considerable side effects, and due to the use of the antibiotic colistin-resistant *A. baumannii* strains have been reported too (Qureshi et al., 2015; Urban et al., 2001). Even if *A. baumannii* causes minority of all infections associated with gram-negative pathogen, the high MDR rates has caused a higher mortality rate compared to any other nosocomial pathogen. When considering all this information, it is clear that there is an urgent need to develop new treatment methods against MDR pathogens, especially for *A. baumannii*. One promising method could be phage therapy, which utilizes bacteriophages to treat bacterial infections.

Bacteriophages or shortly phages are viruses, which infect bacteria. They specifically infect certain bacteria and are known not to affect the body's normal flora or human cells. Phages have been used as a treatment method against bacterial infections since discovering them in the mid-1910s (Abedon et al., 2011). As antibiotics became more common in the 1940s, less attention was paid towards phage therapy research, especially in Western countries. However, the interest towards phages as a treatment method has been revived after the crisis posed by MDR bacteria.

Phage therapy has been successfully used to cure *A. baumannii* infections, when several antibiotic medications did not help. In one of the cases, the cocktail of nine phages was used to treat a critically ill patient with *A. baumannii* infection related to necrotizing pancreatitis that had led the patient to fall into a coma. After the phage treatment, the patient soon awoke from the coma, and fully recovered (Schooley et al., 2017). In the other case, the phage therapy was applied in combination with antibiotics to a tibial infection with *A. baumannii* and *K. pneumoniae* infections resulting in rapid tissue healing. The treatment saved the patient’s leg from amputation (Nir-Paz et al., 2019).

Phages can be isolated from various sources. As phages need a host bacterium to multiply, they can be found most easily from where the bacteria are commonly found. Good sources for phages are water and soil samples, and when isolating phages for clinical strains, the best place to look at is hospital wastewater. Isolating phages in the laboratory via enrichment might be beneficial for therapy purposes for few reasons. Firstly, enrichment happens to favor those phages, which has greater virulence against

the host. Secondly, when similar conditions were used for isolation process, and phage preparation for patients, phages that can be produced easier in laboratory conditions are favored (Abedon et al., 2011).

Before a newly isolated phage can be accepted to be used in therapy, some requirements must be fulfilled. Firstly, the phage needs to be virulent, which means it lyses the host bacteria in the end of the lytic infection cycle, and releases new phages (Abedon et al., 2011). Virulent, also called lytic, phages have an ability to trigger new infection cycles, which leads in exponential growth of the phage population until the host is no longer available. Temperate phages cannot be used as they might follow the lysogenic lifecycle where the phage genome is integrated into the host genome. Thus, the infection of these phages does not destroy the host as effectively, and it may also allow the spread of harmful properties, for example antibiotic resistance genes, through horizontal gene transfer. The lifecycle of the phage can be determined from the genomic data where integrases, transposases or lysogeny repressors strongly indicate lysogenic behavior (Weber-Dąbrowska et al., 2016). Secondly, phages cannot carry any antibiotic resistance genes or genes encoding bacterial toxins.

Phages infecting *A. baumannii* have been isolated previously from various sources but most typically from the hospital wastewater. Phage isolation has been more successful from outside Western countries, because *A. baumannii* is more common there. However, antibiotic resistance is a major problem globally and effective therapy phages are needed all over the world. One of the properties of the phages is that they typically infect strains from the same origin more efficiently than strains isolated from other areas. This poses the challenge of finding suitable phages for therapeutic use. This thesis is focused on finding phages from Beninese water samples to be used in phage therapy, and especially to find phages which infect the clinical *A. baumannii* strains isolated from Finnish patients.

MATERIALS AND METHODS

Bacterial strains and growth conditions

All bacteria used in this study were clinical *Acinetobacter* strains (Table S1). The strains were mainly collected from Finnish patients, but the origins of the infections were unknown. The Skurnik lab storage number was used to identify strains. The bacteria were cultured at 37 °C in Luria broth (LB) liquid

medium, 1.5% (w/v) LB agar plates or 0.4% (w/v) LB soft agar medium solidified on LB agar plates (Sambrook & Russell, 2001). The corresponding brain heart infusion media was used to culture *Acinetobacter junii* strain #5567, and *Acinetobacter ursingii* strains #5569, #5572, #5913, and #5932. For overnight cultures, one single colony was picked from an agar plate, inoculated in 5 ml of suitable liquid medium, and then incubated whilst shaking overnight.

Phage titration methods

Double layer overlay method (Sambrook & Russell, 2001) was used to determine titer of the phage lysates, to produce semiconfluent plates, and for plaque purification. The host bacteria were cultured until the logarithmic growth phase, followed by mixing $45 \times OD_{600}^{-1}$ µl bacteria and 50 µl of appropriate 10-fold dilution of phage lysate with 3 ml molten soft-agar tempered to 55 °C. The mixture was vortexed, poured onto a room temperature LB plate, and left to solidify for 30 minutes. An equal volume of growth medium was used, instead of the phage lysate, as a negative control.

No phage lysate was added into soft agar before pouring it onto a plate to test phage infectivity by the drop test. Instead, 10 µl droplets of different phage dilutions were pipetted on top of hardened soft agar and left to dry at least for 60 minutes. All plates were incubated overnight at 37 °C.

Optimizing Bioscreen conditions for *Acinetobacter*

Bioscreen FP-1100-C (Oy Growth Curves Ab Ltd) plate reader was used to screen enrichment cultures and in host range tests. Before the method could be used, measurement conditions had to be optimized for *Acinetobacter*. The conditions were optimized using *Acinetobacter pittii* #5565, which is the host of the phage vB_ApiM_fHyAci03 (fHy-Aci03) (Pulkkinen et al., 2019) and four randomly selected *A. baumannii* strains from the available collection. *A. baumannii* strains used were #5542, #5706, # 5911 and #5934.

Preliminary tests were made using different dilutions of bacteria to evaluate in which rate the bacterium is growing in liquid medium in the measurement conditions. To set up the measurement, 100 µl of diluted

overnight cultures were pipetted in duplicates on honeycomb plates. Dilutions of 1:50, 1:100, 1:500 and 1:10000 were made from each strain. Equal volume of growth medium was used as a negative control. Plates were incubated at 37 °C for 12 hours with continuous, medium amplitude and normal speed shaking. Turbidity of the wells were measured with a wavelength of 600 nm once in every 60 minutes. Arithmetic mean of parallel wells was calculated to make results more reliable and to calculate growth curves.

Testing the effectiveness of the phage infection, *A. pittii* phage fHy-Aci03 and its host #5565 was used because no lytic *A. baumannii* phages were available in the Skurnik lab collection at that point. Based on the preliminary tests, dilutions of 1:50 and 1:100 of overnight cultures were considered most suitable. In addition, one new dilution of 1:200 was used. Each dilution was used with phage concentrations of 10^6 pfu ml^{-1} and 10^7 pfu ml^{-1}. First, 10 µl of phage lysate was aliquot into the honeycomb plate, and after that, 100 µl of diluted bacteria was added into wells. The combinations were made in triplicates. As a negative control, 10 µl growth medium was used instead of the phage lysate. Same conditions were used as those in the preliminary tests, but the measurement time was only 6 hours. To summarize the results, arithmetic mean of the parallel wells was calculated, and the growth curves were drawn based on the mean values of each time point.

Phage isolation by enrichment cultures and plaque purification

In order to find phages for *A. baumannii*, 47 Beninese water samples were received from Doctor Victorien Tamègnon, Docent Kaisa Haukka and Professor Anu Kantele, and it included 34 hospital wastewater samples from Hospital de Zone Calavi, Hospital Menontin, and Hospital Saint Jean. The rest were environmental water samples. These samples were pooled to four cocktails, which resulted in three hospital wastewater cocktails (Hospital I – Hospital III) and one environmental cocktail (Environment I) (Table S2).

Isolation of the phages were carried out using enrichment cultures and plaque purification (Sambrook & Russell, 2001). Enrichment was made separately against 23 clinical *A. baumannii* strains (Table S1). Each strain was cultured with each water cocktail. For enrichment cultures, 1.5 ml of the pooled water sample was mixed with 45 µl of bacteria culture (OD_{600} = 1) and 4.5 ml of the growth medium. Cultures

were incubated overnight at 37 °C, whilst shaking. Remaining bacteria were lysed by adding 150 µl of chloroform into the tube, followed by a 10-minute incubation in the rocking platform at the ambient temperature. After centrifugation at 5000 rpm for 10 minutes at the ambient temperature, the supernatant was filtered through 0.22 µm filter (Minisart®, Sartorius). Enriched cultures were screened with the Bioscreen plate reader using previously optimized conditions. In short, 10 µl of undiluted enrichment cultures were aliquoted into a honeycomb plate in triplicates, and 100 µl of 1:100 diluted bacteria culture of enrichment host was added on top. Measurement was carried out using the same settings as when testing the effectiveness of the phage infection, and the results were handled similarly. Positive and intermediate reactions (Fig. S1) were confirmed with the double layer overlay method.

Three rounds of plaque purifications were made for those enrichment samples, which showed the presence of phages. One single plaque was picked from the titration plate and incubated in 500 µl of SMG-buffer (100 mM NaCl, 10mM $MgSO_4$, 50 mM Tris-HCl, pH 7.5, 0.01% (w/v) gelatin) for 1 hour, and then plated according to the double layer overlay method. For latter rounds, phage from the previous purification round was used as the starting material.

Phage lysates

After plaque purification, new lysates were produced with semiconfluent plates. To dissolve the phages, 3 ml of SMG-buffer was added on top of the semiconfluent plate, and incubated at ambient temperature for 2 hours. The soft-agar and buffer were collected with L-shaped rod into the falcon tube and 150 µl of chloroform was added. Tubes were incubated for 10 minutes at the ambient temperature in the rocking platform, and centrifuged at 5000 rpm for 10 minutes at the ambient temperature. Supernatants were filtered through 0.22 µm filter (Minisart® Sartorius), and 1.2 ml of 40% (w/v) sucrose were added.

Host range screening

Host range of the phages were determined in the liquid culture using the Bioscreen plate reader against 57 clinical *Acinetobacter* strains (Table S1) using optimized conditions. Briefly, phage lysates were

diluted to a concentration of 10^7 pfu ml^{-1} and then distributed onto a honeycomb plate in 10 µl aliquots. The overnight cultures of each strain were diluted to 1:100 and 100 µl of the dilutions were pipetted on the top of the phage lysates into three parallel wells. The Bioscreen was used under the same settings as before and means of the parallel wells were calculated. Positive and intermediate reactions (Fig. S1) were confirmed using the drop test method.

DNA isolation and sequencing

The DNA was isolated from the unpurified high-titer phage lysate (10^{10} pfu ml^{-1}) using phenol-chloroform extraction and ethanol precipitation (Sambrook & Russell, 2001). To degrade bacterial DNA and RNA, 1.3 µl of Dnase I (1 U $µl^{-1}$) and 4 µl of RNase A (1 mg ml^{-1}) were added to 400 µl of phage lysate. The mixture was incubated at 37 °C for 30 minutes. Phage capsid was then hydrolyzed by adding 16 µl of EDTA, 1.2 µl of protease K (20 mg ml^{-1}) and 20 µl of 10% (w/v) SDS. The next mixture was incubated at 56 °C for 60 minutes. The mixture was cooled to the room temperature. After that 1 volume of phenol was added and the tube was incubated in the rocking platform for 15 minutes. After centrifugation at 13400 rpm for 15 minutes at the ambient temperature, the aqueous upper phase was transferred into a new tube. Phenol extraction was repeated once, until no whitish precipitate appeared between phases. Extraction was repeated once as stated above with 1 volume of chloroform. To precipitate DNA, 0.1 volume 3M NaOAc (pH 7.0) and 2 volumes absolute ethanol was added, and the tube was turned gently by hand up and down until a DNA thread became visible. The thread was transferred with a pipette tip into a new tube containing 1 ml of 70% (v/v) ethanol. After pelleting DNA by centrifuging the tube at 13400 rpm for 20 minutes at the ambient temperature, supernatant was removed and the pellet was air-dried. Finally, the DNA was dissolved into 100 µl of TE-buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0).

The DNA concentrations were measured with the Qubit™ 4 Fluorometer device (Invitrogen, ThermoFisher Scientific) with the Qubit™ dsDNA BR Assay Kit (ThermoFisher Scientific) using the manufacturer's protocol. Quality of the DNA was checked with agarose gel electrophoresis to make sure that it was intact. The gel was prepared by diluting 0.8% (w/v) of LE Agarose (SeaKem™) in the TAE-

buffer (40 mM Tris, 20 mM acetic acid, 1mM EDTA). Sequencing was made by Novogene (https://en.novogene.com/) using the Illumina based method.

Genome analyzation

For the de novo assembly of the phage genomes, 50,000 reads subset was made from both forward and reverse sequence files using the Chipster v4 (Kallio et al., 2011). Assembly of the genomes was made by using the A5-miseq integrated pipeline for the de novo assembly of microbial genomes (Coil et al., 2015). The BLASTn 2.10.1+ (Nucleotide Basic Local Alignment Search Tool) (Zhang et al., 2000) search under standard settings was used to identify phage genomes among the resulting contigs. PhageTerm (Garneau et al., 2017) was used to predict physical ends of the phage genomes. The genomes were trimmed and organized manually based on the PhageTerm results. Assemblies were confirmed using Geneious Prime 2020.1.2 (https://www.geneious.com/) by mapping all original reads back to the de novo assemblies. Preliminary annotations were made using RAST 2.0 (Rapid Annotation using Subsystem Technology) (Aziz et al., 2008; Brettin et al., 2015; Overbeek et al., 2014) after which the annotations were checked and edited manually using Artemis 18.1.0 (Berriman & Rutherford, 2003), BLASTp 2.10.1 (Protein Basic Local Alignment Search Tool) (Altschul et al., 1997) and HHpred (Zimmermann et al., 2018).

The sizes of the RNA polymerases were estimated using Expasy (https://www.expasy.org/). The presence of tRNA genes were analyzed using Aragorn v1.2.38 (Laslett & Canback, 2004). VirulenceFinder 2.0 (Joensen et al., 2014) was used to screen bacterial toxins while ResFinder 4.0 (Bortolaia et al., 2020) and CARD 3.0.7 (Alcock et al., 2020) were used to screen antibiotic resistance genes.

Genomes were aligned using the Geneious Prime alignment tool under the standard settings. Based on the alignment, the least similar protein that the phages shared and one conserved structural protein were selected to compare the protein level phylogenetic relationships to the genome-wide phylogeny. The closest relatives of the phages were identified with BLASTn searches for whole genome sequences and matches with over 90% query coverage were used for the construction of the phylogenetic trees (Table

S3). Duplicates between the phages were removed. Annotation of the genomes were checked using Artemis and required edits were made based on the BLASTp search results.

A genome-wide phylogenetic tree was constructed with VICTOR (Virus Classification and Tree Building Online Resource) using the Genome-BLAST Distance Phylogeny (Meier-Kolthoff et al., 2013) and standard settings recommended for the prokaryotic viruses (Meier-Kolthoff & Göker, 2017). Phylogenetic trees for tailspike proteins and capsid proteins were built with the GGDC web server (http://ggdc.dsmz.de/) (Meier-Kolthoff et al., 2013), which uses the DSMZ phylogenomics pipeline (Meier-Kolthoff et al., 2014).

RESULTS

Optimal Bioscreen conditions for *Acinetobacter*

Bioscreen conditions were optimized from *Acinetobacter* to test phage infectivity in liquid. Preliminary tests confirmed that *A. pittii* and *A. baumannii* grew approximately at the similar rate (Fig. S2), which indicates that the same conditions can be used for both species.

Results from the phage infectivity test are presented in Fig. 1. Combinations of the phage with dilution of 1:50 made from the overnight culture did not lyse the bacteria clearly enough or the lysis was too slow. The growth curves for 1:200 dilution with phage concentration of 10^6 pfu ml^{-1} and dilution of 1:100 with 10^7 pfu ml^{-1} were almost identical resulting in the clearest reaction. Dilution of 1:100 with phage concentration of 10^7 pfu ml^{-1} was considered to be the best combination, because in the case of dilution of 1:200, the bacteria without the phage did not grow quickly enough. Lysis can be seen clearly after three to four hours.

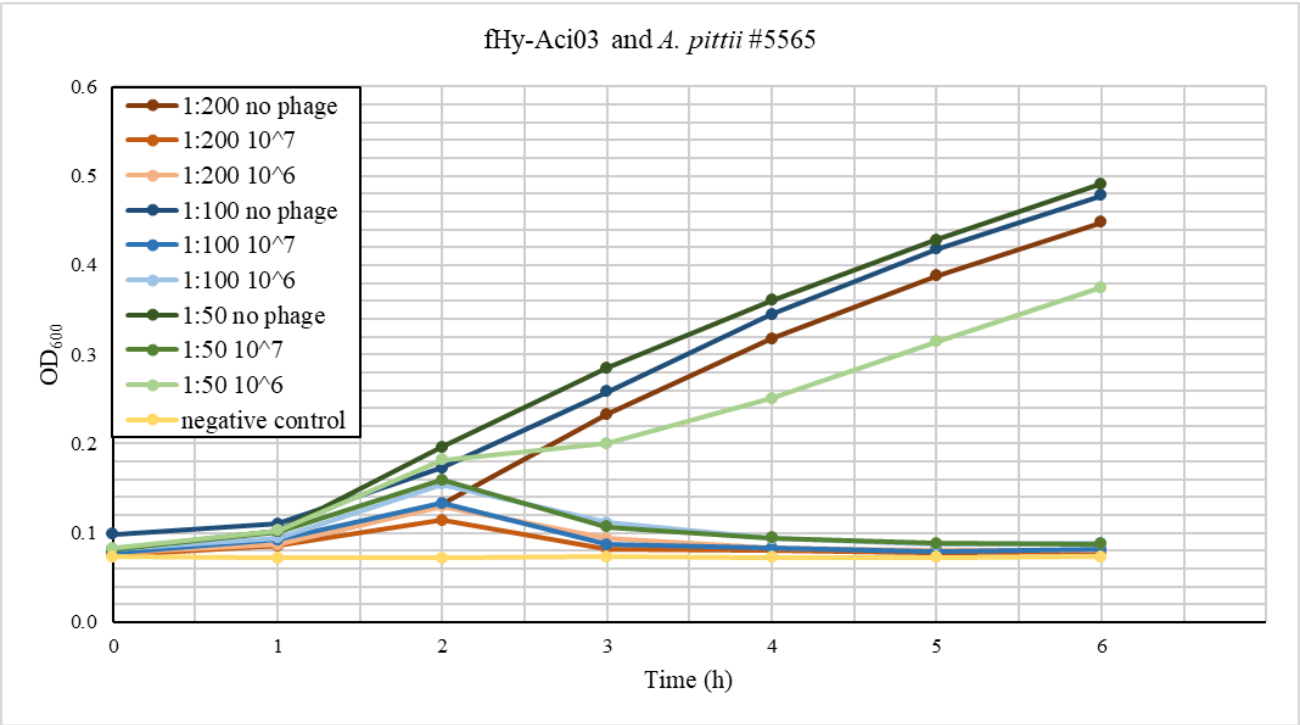

Fig. 1. Growth curves for phage fHy-Aci03 and *A. pittii* #5565. The curves are drawn based on the Bioscreen results from the phage infectivity test and the arithmetic mean of three parallel wells were calculated to make results more reliable. In the legend box the first stated value is the dilution of overnight culture and the second value is the phage concentration.

Phage isolation

Enrichment was made against 23 clinical *A. baumannii* strains using four cocktails pooled from the Beninese water samples, and cultures were screened using Bioscreen, after which the positive and intermediate reactions were confirmed on titration plates. The phage activity was detected from cocktail Hospital II against strains #5542, #5707, #5910 and #6898. Other three cocktails produced a positive reaction only against strain #6898. One phage was plaque purified from each positive sample (Table 1).

Out of seven phages isolated from the samples, the first two found were described and characterized more in detail. Phages vB_AbaA_fBenAci001 (fBen-Aci001) and vB_AbaA_fBenAci002 (fBen-Aci002) were isolated against *A. baumannii* strains #5542 and #5707 respectively. In soft agar plates, fBen-Aci001 forms 3 mm diameter clear plaques with a halo on its isolation host. The plaques of fBen-

Aci002 were otherwise similar but bigger with a 6 mm diameter. When increasing the incubation time, the halos grew while the clear areas remained the same size (Fig. S3).

Table 1. Phages isolated from the Beninese wastewater samples.

Phage	**Isolation host** ***A. baumannii***	**Cocktail**
fBen-Aci001	#5542	Hospital II
fBen-Aci002	#5707	Hospital II
fBen-Aci003	#5910	Hospital II
fBen-Aci004	#6898	Hospital I
fBen-Aci005	#6898	Hospital II
fBen-Aci006	#6898	Hospital III
fBen-Aci007	#6898	Environment I

Detailed information about the cocktails is available in Table S2.

Host range

The host range of phages fBen-Aci001 and fBen-Aci002 were determined against 57 clinical *Acinetobacter* strains representing eight different species. Twenty-three different *A. baumannii* strains were included in the 57 tested strains. Both phages had a very narrow host range. The phage fBen-Aci001 infected only the original isolation host, which represents 4% of tested *A. baumannii* strains. The phage fBen-Aci002 was able to infect one *A. baumannii* strain in addition to the original isolation host, which means 9% of *A. baumannii* strains. Infected strains were different between the two phages. No other *Acinetobacter* species than *A. baumannii* were infected (Table S4).

Genome analyses

For both phages, over 99.6% of the 100,000 sequence reads subset were used in the assembly resulting in the median depth of the coverage around 370 reads. For the fBen-Aci001 assembly resulted in six

contigs, out of which one was over 40 kbp in size, the others being below 800 bp. The BLASTn searches identified small contigs as genomic sequences of *A. baumannii*. Based on this information, it was concluded that the small contigs are contaminants from the host bacteria, and the work was continued only with over 40 kbp contig, which is most likely the phage genome. For the phage fBen-Aci002, only one contig was identified.

The genomic characteristics of the phages are listed in Table 2. Based on the whole genome alignment (Fig. 2), phages shared 82.2% identity with each other. Both of them had around 41 kbp sized genomes and GC content of 39.2. The number of the predicted genes were 50 for the fBen-Aci001 and 53 for the fBen-Aci002. For both phages, around half of the genes were hypothetical proteins whose functions are unknown. All genes were in forward orientation. Direct terminal repeats were identified from both phages using the PhageTerm. The length of repeat regions were 394 amino acids and 375 amino acids for fBen-Aci001 and fBen-Aci002 respectively. An RNA polymerase with a size of 90 kDa was identified from both phages.

Almost all genes for which the function could be predicted were the same between phages fBen-Aci001 and fBen-Aci002. However, the sequences of the shared genes differed from each other, as shown in the alignment picture (Fig. 2). Although, the genes were annotated to be functionally similar, 100% identity in their sequence was only very local. The tailspike protein genes were the least similar gene that the phages shared. The most typical difference between the genomes of the phages was a hypothetical protein that were present in only one of the phages. In particular, phage fBen-Aci002 had two interesting genes that were not present in phage fBen-Aci001. One of the hypothetical proteins in the early regions of fBen-Aci002 had short 39 amino acids sequence belonging to the DNA polymerase A superfamily. In addition, a putative homing endonuclease locating downstream from the DNA polymerase I could be identified for fBen-Aci002. The hypothetical proteins at the same sites in the genome of fBen-Aci001 had completely different nucleotide and amino acid sequences compered to ones found in fBen-Aci002, and any function could not be predicted for them.

The Aragorn software did not predict any tRNA genes for either of the phages. No bacterial toxins or antibiotic resistance coding genes were found. No known genes indication of temperate lifecycle (integrases, transposases or lysogeny repressors) was found, which indicates phages being lytic.

Sequences of phages fBen-Aci001 and fBen-Aci002 have been submitted to the GenBank under accession numbers MW056501 and MW056502, respectively.

VICTOR was used to construct the phylogenetic tree of the whole genome sequence. The Genome-BLAST Distance Phylogeny tree using the distance formula D0 is presented in Fig. 3. It revealed that all the phages belonged to the *Autographiviridae* family and the *Friunavirus* genus while falling into 15 species. Phages fBen-Aci001 and fBen-Aci002 were classified into the different species with each other and no other phages were identified to be the same species with either of them. The least conserved shared protein and the one conserved structural protein were used to build protein level phylogenetic trees. The proteins used were tailspike proteins (Fig. 4), which were the least conserved ones and capsid protein (Fig. 5), which was the highly conserved one. The trees were built with the GGDC web server, which uses the DSMZ phylogenomics pipeline. When comparing the protein level phylogenetic trees with the tree built based on the whole genome sequences, no clear correlation was seen.

Table 2. Overview of the genomes of fBen-Aci001 and fBen-Aci002

	fBen-Aci001	**fBen-Aci002**
Accession number	MW056501	MW056502
Family	*Autographiviridae*	*Autographiviridae*
Genus	*Friunavirus*	*Friunavirus*
Genome size (bp)	40,535	41,953
GC content (%)	39.24	39.22
Predicted genes	50	53
Hypothetical proteins	26	27
Hypothetical proteins (%)	52.0	50.9
Length of direct terminal repeats (amino acids)	394	375
tRNA genes	None	None
Bacterial toxin genes	None	None
Antibiotic resistance genes	None	None
Genes indicating temperate lifecycle	None	None
Identity with fBen-Aci001 (%)	100	82.2

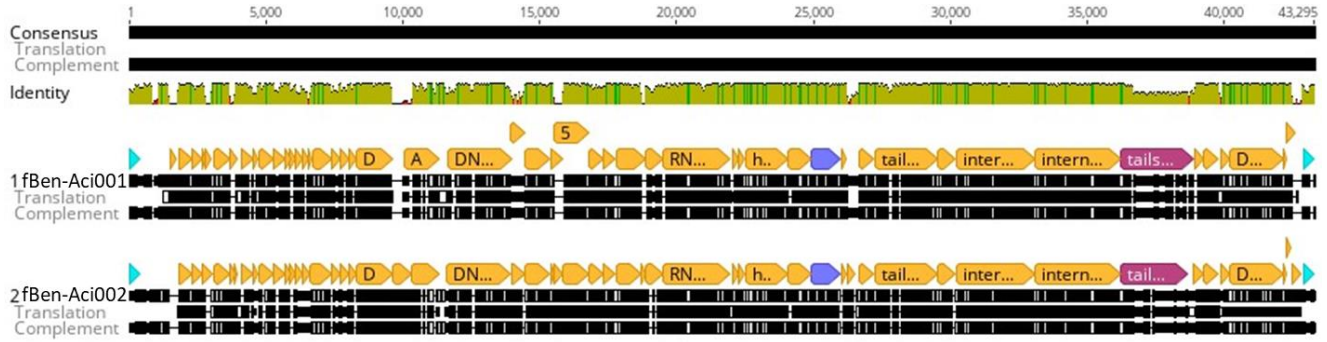

Fig. 2. The genome-wide alignment of the phage genomes fBen-Aci001 and fBen-Aci002. The alignment was made with the Geneious Prime alignment tool. The mean pairwise identity over the genomes can be seen from the height and the color (green, 100% identity; greeny-brown, 30% to 100% identity; red, below 30% identity) of the identity bar. The genes are marked with orange arrows with the exception of the capsid protein genes and tailspike protein genes, which are marked using blue and purple arrows, respectively. The repeat regions are marked with turquoise arrows.

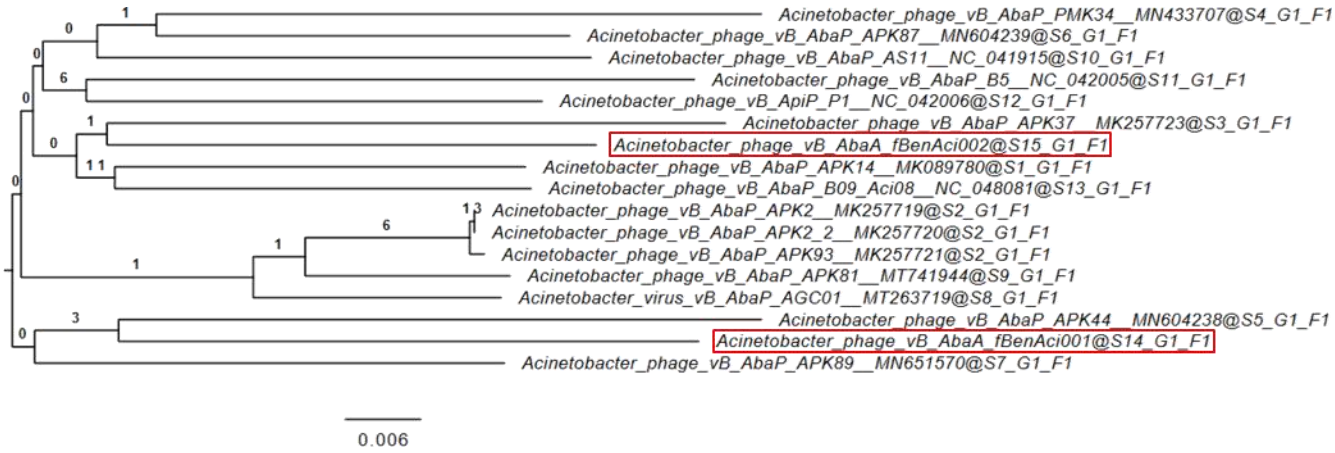

Fig. 3. The phylogenetic tree of the phages fBen-Aci001 and fBen-Aci002 and 15 closest relatives found with the BLASTn searches. The Genome-BLAST Distance Phylogeny tree using the distance formula D0 was constructed using VICTOR. S, species; G, genus; F, family

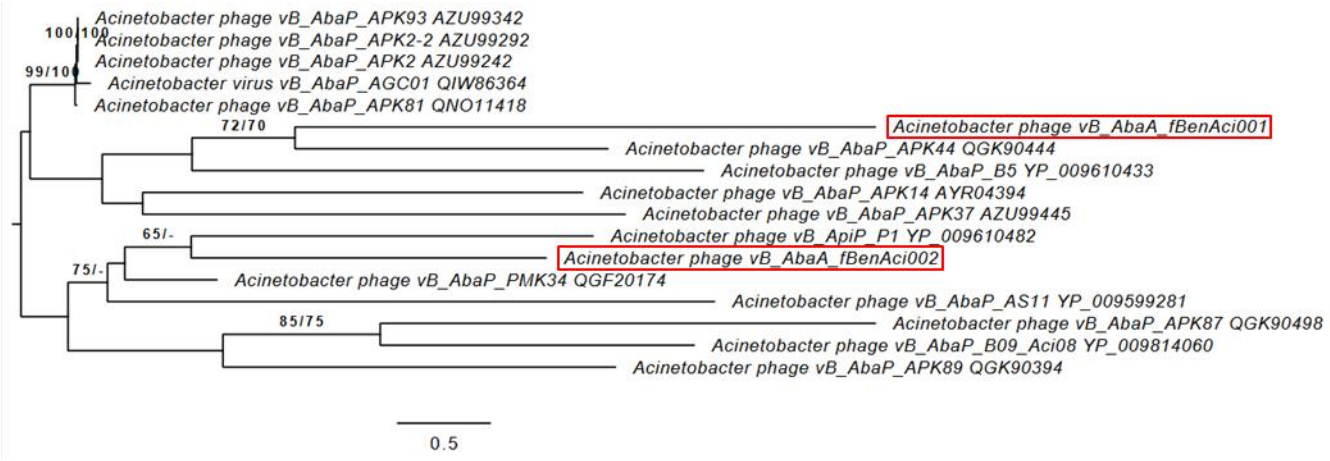

Fig. 4. The phylogenetic tree of the tailspike proteins. The tree was calculated using amino acid sequences of tailspike proteins with the GGDC web server, which uses the DSMZ phylogenomics pipeline.

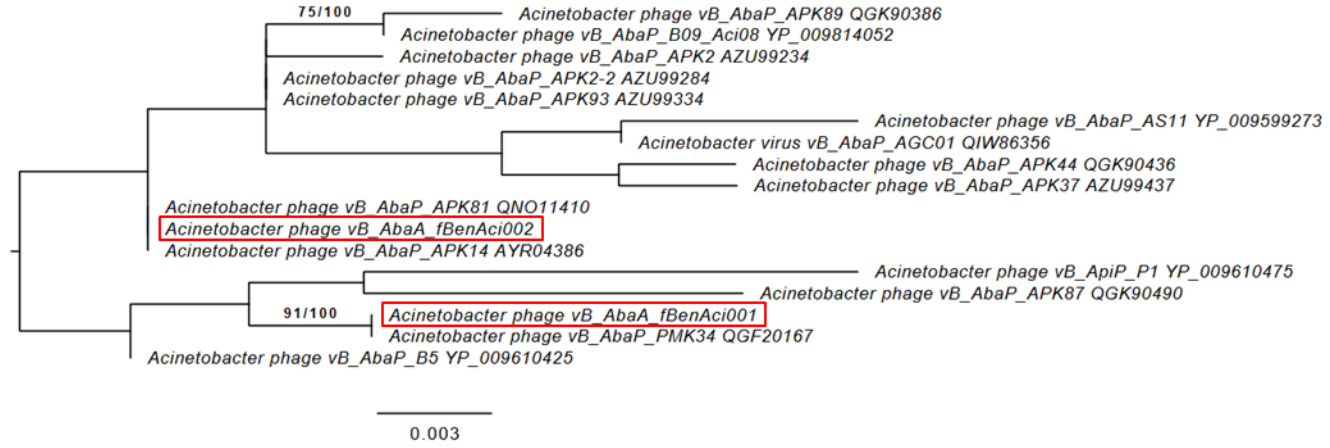

Fig. 5. The phylogenetic tree of the capsid proteins. The tree was calculated using amino acid sequences of capsid proteins with the GGDC web server, which uses the DSMZ phylogenomics pipeline.

DISCUSSION

In this thesis, seven phages infecting clinical *A. baumannii* strains were isolated from the Beninese water samples, and the first two phages that were found, were characterized more in detail. Phages fBen-Aci001 and fBen-Aci002 were identified as belonging to the *Autographiviridae* family and the *Friunavirus* genus. They are separate species, and they are the only phages so far that have been characterized from

their respective species. The sequences of the phages are available in the GenBank database under accession numbers MW056501 (fBen-Aci001) and MW056502 (fBen-Aci002).

Genomic characteristics of the phages

When comparing the genomes of the phages with each other, 82.2% identity were seen. The genomes of the phages fBen-Aci001 and fBen-Aci002 were linear, terminally redundant dsDNA, and approximately 41 kbp in size. These all are characteristics of the *Autographiviridae* family. The other characteristic of the *Autographiviridae* family is that all of the phages encode a large, over 100 kDa, single subunit RNA polymerase, which is responsible for the middle and late transcription (Turner et al., 2019). The RNA polymerase was identified from both of the phages, but the size of the proteins were only 91 kDa. Aragorn did not predict tRNA genes from either of the genomes. That indicates that the phages are dependent on the hosts tRNA molecules.

The GC content of *Friunavirus* phages is typically around 39.3 (Lai et al., 2016; Popova et al., 2017), while the median GC content of *A. baumannii* is 39 (National Center for Biotechnology Information). The GC content of the phages characterized in this thesis were 39.2 that is similar than the GC content of the other phages from the same genus, and it is very close to the host's GC content. As it is typical that the phages and their hosts have similar GC content, this result can be considered as normal and no further studies were required.

The phages fBen-Aci001 and fBen-Aci002 can be used fairly safely in the phage therapy, as no known genes indicating the temperate lifecycle were identified. In addition, there were no antibiotic resistance genes or genes encoding bacterial toxins within the genomes. It is worth of noting that the function of around half of the annotated genes were unknown. This is typically the case when annotating the phage genomes as a lot is still unknown about the phages and their properties. However, other phages from the *Friunavirus* genus has been used successfully in phage therapy trials (Ho et al., 2016; Wu et al., 2018). All this supports the conclusion that the phages characterized in this thesis are safe to use.

Host range

The phages characterized in this thesis had a very narrow host range which is considered to be the typical feature of the *A. baumannii* phages (Huang et al., 2013; Lai et al., 2016; Popova et al., 2017). These phages infected 4 to 9% of the tested *A. baumannii* strains, but no other species of *Acinetobacter*. Phage fBen-Aci001 infected only its isolation host. The capability to infect species from other genera were not tested, but based on the previous studies of the *Friunavirus* phages (Grygorcewicz et al., 2020; Huang et al., 2013; Lin et al., 2010), it would be reasonable to assume that the phages will infect only the members of the *Acinetobacter* genus and more specifically *A. baumannii*.

Based on the previous studies, the *Friunavirus* phages have varying capability to infect *A. baumannii* strains, infecting 3 to 50% of the strains (Grygorcewicz et al., 2020; Huang et al., 2013; Wu et al., 2018). In most of the studies that showed the broad (over 30%) host range, all the strains were isolated from the same hospital (Grygorcewicz et al., 2020; Yuan et al., 2020) or from an otherwise limited area (Wu et al., 2018). Each hospital most likely has one or only few consistent nosocomial strains and these strains are widely spread within the hospital. Therefore, in those hospitals where patients got infected in the hospital itself, the strains isolated from the different patients are typically similar. It is also known that if the phage infects one strain, it most likely infects other strains that are similar.

When testing phage infectivity against strains from variable origins, the phages typically infect under 10% of tested strains (Popova et al., 2017) or just the isolation host (Abdelkader et al., 2020). The strains used in this thesis were isolated mainly in Finland from patients, who most likely got the infections from various foreign locations as *A. baumannii* is not known to spread in Finland. Therefore, phages fBen-Aci001 and fBen-Aci002 infected only 4 to 9% of strains resulting a narrow host range. This reduces the usability of these phages in the phage therapy.

Even if the host ranges were narrow, the phages infected different strains with each other. The main factor influencing the host spectrum of the phage is the compatibility with the receptors on the surface of the target bacterium. This type of phages uses the tailspike proteins to recognize the receptors in the host cell surface. The different structures of the tailspike lead to the identification of different receptors on the surface of the bacterial cells. In this thesis, especially the C-terminal end of the tailspike proteins had low similarity, while the N-terminal end was much more alike. The finding is similar than reported

previously for the *A. baumannii* phages φAB1 and φAB6 (Lai et al., 2016). It is likely that the C-terminal end is used in the host recognition as there is a lot of variation in the structures. The N-terminal part attaches to the other structural proteins, so the structure must be constant in order to fit the parts perfectly together when the phage is assembled.

The phages infecting gram-negative bacteria often use the lipopolysaccharides or the capsular polysaccharides as their receptor. The capsular polysaccharide has been suggested to be the receptor for the *A. baumannii* phages that has depolymerase activity in their tailspike protein. Some members of the *Friunavirus* genus has this property (Popova et al., 2017). There are more than 100 distinct capsule types identified for *A. baumannii* (Popova et al., 2017) and if assuming that one phage can infect only one capsule type, it can be understood why the host spectrum of such phages is often so narrow. However, not all the phages from the same genus had the depolymerase activity in their tailspike protein (Lai et al., 2016). Also, in addition to the receptor recognition, many other factors, such as the prophages or the other resistance mechanisms, might affect the host range. Based on this information the exact reason cannot be concluded, why the phages were infecting the different strains.

Other properties of the phages

The morphologies of the phages were not examined, but it can be assumed that the phage particles are similar to the other members of the *Autographiviridae* family having a small icosahedral head and a short tail.

The plaques of the phages fBen-Aci001 and fBen-Aci002 had a halo, which became wider after prolonged incubation, while the size of the lysis zone did not change. Generally, the halo has been associated with the virion-associated polysaccharide depolymerases, which degrades the capsular polysaccharides of the host (Cornelissen et al., 2011). It is known that some of the phages from the genus has depolymerase activity in their tailspike protein (Lai et al., 2016; Popova et al., 2017). In the earlier studies, the presence of the phages has been observed from the halo zones together with the viable bacteria (Cornelissen et al., 2011; Huang et al., 2013). It has been suggested that when the bacteria enter the stationary growth phase and the phage replication slows down, the tailspikes are still functioning. The phages diffuse away from the lysis zone which increases the halo zone (Cornelissen et al., 2011). It

is also known that *A. baumannii* is more susceptible to endolysins compared to the other gram-negative bacteria, though the reason behind this phenomenon is still unknown (Gerstmans et al., 2018). In this thesis, the reason for the halo and its expansion is most likely similar as described in the other studies, but this was not confirmed.

In this thesis the phylogenetic trees based on the tailspike proteins and the capsid proteins were built, in addition to the one that was built using VICTOR. When comparing the phage genomes with each other, they are mosaic-like having some common features with one phage and other common features with another phage. In addition, there is not a single protein that can be found from every phage, which could be used to construct phylogenetic trees, similar to the 16S RNA of bacteria. For that reason, the phylogenetic trees of the phages are normally constructed based on the genome-wide information and the whole genome sequences. As VICTOR is optimized for the phage classification and the tree building, it is more reliable to construct the phylogenetic trees for phages with it than with some other tools. The results in this thesis demonstrated that the phage genomes have the mosaic-like features, because the trees built based on the single proteins were completely different from each other and different than the one built with VICTOR using the whole-genome sequences.

Properties of the host strain of fBen-Aci001

A. baumannii strain #5542, which was the isolation host of fBen-Aci001 needed to be freshly cultured (less than two days) in order for the phage fBen-Aci001 to infect the strain efficiently. The host strains of fBen-Aci002 were susceptible for the phage infection after the longer storing time. This happened when the bacteria were grown without the phage and therefore, the reason was not the formation of the phage resistant cells.

The structures in the surface of the bacteria is known to affect the phage sensitivity. In addition, the biofilm formation and the antibiotic resistance can also impact the phage sensitivity. According to the earlier study, it is possible that the metabolic switch or the phase variation takes a place in the subpopulation of the bacteria and as a result the surface structures might change. The phase variation was reported to be strain specific and affect for example the biofilm formation and the antibiotic resistance (Ahmad et al., 2019). Therefore, it is reasonable to assume that the phage sensitivity in this

thesis was affected due to the phase variation. However, the phenomenon needs to be studied further to fully understand why the phage sensitivity of the strain changed when stored in the culture plate. This property of the strain #5542 was taken into account when conducting the experiments.

Future prospects

In this thesis it was rather difficult to make comprehensive conclusions as so much about the phages is still unknown. Further studies of the tailspike proteins could provide a lot of useful information about the properties of the phages that are still uncertain. If the enzymatic activity of the tailspikes were known, more conclusions could be made about the expansion of the halo zones. This information could also help to understand differences in the host range of the phages. In addition, if the receptor that the phage fBen-Aci001 uses were known, it would help to make conclusions about the phage sensitivity of the host #5542.

The Bioscreen conditions optimized in this thesis can be beneficial in the future when these phages are screened towards new patient isolates or in the isolation process of the new *Acinetobacter* phages. The conditions can be used to screen new phages at least for *A. baumannii* and *A. pittii*, but not necessarily for all the *Acinetobacter* species. During the host range screening, all *A. baumannii* and *A. pittii* strains grew well in the optimized Bioscreen conditions, but some strains of *A. junii* and *A. ursingii* grew slower under these conditions, so the conditions are not optimal for their growth. However, most of the nosocomial infections related to the *Acinetobacter* species are caused by *A. baumannii* while the infections attributed to *A. junii* and *A. ursingii* are rare, and they are usually more susceptible to antibiotics (DeLong et al., 2014). Therefore, the fact that the conditions are not optimal for *A. junii* and *A. ursingii* is not a major concern.

For the rest of the five phages, one has been sequenced since and it was similar to the phages described in this thesis. The sequence of fBen-Aci003 has been submitted to GenBank under the accession number MW056503. Other four phages have shown some evidence of temperate lifecycle, which makes them unsuitable for phage therapy. An indication of this is that the plaques of the phages are small and turbid, and the titer of the phage lysates remains low (10^5 pfu ml^{-1}). Also, the host of these phages, *A. baumannii* #6898, has acted as a host for the other temperate phages previously isolated in the Skurnik lab (Badawy

et al., 2020). This might indicate that the strain #6898 lacks some resistant mechanisms or prophage, which makes it sensitive to the temperate phage infections.

Conclusions

In conclusion, the phages fBen-Aci001 and fBen-Aci002 appeared to be suitable to be used in phage therapy, as no signs of lysogeny or genes encoding antibiotic-resistance or bacterial toxins were identified. All the lytic phages found in this thesis were isolated from the hospital wastewater and several phages were isolated in a relatively short time using relatively small number of strains. Therefore, wastewaters from Beninese hospitals seems to be a good source for the *A. baumannii* phages. Typically, phages have more potential to infect strains that are isolated from the same origin, so it was very promising to find phages from the Beninese water samples that were infecting the MDR strains isolated from Finnish patients.

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SUPLEMENTARY MATERIAL

Table S1: The *Acinetobacter* strains used in the thesis

Table S2. The cocktails made from the Beninese water samples

Table S3. The GeneBank accession numbers and the protein ids of the phages used in the phylogenetic trees

Table S4. The detailed results from the host range screening

Fig. S1. An example of the intermediate and the positive reactions of the Bioscreen results

Fig. S2. An example of the growth curves of *A. baumannii* and *A. pittii*

Fig. S3. The double layer overlay plates

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Table S1: The *Acinetobacter* strains used in the thesis

Species	**Storage number in Skurnik lab collection #**	**Resistance**	**Origin**	**Growth medium**	**Reference**
Acinetobacter baumannii	5542*	MDR	Not known	LB	Huslab
	5568*		Blood	LB	Huslab
	5570*		Blood	LB	Huslab
	5596*	MDR	Bronchus	LB	Huslab
	5597*	MDR	Rectum	LB	Huslab
	5706*		Tooth removal hole	LB	Huslab
	5707*	MDR	Hip surgical wound	LB	Huslab
	5729*	MDR	Rectum	LB	Huslab
	5730*	MDR	Rectum	LB	Huslab
	5731*		Middle ear	LB	Huslab
	5907*		Trachea	LB	Huslab
	5910*	MDR	Skin	LB	Huslab
	5911*	MDR	Rectal mucus	LB	Huslab
	5919*	MDR	Rectal mucus	LB	Huslab
	5920*	MDR	Not known	LB	Huslab
	5923*	MDR	Wound pus	LB	Huslab
	5924*		Blood	LB	Huslab
	5933*		Urine	LB	Huslab
	5934*		Not known	LB	Huslab
	6594*	MDR	Bronchus	LB	Huslab
	6597*	MDR	Not known	LB	DSM 106838
	6898*	MDR	Sacral chronic wound	LB	Huslab
	6906*		Trachea	LB	Germany

Acinetobacter calcoaceticus	5922		Blood	LB	Huslab
	5935		Not known	LB	Huslab
Acinetobacter junii	5567	MDR	Stool	BHI	Huslab
Acinetobacter lwoffii	5912		Blood	LB	Huslab
	5921		Blood	LB	Huslab
	5928		Blood	LB	Huslab
Acinetobacter nosocomialis	5901		Foot surgical wound	LB	Huslab
	5904		Peritoneal abscess	LB	Huslab
	5929		Urine	LB	Huslab
Acinetobacter pittii	5565		Blood	LB	Huslab
	5566		Blood	LB	Huslab
	5573		Blood	LB	Huslab
	5673		Blood	LB	Huslab
	5674		Wound pus	LB	Huslab
	5728		Wound pus	LB	Huslab
	5902		Not known	LB	Huslab
	5903		Not known	LB	Huslab
	5905		Leg wound pus	LB	Huslab
	5906		Urine	LB	Huslab
	5908		Cerebrospinal fluid	LB	Huslab
	5909		Blood	LB	Huslab
	5914		Blood	LB	Huslab
	5917		Blood	LB	Huslab
	5918		Blood	LB	Huslab
	5925		Blood	LB	Huslab
	5930		Urine	LB	Huslab
	5931		Urine	LB	Huslab

Acinetobacter radioresistens	5915	Blood	LB	Huslab
	5916	Blood	LB	Huslab
Acinetobacter ursingii	5569	Not known	BHI	Huslab
	5572	Not known	BHI	Huslab
	5913	Blood	BHI	Huslab
	5927	Skin	LB	Huslab
	5932	Urine	BHI	Huslab

*, used as an enrichment host; MDR, strain is confirmed to be multi drug resistant; LB, Luria broth; BHI, brain heart infusion broth

Table S2. The cocktails made from the Beninese water samples

Cocktail	**Sample**	**Type of sample**	**Site of sample**
Hospital I	BH01	Hospital wastewater	Neonatology septic tank
	BH03	Hospital wastewater	Surgery room septic tank
	BH05	Hospital wastewater	Not known
	BH06	Hospital wastewater	Mortuary ward septic tank
	BH07	Hospital wastewater	Laundry room, open pit sump
	BH08	Hospital sludge	Hospital main sump
	BH09	Hospital wastewater	Surgery room of demanding operation sump
	BH11	Hospital wastewater	Open well next to the hospital
	BH12	Hospital wastewater	Tap water
Hospital II	BH27	Hospital wastewater	Central septic tank for intensive care unit
	BH28	Hospital wastewater	Puddle
	BH29	Hospital wastewater	Surgery unit open tank
	BH30	Hospital wastewater	Surgery unit 1st peripheral septic tank
	BH31	Hospital wastewater	Surgery unit 2nd peripheral septic tank
	BH33	Hospital wastewater	Surgery unit 3rd peripheral septic tank
	BH 34	Hospital wastewater	Surgery unit central septic tank
	BH35	Hospital wastewater	Small surgery unit septic tank
	BH36	Hospital wastewater	Maternity ward septic tank
	BH37	Hospital wastewater	Central septic tank

	BH38	Hospital wastewater	Central septic tank, surface layer
	BH39	Hospital wastewater	Septic tank collecting waters from all laboratories
Hospital III	BH44	Hospital wastewater	Central septic tank from 10 cm depth
	BH45	Hospital wastewater	Central septic tank from 1 m depth
	BH46	Hospital wastewater	Pediatric clinic septic tank from 20 cm depth
	BH47	Hospital wastewater	Septic tank next to vaccination room
	BH48	Hospital wastewater	Water surrounding the surgery room septic tank
	BH49	Hospital wastewater	Maternity clinic surgery room septic tank
	BH50	Hospital wastewater	Toilet water
	BH51	Hospital wastewater	Septic tank
	BH58	Hospital wastewater	Hospital septic tank
	BH59	Hospital wastewater	Hospital septic tank
	BH60	Hospital wastewater	Hospital septic tank
	BH61	Hospital wastewater	Hospital septic tank
	BH62	Hospital wastewater	Hospital septic tank
Environment I	BSE74	Environment	Zoun river
	BSE 94	Environment	Winery
	BSE 93	Environment	Drinking water
	BSE 89	Environment	Not known
	BSE 88	Environment	Not known
	BSE 79	Environment	Bedrock river
	BSE 61	Environment	Well water
	BSE 60	Environment	Public well
	BSE 59	Environment	Public well
	BSE 44	Environment	Well water
	BSE 100	Environment	Caiman river
	BCE 1	Environment	Biosecurity farm
	BCE 8	Environment	Farm site

Table S3. The GeneBank accession numbers and the protein ids of the phages used in the phylogenetic trees

Phage	Accession number	Protein id of tailspike protein	Protein id of capsid protein	Notes
Acinetobacter phage vB_AbaP_APK2-2	MK257720.1	AZU99292.1	AZU99284.1	
Acinetobacter virus vB_AbaP_AGC01	MT263719.1	QIW86364.1	QIW86356.1	Tailspike and capsid protein reannotated
Acinetobacter phage vB_AbaP_B09_Aci08	NC_048081.1	YP_009814060.1	YP_009814052.1	Tailspike and capsid protein reannotated
Acinetobacter phage vB_AbaP_APK14	MK089780.1	AYR04394.1	AYR04386.1	
Acinetobacter phage vB_AbaP_APK37	MK257723.1	AZU99445.1	AZU99437.1	
Acinetobacter phage vB_AbaP_APK89	MN651570.1	QGK90394.1	QGK90386.1	
Acinetobacter phage vB_AbaP_APK2	MK257719.1	AZU99242.1	AZU99234.1	
Acinetobacter phage vB_AbaP_APK93	MK257721.1	AZU99342.1	AZU99334.1	
Acinetobacter phage vB_AbaP_PMK34	MN433707.1	QGF20174.1	QGF20167.1	Tailspike annotated as tail fiber protein
Acinetobacter phage vB_AbaP_APK44	MN604238.1	QGK90444.1	QGK90436.1	
Acinetobacter phage vB_AbaP_APK81	MT741944.1	QNO11418.1	QNO11410.1	
Acinetobacter phage vB_AbaP_AS11	NC_041915.1	YP_009599281.1	YP_009599273.1	
Acinetobacter phage vB_ApiP_P1	NC_042006.1	YP_009610482.1	YP_009610475.1	Tailspike named as tail fiber protein

Acinetobacter phage vB_AbaP_B5	NC_042005.1	YP_009610433.1	YP_009610425.1	Tailspike named as tail fiber protein
Acinetobacter phage vB_AbaP_APK87	MN604239.1	QGK90498.1	QGK90490.1	
Acinetobacter phage vB_AbaA_fBenAci001	MW056501.1	QOV07748.1	QOV07741.1	
Acinetobacter phage vB_AbaA_fBenAci002	MW056502.1	QOV07800.1	QOV07792.1	

BLASTn search made 2nd of Oct 2020. Acinetobacter phage vB_AbaA_fBenAci001 and Acinetobacter phage vB_AbaA_fBenAci002 were added to the table after sequences became public on 4th of Nov 2020. The protein ids for the tailspike protein and the capsid protein were searched from the whole genome sequences. For two of the phages the correct proteins were reannotated based on the protein sequence homology.

Table S4. The detailed results from the host range screening

Storage number in Skurnik lab collection #		**fBen-Aci001**	**fBen-Aci002**
A. baumannii	5542	+*	-
	5568	-	-
	5570	-	-
	5596	-	-
	5597	-	-
	5706	-	-
	5707	-	+*
	5729	-	-
	5730	-	-
	5731	-	-
	5907	-	-
	5910	-	-
	5911	-	-
	5919	-	-
	5920	-	+

	5923	-	-
	5924	-	-
	5933	-	-
	5934	-	-
	6594	-	-
	6597	-	-
	6898	-	-
	6906	-	-
A. calcoaceticus	5922	-	-
	5935	-	-
A. junii	5567	-	-
A. lwoffii	5912	-	-
	5921	-	-
	5928	-	-
A. nosocomialis	5901	-	-
	5904	-	-
	5929	-	-
A. pittii	5565	-	-
	5566	-	-
	5573	-	-
	5673	-	-
	5674	-	-
	5728	-	-
	5902	-	-
	5903	-	-
	5905	-	-
	5906	-	-
	5908	-	-
	5909	-	-
	5914	-	-
	5917	-	-

	5918	-	-
	5925	-	-
	5930	-	-
	5931	-	-
A. radioresistens	5915	-	-
	5916	-	-
A. ursingii	5569	-	-
	5572	-	-
	5913	-	-
	5927	-	-
	5932	-	-

The results from the host range screening after confirming the results with the drop test method.
+, infection; +*, isolation host; -, no infection

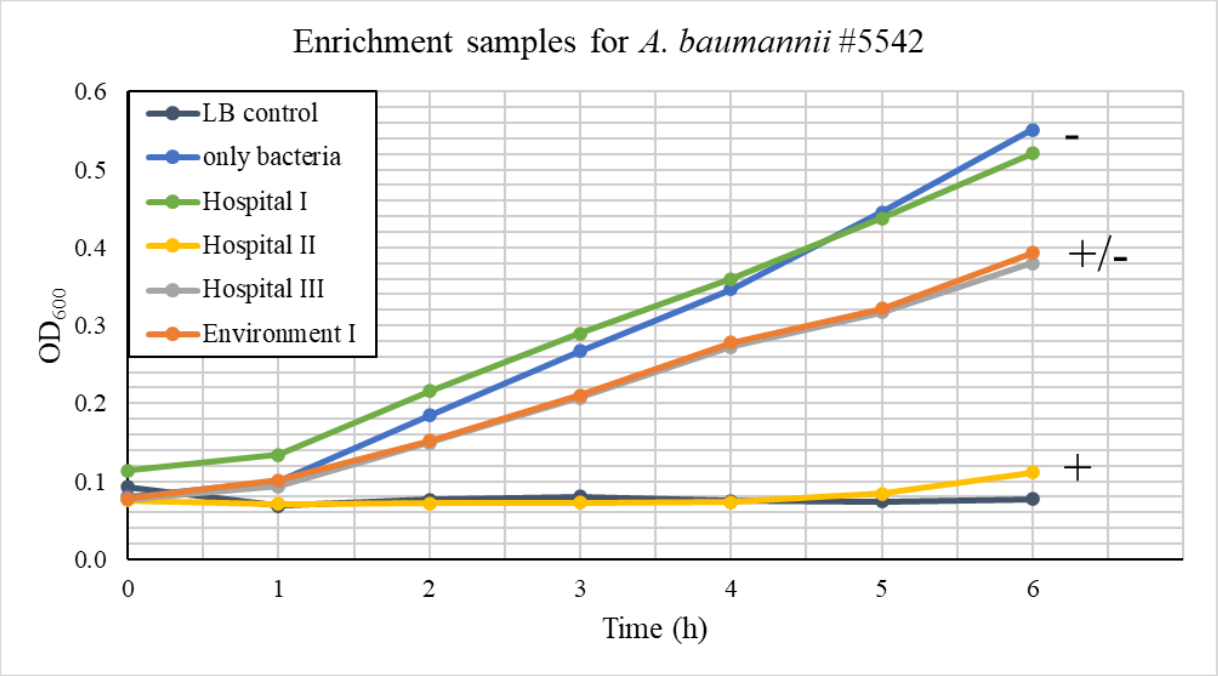

Fig. S1. An example of the intermediate and the positive reactions of the Bioscreen results. The growth curves are drawn based on the arithmetic mean of the parallel wells.
+, positive; +/-, intermediate; -, negative

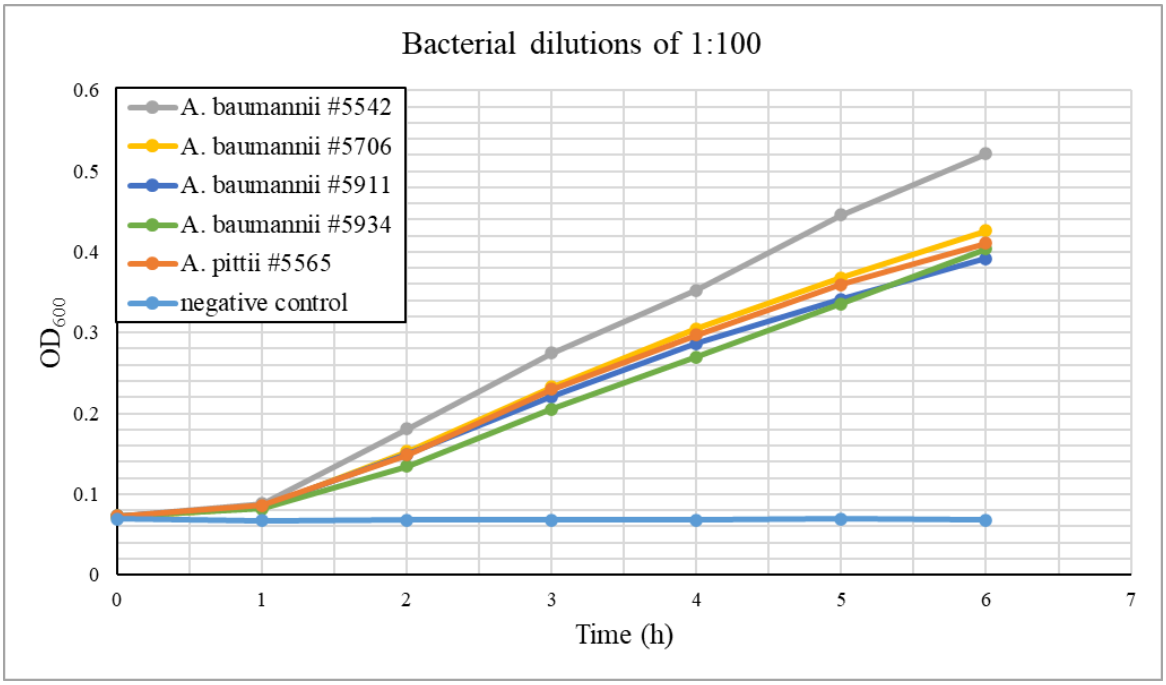

Fig. S2. An example of the growth curves of *A. baumannii* and *A.pittii*. Dilution of 1:100 made from the overnight cultures was used to demonstrate that both species grew in similar rate in the preliminary Bioscreen tests. The curves are drawn based on the arithmetic mean of the parallel wells.

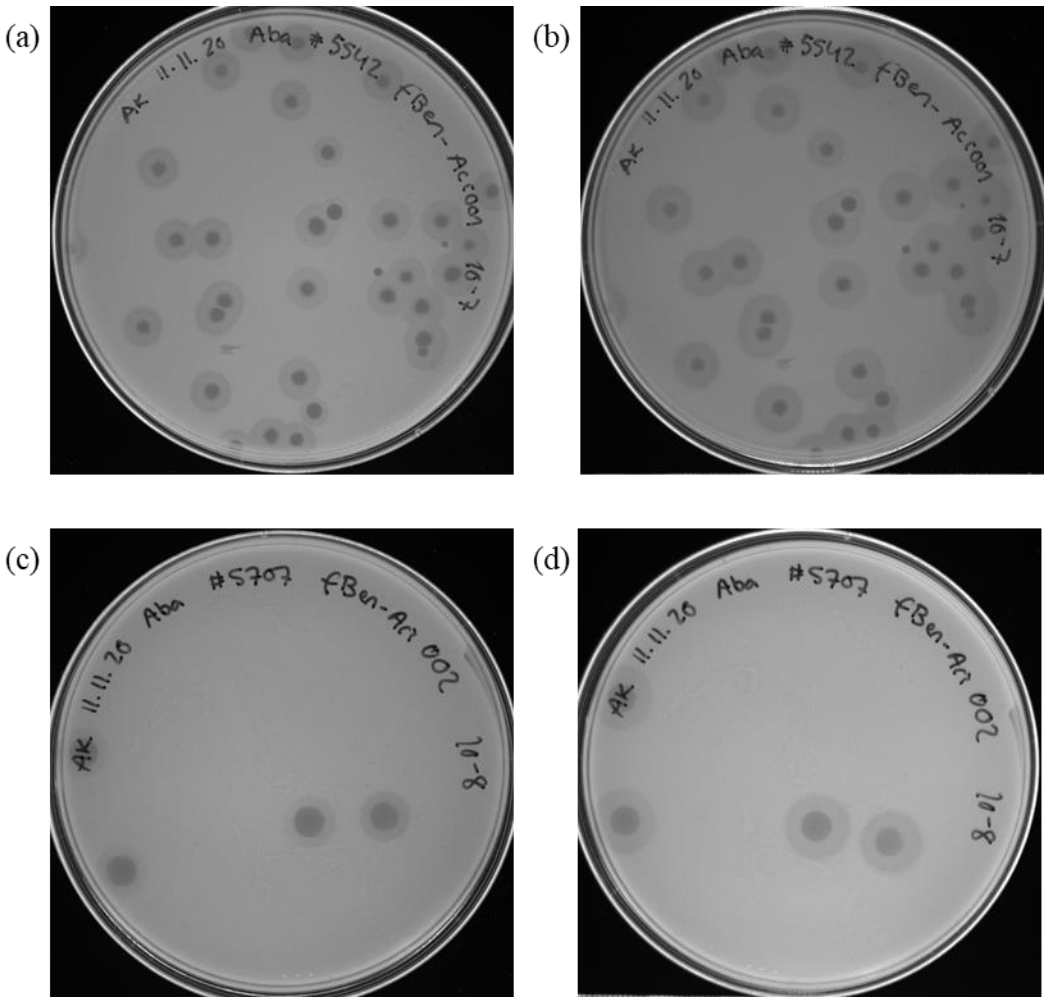

Fig S3. The double layer overlay plates. (a) The phage fBen_Aci001 after 16 hours of incubation. (b) The phage fBen_Aci001 after 24 hours of incubation. (c) The phage fBen_Aci002 after 16 hours of incubation. (d) The phage fBen_Aci002 after 24 hours of incubation.