Isolation, Purification, and Characterization of Mycobacteriophage HerbSipe

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The goal of this project was to isolate and purify a novel bacteriophage, HerbSipe, from the environment to a point that its genetic material can be sequenced. The research was undertaken as a part of the National Genomics Research Initiative, a project for the Howard Hughes Medical Institute (HHMI).

INTRODUCTION

Bacteriophages that infect mycobacteria were examined since the properties of the bacterium that causes tuberculosis, *Mycobacterium tuberculosis*, can be better understood through bacteriophage studies. Properties such as antibiotic resistance, for example, can be observed. An example of a technique that could do this is engineering a bacteriophage to carry a gene for a protein that fluoresces. The bacteriophage can infect bacteria growing in the presence of antibiotics. Bacteria that are not resistant are killed off, but bacteria that are resistant live and fluoresce because of the bacteriophage. This is clinically usefully because tuberculosis cases with drug-resistant strains could possibly be identified more quickly and cheaply. Cultures of the bacteria causing the disease can be isolated and exposed to the bacteriophage while growing in the presence of antibiotics.[2] This project, however, was concerned with isolating and purifying a novel bacteriophage from the environment. There are an estimated 10^31 different bacteriophages worldwide. Mycobacteriophage HerbSipe was isolated from soil samples taken from the campus of Hampden-Sydney College in Hampden-Sydney, Virginia.

MATERIALS AND METHODS

Collection of Soil Sample A 50mL conical tube is filled with soil from the location of choice. GPS coordinates of the location are recorded using the Android app GPS Test (version 1.2.4) and weather conditions are determined using http://www.weather.com. Every attempt at soil sample collection will be drawn from a different location with different GPS coordinates.

Direct Plating Half of the soil sample is flooded with Phage Buffer (PB), vortexed, and allowed to sit for 20 minutes. 4 infections are made for this procedure: the soil sample, D29 (a known and isolated bacteriophage), MW (a known and isolated bacteriophage), and PB alone. A phage filtrate is made with the soil sample using a syringe and filter media. 0.5mL of *Mycobaterium smegmatis* is mixed with 50µL of my sample, MW, and PB in 3 separate test tubes. 0.5mL of *M. smegmatis* is mixed with 10µL of D29 in a separate test tube. The tubes are incubated for 15-30 minutes. 1.0mL $CaCl_2$ is added to a premade bottle of top agar (TA). 4.5mL of TA is added to each of the 4 test tubes. Tubes are mixed thoroughly and the solutions in each are plated on 4 separate agar plates. The plates are inverted when top agar solidifies and they are incubated at 37°C for 24 hours. The presence of phage is identified by plaques (clearings) on the lawn of bacteria on each plate.

Spot Testing to Verify Plaques as Bacteriophage A layer of TA (with 1.0mL $CaCl_2$ and 0.5mL *M. smegmatis*) is placed on an agar plate with a preformed 3x3 grid. 5µL samples from the direct plating procedure and from the D29 and MW lysates are made up by placing 100µL PB into microcentrifuge tubes and then swabbing a plaque with a pipette tip on the direct plate. The tip (now with bacteriophage) is placed into the microcentrifuge tube with 100µL PB. A 5µL sample is taken from this tube and placed on top of a grid box on the agar plate. This is done for however many suspected plaques are present on the direct plating plate. The plates are incubated for 24 hours. Confirmation of bacteriophage presence is given by plaques forming on the designated grid space.

Enrichment, Dilution, and Plaque Screening The other half of the soil sample is placed into a disposable flask with 40mL of deionized water, 5mL 10x 7H9/Glycerol Broth, 5mL AD supplement, 0.5mL 100mM $CaCl_2$, and 5mL *M. smegmatis* solution. The flask is thoroughly vortexed and is placed into incubating shaker (37°C) for 24 hours. The contents of the flask are transferred to a 50mL conical tube and spun at 3000rpm for 10 minutes. Using a syringe with sterile filter media, the sample is filtered into a sterile conical tube to create phage filtrate. The filtrate is diluted into microcentrifuge tubes containing PB. 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions are made in microcentrifuge tubes. The same is done for a D29 and MW lysate. Test

tubes with 0.5mL *M. smegmatis* are infected with 50µL of each dilution tube (one is infected with PB alone). Each test tube is vortexed and allowed to incubate for 15-30 minutes. 4.5mL of TA (with $CaCl_2$) is added each test tube and mixed well. The contents of each test tube are then plated on labeled agar plates. The plates are allowed to dry and inverted. The plates are placed in an incubator (37°C) for 24 hours. A spot test is done on any presenting plaques from these plates.

Plaque Streak Protocol to Purify the Phage A medium-sized pipette tip is removed. A control agar plate is opened, and the pipette tip is used to gently streak back and forth across the top third of the agar plate without lifting from the agar. With a new pipette tip, beginning in the area streaked in the previous step, streak the adjacent unstreaked portion (≈1/3) of the agar making sure to only overlap the previous streaks with a few strokes. A new pipette tip is obtained and the last unstreaked portion of the agar plate is streaked with the overlap to the previously streaked portion being only by a few strokes. A new pipette tip is swabbed in a plaque from a previous plate (spot test or plaque screening) and streaked in the same fashion on a second agar plate. Two new pipette tips are then used to sequentially streak the remaining thirds of the second plate (ensuring not to reswab the plaque from the spot test/plaque screening plate). The least dilute area on both agar plates is identified by a marking. For both plates, 4.5mL of TA added to a 0.5mL aliquot of *M. smegmatis* is carefully dispensed slowly on to the most dilute point of the streaked plate. The mixture is allowed to spread across the plate by gently tapping or tipping. The TA is allowed to harden and both plates are inverted and placed in an incubator (37°C) for 24 hours. This procedure is repeated for individual plaques visible on plaque streak protocol plate to further purify the phage.

Phage-Titer Assay to Determine Concentration of Phage Particles in Solution 100µL of PB is placed into a microcentrifuge tube. A sterile pipette tip is used to touch the center of a purative plaque on the 10^{-2} plaque screening plate (due to its visible presence of pure plaques). This tip is then placed into the microcentrifuge tube containing 100µL PB, stirred around, removed, and disposed of (10^{0} phage sample). The microcentrifuge tube is then vortexed. 4 microcentrifuge tubes are arranged in a rack and labeled -1, -2, -3, and -4 corresponding to the phage dilution that will go in each. 90µL of PB is added to each of these microcentrifuge tubes. 10µL of the 10^{0} phage sample is added to the tube labeled -1, and this tube is vortexed. Successive dilutions are continued until the -4 tube is reached. Culture tubes containing 0.5mL *M. smegmatis* are labeled with the dilution factor. The appropriately labeled culture tubes are infected with 10µL of each dilution. A negative control culture tube is infected with 10µL of PB. The phage is allowed to infect the bacteria for 30 minutes. 6 agar plates are labeled with the sample-identifying data. 4.5mL of TA (+$CaCl_2$) is added to a culture tube. The culture tube is mixed by gently twisting and poured onto the corresponding agar plate. The plate is gently swirled. This is done for all corresponding culture tubes and plates. The plates are allowed to sit for at least 20 minutes. They are inverted and placed in 37° incubator for 24 hours. The resulting plaques are counted on a plate with a range of 20-200 plaques. The titer is calculated according to the formula (where *pfu* ≡ plaque forming units):

Formula 1

$$Titer\ \left(\frac{pfu}{mL}\right) = \frac{pfu}{\#mL} \times \frac{1000\mu L}{mL} \times Dilution\ Factor$$

Final Plaque Purification to Purify Higher Numbers of Filter-Sterilized Phage A plate is chosen from the most recent titer on which there is a single phage population and the bacterial lawn is nearly cleared. 8mL of PB is added to this plate, and the plate is swirled gently. The plate is allowed to site for 2-4 hours at room temperature or overnight at 4°C. A pipette is used to aliquot the newly created phage solution from this plate and into a 0.22µm filter syringe. The phage solution is filtered into a sterile 15mL centrifuge tube. Aspirate 4.5mL of TA into a pipette and add it to a 0.5mL aliquot of *M. smegmatis* in a culture tube. The culture tube is twisted gently, and the TA-bacteria mixture is added to an agar plate labeled with a grid designating phage dilutions from 10^{-1} to 10^{-10} and a negative (PB only) control. The plate is gently swirled and the TA is allowed to harden for 10 minutes. 90µL of PB is added 10 microcentrifuge tubes labeled from -1 to -10. 10µL of the undiluted phage sample is added to the -1 tube and vortexed. Successive dilutions are continued until the -10 tube is reached. 5µL of each phage dilution sample (and the negative control) is transferred to the corresponding blocks on the agar plate grid. Droplets are allowed to soak into the agar until no apparent liquid remains on the agar. The plate is inverted and placed into the 37°C incubator for 24 hours. Plaques are counted on the grid square plate that has between 5-50 plaques. Formula 1 is used to obtain a titer from this number. Full plates of each dilution can also be made to assist determining a titer.

The approximate number of pfu that will be required for complete bacterial lysis on an agar plate is calculated according to the following formula:

Formula 2

$$pfu_{\max web} = \frac{Area\ of\ Agar\ Plate\ (mm^2)}{Area\ of\ a\ Plaque\ (mm^2)}$$

The dilution needed for an infection that will yield an optimal webbing pattern on an agar plate is calculated according to the following formula:

Formula 3

$$Volume\ (mL)_{phage\ stock} = \frac{pfu_{\max web}}{pfu/mL_{phage\ stock}}$$

A web pattern is defined as an almost complete clearing of bacterial cells on an agar plate but enough to still tell the presence of plaques. The web is the result of the remaining cells around the circular plaques. The range of pfu being tested in the empirical assay is then calculated. The volume calculated in formula 3 is multiplied by 2 and 4 and divided by 2 and 4 to give a five plate range including the original calculated volume. Microcentrifuge tubes are arranged on a rack and labeled according to dilution. Serial, 10-fold dilutions of the phage stock solution are carried out however dilute the calculated range calls for. 5 culture tubes are infected with the specific volumes and dilutions as indicated from the calculated range. 1 culture tube is infected with 50µL PB as a negative control. The phage are allowed to infect the bacteria at room temperature for 30 minutes. 4.5mL of TA (+$CaCl_2$) is individually added to each culture tube. The tubes are twisted and poured onto 6 agar agar plates labeled with the phage sample volume and dilution. The TA is allowed to harden and the plates are incubated at 37° for 24 hours. The volume-dilution combination that yields the best web pattern of phage infected *M. smegmatis* growth is determined. This combination is plated on 10 separate agar plates each with 4.5mL TA (+$CaCl_2$) and 0.5mL *M. smegmatis*. These 10 plates are incubated at 37°C for 24 hours. If no web pattern present, the procedure is repeated with modified combinations.

Harvesting the Bacteriophage Lysate for DNA Characterization 8mL of PB is added to 10 web plates and is made to cover the entire surface of the agar. The plates are allowed to sit for 2-4 hours at room temperature or overnight at 4°C. The PB (now containing phage) is removed from the agar plates and placed into 50mL conical tubes. These tubes balanced and centrifuged at 2500×g for 20 minutes the pellet cell debris. The supernatant is transferred into a 50mL filter sterilization unit, and a vacuum pump is used to filter sterilize the lysate. A titer is calculated for the lysate by performing serial dilutions and infecting 10 agar plates of *M. smegmatis* (0.5mL of cells in 4.5mL of TA) with 50µL of each dilution. The tubes containing the filter-sterilized lysate are stored at 4°C.

Isolate and Purify Phage Genomic DNA 10mL of phage lysate is transferred to an Oak Ridge Tube. 40µL nuclease mix is added, and the tube is mixed thoroughly. The tube is incubated for 30 minutes at 37°C. The tube is allowed to sit at room temperature for an hour. 4mL of phage precipitation solution is added to the nuclease-treated lysate, and the tube is mixed thoroughly by inversion. The tube is incubated overnight at 4°C and then centrifuged at 4000×g for 40 minutes. The supernatant is decanted, and the tube is inverted over a paper towel for 2-3 minutes to drain the excess liquid from the pellet. 0.5mL of ddH_2O is added to the tube and the pellet is resuspended by repeatedly pipetting up and down. The tube is allowed to sit at room temperature for 5-10 minutes. 2mL of warmed (37°C) DNA clean-up resin is added to the tube. The phage particles are uncoated by gently pipetting up and down. A DNA isolation column provided with a Promega Wizard DNA Clean-up System is attached to a syringe. 1.25mL water-resin-phage-genomic-DNA solution is added to the syringe using a pipette. The solution is pushed through the column using the syringe plunger and is removed from the syringe. The plunger is then removed from the syringe and the column reattached. 2mL of 80% isopropanol is added to the syringe and pushed through the column with the plunger. This elution procedure is repeated with the remaining water-resin-phage-genomic-DNA solution in the tube with a second syringe and column. The columns are removed from their syringes and are centrifuged (in microcentrifuge tubes) for 5 minutes at maximum speed to remove isopropanol. The columns are transferred to clean microcentrifuge tubes and are centrifuged for 1 minute at maximum speed to remove any excess alcohol. The columns are transferred to clean microcentrifuge tubes. 50µL of 80°C TE buffer is added to the resin in the column, and TE is allowed to sit on the column for 30-60 seconds to dissolve the DNA. The columns are centrifuged for 1 minute to elute the purified phage-genomic DNA. The DNA samples are combined into one tube. The concentration of the DNA is determined using an EPOCH Spectrophotometer from BioTech. The DNA is stored at 4°C.

Purification of Genomic DNA by Ethanol Precipitation to Obtain Higher DNA Concentrations All microcentrifuge tubes containing DNA sample are combined into a single microcentrifuge tube. Double the volume of this

sample of cold 100% ethanol is added to this tube. 1/10 of the original sample volume of 3M NaOAc is added to the tube. The tube is mixed by quickly inverting several times. The tube is placed at -80°C overnight to facilitate precipitation. The tube is spun at 13-14k rpm in a tabletop centrifuge for 10 minutes. The supernatant liquid is removed from the tube by pouring, pipetting, or vacuuming. 500µL of 70% ethanol is added to the tube. The pipette is aimed just above the pellet to attempt to dislodge it. The tube is inverted several times to thoroughly wash. The tube is spun at 13-14k rpm for 5 minutes. The supernatant liquid is removed. The pellet is air dried just until the ethanol is no longer visible but the pellet does not appear dry (5-10 minutes). The appropriate amount of ddH_2O is added to the tube to fully resuspend the pellet. The concentration of DNA is determined using an EPOCH Spectrometer from BioTech. The DNA is stored at 4°C.

Restrict and Analyze Phage-Genomic DNA The DNA sample is mixed by flicking the closed tube, which is incubated at 65°C for 10 minutes. Afterwards, the tube is immediately placed on ice and in a centrifuge for a quick spin (less than 1 minute). The reactions are set up as shown in Table 1.

Tables 1. DNA Restriction Reactions

	1	2	3	4	5	6
10X Reaction Buffer	2µL	2µL	2µL	2µL	2µL	2µL
Phage Genomic DNA	0.5µg	0.5µg	0.5µg	0.5µg	0.5µg	0.5µg
10X BSA	2µL	2µL	2µL	2µL	2µL	2µL
BamH1	-	10U	-	-	-	-
Cla1	-	-	10U	-	-	-
EcoR1	-	-	-	10U	-	-
HaeIII	-	-	-	-	10U	-
HindIII	-	-	-	-	-	10U
ddH_2O	To 20µL	To 20µL	To 20µL	To 20µL	To 20µL	To 20µL

Each tube is mixed gently but well by flicking, and the tubes are quick spun and incubated in a 37°C water bath for at least 2 hours. The tubes are quick spun and placed on ice.

Once the gel apparatus is set up, 0.8% (weight/volume) agarose gel is prepared in an amount enough to fill the gel former. The appropriate mass of agarose powder is transferred to an Erlenmeyer flask where an appropriate volume of 1X TBE buffer is added to the powder, and the mixture is swirled to mix. The mixture is heated in the microwave until it boils (1-2 minutes). The flask is removed from the microwave and swirled to mix. The solution should be clear of clumps of agarose; otherwise, the flask is microwaved again. The volume of the solution is brought back to the original volume with ddH_2O, and the solution is swirled to mix. Ethidium bromide is added to the mixture for a final concentration of 0.5mg/mL in the agarose solution. The agarose containing 0.5mg/mL EtBr is carefully poured onto the prepared gel plate while cautiously monitoring bubble formation. The comb is immediately inserted to create wells in the gel. The gel solution is allowed to cool and harden (20-30 minutes), and it is then transferred to an electrophoresis chamber containing 1X TBE buffer. The DNA standard is prepared by placing 0.5µg 1kb DNA ladder, 1µL 10X restriction enzyme buffer, and ddH_2O to 10µL. The tube containing the standard is mixed gently by quick spinning. 2µL of tracking dye is added to each of the 6 reaction tubes and the standard tube. The tubes are mixed gently by quick spinning. All tubes are placed in a 65°C water bath for 5 minutes. The tubes are immediately placed on ice to cool and mixed by quick spinning. The gel is loaded with 10µL of each sample. The order in which the wells are loaded is noted. The electrodes are set to run at 100V. The electrophoresis is allowed to run until the bromophenol blue (BPB) is within 1-2cm of the end closest to the anode (approximately 30-45 minutes). The power supply is

turned off. The gel plate is removed from the electrophoresis chamber. The gel is placed on an ultraviolet light table, photographed, and compared to gels of other restricted mycobacteriophages.

Analyze Phage Using Electron Microscopy 0.5-1.0mL of high-titer phage lysate is transferred to a sterile microcentrifuge tube. The tube is centrifuged for 1 hour at 4°C at 10,000×g. 20-50µL of supernatant is removed without disturbing the pellet. 100µL of fresh phage buffer is added and the contents are gently mixed using the pipette tip. The concentrated phage sample is stored for at least one hour but no more than 48 hours at 4°C to allow the pellet to completely dissolve.

The designated lab counter is covered with plastic faced paper to create a clean area. The cover paper is removed from a 5×5cm piece of parafilm into the lid of a petri dish. A PELCO tab or a small piece of double-sided tape (1-2cm) is placed onto the parafilm, and the liner is removed to expose the adhesive. A fresh electron microscope grid is placed so that the very edge of the grid is touching the adhesive. 10µL of phage preparation is placed on to the grid and is allowed to sit for a time dependent on the phage-titer.

Table 2. Times for Phage Preparation Sitting on Gird

Phage Titer	Approximate Time
10^6-10^7	5-7 minutes
10^8	5 minutes
≥10^9	2 minutes

If the titer is unknown, the sitting time of 5-7 minutes is used. A small wedge of filter paper (2-3cm) is used to wick off the excess fluid. 10µL of ddH_2O is pipetted on to the grid. The water is allowed to sit for 2 minutes and wicked off with a small wedge of filter paper. 10µL of ddH_2O is pipetted onto the gird again and the cleaning process is repeated. 10µL of 2.0% uranyl acetate is added to the grid and allowed to stain for 2 minutes. The excess stain is wicked off and the grid is allowed to air dry for at least 1 hour. The grid is observed with a transmission electron microscope to observe the phage morphology.

Archiving Phage Lysate

300µL of phage lysate was placed into a cryotube. 600µL of 75% glycerol solution was added to the cryotube. The solution is mixed by pipetting up and down. The cryotube is placed in the freezer and/or shipped (on ice) to the desired location.

RESULTS

Due to lack of phage in the first soil sample (collected from the Wilson Trail on the Hampden-Sydney College campus), a second sample was collected from Venable Lawn also at Hampden-Sydney College. This sample contains HerbSipe (Lon: 78° 27' 40.375" W; Lat: 37° 14' 26.533" N; Temperature: 30°C; Weather: Sunny and clear).

Visible plaques caused by HerbSipe were detected on soil sample plates with 10^{-1}, and 10^{-2} dilutions plated. Spot test confirmed presence of phage. Contamination present in first trial. Second trial contained no contamination and yielded individual plaques from HerbSipe which were picked from and purified a second time. Then titer was calculated from 10^{-4} phage-titer assay plate.

Table 3. HerbSipe Titer Calculation Values from Phage Titer-Assay Using the values from Table 1 in Formula 1, a titer of 5×10^7 pfu/ml was calculated.

#pfu	Dilution	Sample Volume (µL)
50	-4	10

Final plaque purification spot test plate yielded plaques in the 10^{-6}-10^{-9} spots. These four dilutions were individually plated to get a countable number of plaques. The first attempt resulted in contamination most likely stemming from my PB and/or $CaCl_2$ stock. The second attempt was successful. The plate from the 10^{-6} dilution displayed many plaques. The 10^{-7} plate displayed a countable number of plaques. Therefore, a titer could be calculated.

Table 4. A titer of 6.7×10^{10} pfu/mL was calculated using the values bellow in Formula from Final Plaque Purification procedures.

#pfu	Dilution	Sample Volume (µL)
67	-7	10

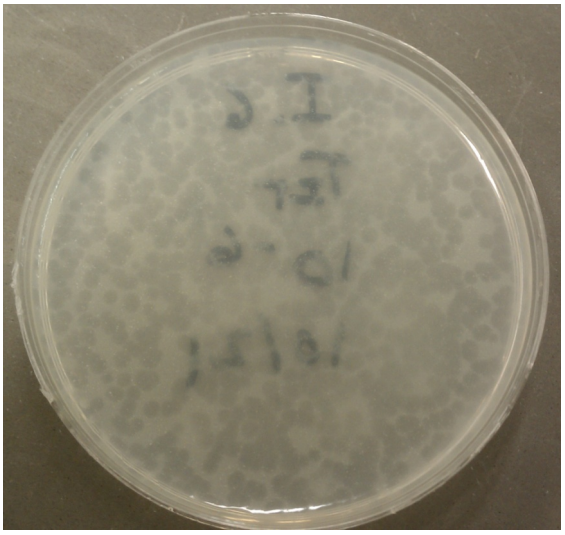


Fig 1. Plaques on 10^{-6} Dilution Plate from Final Plaque Purification

Table 5. $pfu_{max\ web}$ Calculation Values

Radius of Agar Plate	Radius of Plaque	Area of Plaque	Area of Agar Plate
47.5mm	1.5mm	$7.1mm^2$	$5.7 \times 10^3\ mm^2$

Using the value in Table 3 in Formula 2, a $pfu_{max\ web}$ of 802.8 was calculated. Using this value along with the titer value from the final plaque purification in Formula 3, a volume of 1.2×10^{-5}mL phage solution was determined to produce a web pattern. An empirical test range was then produced:

Table 6. Empirical Testing Range

Multiplier to Original Calculated Volume	Plate #	Volume (µL)	Dilution
¼	1	30	10^{-7}
½	2	60	10^{-7}
1	3	12	10^{-6}
2	4	24	10^{-6}
4	5	48	10^{-6}

No plates in this range formed a web plate. 10 plates were infected with 60µL of the 10^{-6} dilution to determine the volume-concentration combination that produces a web plate. This was done by using a 5mL aliquot of *M. smegmatis* with 60µL of the 10^{-5} dilution. This was plated on 10 agar plates 5mL at a time with 45mL of TA. Web plates were observed on all 10 plates. 60mL of filter sterilized lysate was obtained from a harvesting procedure for DNA Characterization. The procedure was repeated due to the high-lysate-volume demand of the DNA precipitation steps. A titer was unable to be calculated due to contamination events stemming from the *M. smegmatis* cells and the phage buffer. This procedure was performed a second time due to low DNA yields resulting from the first harvest.

2 vials were obtained with concentrations of 40 and 50ng/µL in order to Isolate and Purify Phage Genomic DNA of HerbSipe. The procedure was repeated and 2 vials were obtained with concentrations of 33 and 35 ng/µL. The procedure was repeated with the second harvested lysate. 2 vials were obtained with concentrations of 232ng/µL and 154ng/µL.

The next step was the purification of genomic DNA by ethanol precipitation in order to obtain higher DNA concentrations.The single vial obtained from this procedure displayed a concentration of 32ng/µL. This step was omitted in working with the second lysate because of the high concentrations obtained from the DNA isolation and purification step.

The vial containing 40ng/µL DNA was used in restricting and Analyzing Phage-genomic DNA. No bands were observed on the gel besides those on the standard DNA ladder. The vial containing 232ng/µL was subsequently used. Bands were observed as shown in Fig 2.

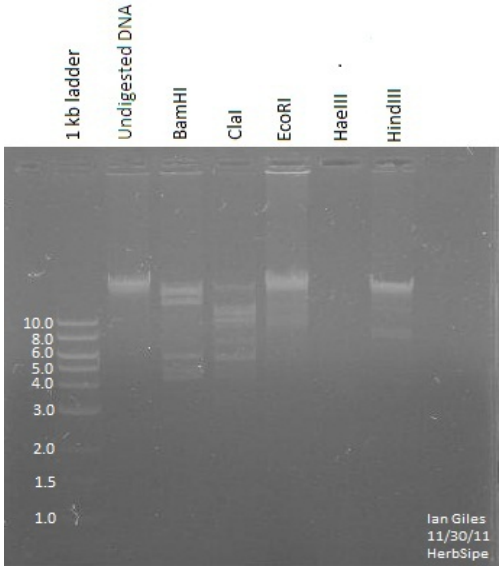
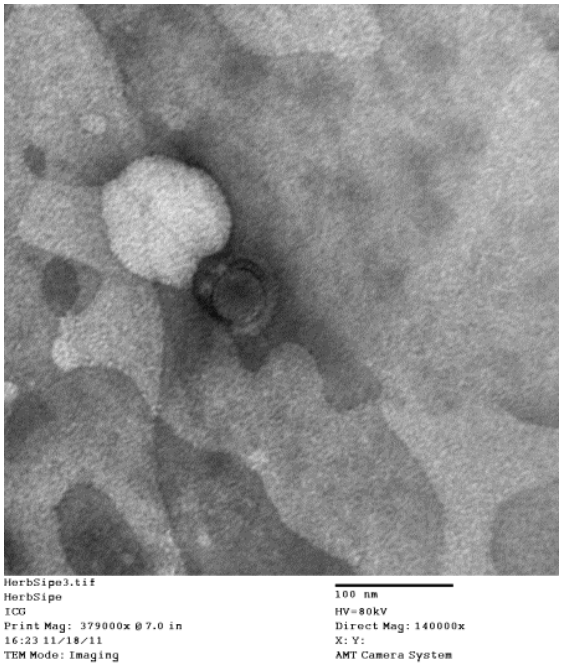
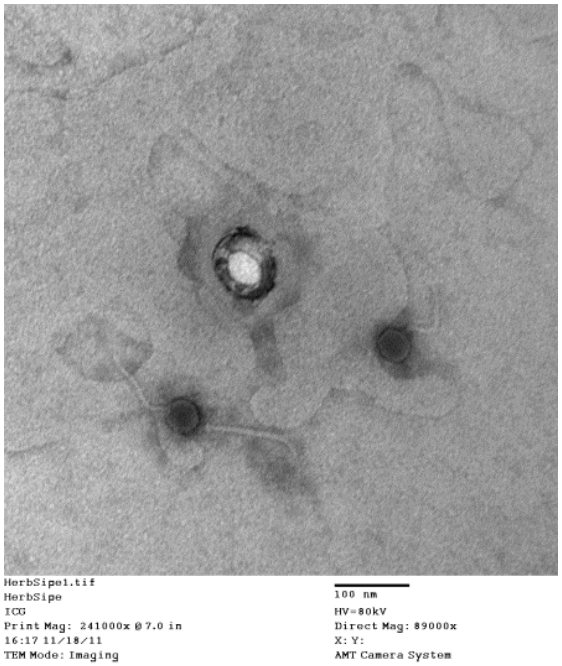


Fig 2. Electrophoresis Gel of Restriction Analysis of HerbSipe

When comparing this gel to a database of restriction profiles of sequenced mycobacteriophages, HerbSipe most closely resembles mycobacteriophage Bxb1. Therefore, HerbSipe would most likely be assigned to cluster A1.





The analysis via electron microscopy successfully displayed the morphology of mycobacteriophage HerbSipe (Fig 3).

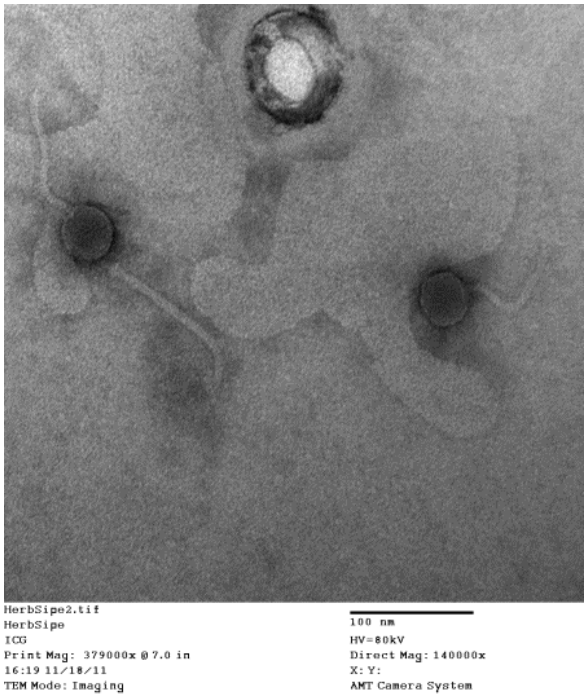


Fig 3. Images taken from electron micrographs of HerbSipe

Upon observation, the diameter of the head is approximately 50nm while the length of the tail is approximately 120nm. It is also interesting to note that the tail of HerbSipe tends to curve. The curving of the tail could aid in its attachment to bacterial cells. These data show that HerbSipe is of the Siphoviridae morphotype.

The lysate/glycerol solution was stored at Hampden-Sydney College and was sent to the Science Education Alliance (SEA) laboratory at the Howard Hughes Medical Institute.

DISCUSSION

This project led to the isolation and purification of mycobacteriophage HerbSipe using molecular biology techniques such as plaque streak protocol and spot testing. A stock of the phage lysate is stored in the Biology Department of Hampden-Sydney College and a stock was sent to the SEA laboratory.

Characterization of the phage consisted of performing a restriction analysis on precipitated DNA and analyzing that through electrophoresis and examining the phage through electron microscopy. Both of these techniques allowed for the prediction

that HerbSipe is a novel bacteriophage that is of the Siphoviridae morphotype and is classified in cluster A1 because of its restriction pattern resembling that of mycobacteriophage Bxb1. The results collected from the research project also suggest that evolution of bacteriophages occurs at a very rapid rate.

Though characterization was carried to the point of restriction analysis and prediction of a phage cluster, HerbSipe was unable to be sent for genome sequencing due to financial reasons. Continuation of this project would consist of obtaining a genome sequence of HerbSipe and comparing that to the 248 sequenced phages in the phagesdb.org registry. Sequence comparison would provide more solid evidence for cluster designation and phage evolution

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