INVESTIGATION OF BACTERIOPHAGES OF THE BIRD PATHOGEN, *BORDETELLA AVIUM*

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ABSTRACT

Bordetella avium is a bacterium that causes upper respiratory disease in many avian species. A number of strains of *Bordetella avium* contain two bacteriophages, Ba 1-1 and Ba 1-2. The objective of this research project was to discover which of 27 strains of *B. avium* contained the bacteriophage Ba 1-1, which strains contained Ba 1-2, which contained both, and which contained neither. The strains were cultured and tested for spontaneous lysis and for their ability to infect *B. avium* strain 197N. The results of these tests showed evidence of active phage in at least eight of the strains. Additionally, polymerase chain reaction (PCR) was performed on the bacterial DNA using five different sets of primers, chosen for their specificity to one phage or the other. The results of the PCR indicated phage DNA presence in 24 of the 27 strains. Although few trends were apparent in the results, this experiment laid the groundwork for future experimentation regarding phage presence in *B. avium*.

INTRODUCTION

Bordetella avium is a gram negative, nonfermentable, aerobic and motile bacterium that colonizes the trachea of chickens, turkeys (Figure 1), cockatiels and ostriches and many other avian species [1]. Studies have shown that infection is prevalent in many kinds of birds, which may or may not have the disease. A respiratory disease caused by *B. avium*, known as bordetellosis, directly affects commercially grown turkeys in the United States, Canada and Germany. The infection is not lethal, but infected birds are susceptible to secondary infections, which often prove lethal. The result is severe economic losses in all poultry-producing regions of the world. [1]

Figure 1: Turkey

The study conducted at Governor's School involved 27 strains of *B. avium* from a variety of infected birds containing two different bacteria-specific viruses (bacteriophage). Bacteriophage, most of which are roughly 100-1000 times smaller than the bacteria they infect, consist of a head, tail sheath and tail fibers (Figure 2). Nucleic acid in the form of either DNA or RNA is protected by a protein capsid, also referred to as the head. The tail sheath provides the pathway for the virus' nucleic acid to enter the host cell. The tail fibers bind the virus to the cell. The injected nucleic acid uses the host cell to replicate the virus by making the host cell produce proteins that are encoded in the injected nucleic acid [2].

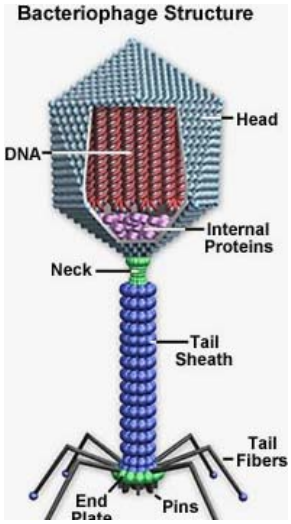


Figure 2: Bacteriophage structure [3]

There are six basic steps in the bacteriophage life cycle of a temperate phage. The first step is attachment, during which the bacteriophage uses its tail fibers to attach onto the cell surface and its DNA is injected into the cell. Temperate phage can enter one of two alternative life cycles, lytic or lysogenic, as illustrated in Figure 3. The lytic cycle starts when the viral DNA is transcribed into mRNA, which is then translated into several types of viral proteins. These proteins have functions that include the synthesis of viral DNA and coat protein, as well as the degradation of host DNA. Although the virus has full control of the host DNA, and it replaces the host DNA with its own DNA, it is the host cell that provides energy, nucleotides and amino acids. The newly synthesized viral proteins carry out viral DNA replication. After synthesis of viral DNA and proteins occurs, assembly of mature viruses can begin. In the first stage of viral assembly, the viral DNA and coat proteins assemble into phage heads. Next, the tail fibers and sheaths are attached to the head. Finally, a phage-induced enzyme lysozyme is synthesized. Lysozyme digests the cell membrane of the host cell and causes the cell to lyse and release mature phage. [2]

Alternatively, phage can follow the lysogenic cycle. During lysogenic infection, the inserted phage DNA integrates into chromosome of host cell, and replicates when host DNA replicates. The integrated phage DNA is called prophage and the host cell containing the phage DNA is a lysogen or lysogenic cell. Phage DNA is inserted into host chromosome at the *att* sites, where the two ends of both phage DNA and host DNA are homologous. This process is called site specific recombination. This function is catalyzed by a recombinase enzyme whose coding gene was identified in both Ba1-1 and Ba1-2. Due to the identical ends of both DNA sequences, synapse and integration of the two DNAs is allowed. However, the genes that code for enzyme excisase, which removes integrated phage DNA from host chromosome, must be repressed in order to maintain the lysogenic state. This repressor gene was identified on the Ba1-1 chromosome. Without it the excised viral DNA would replicate, be transcribed, and enter the lytic cycle.

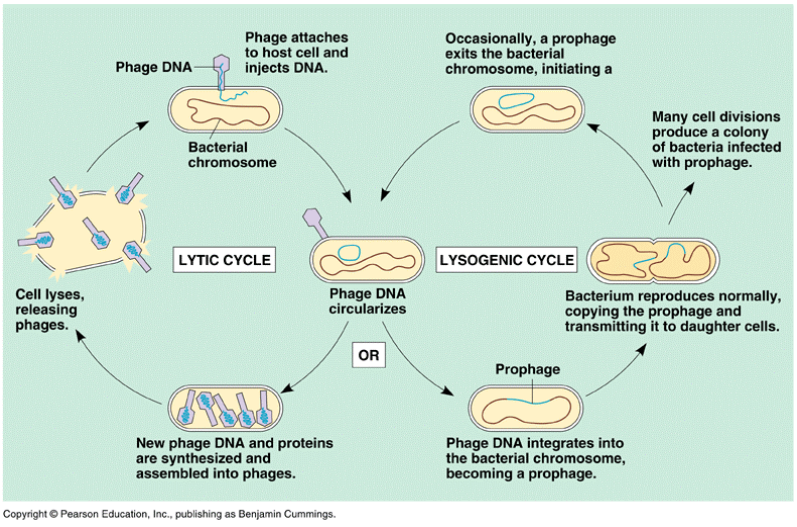
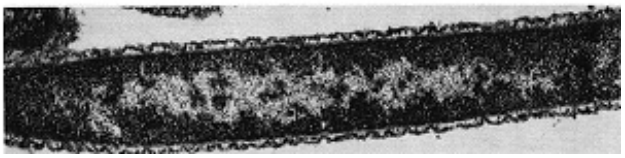


Figure 3: The Cycles of a Temperate Bacteriophage [4]

In order to make comparisons of phage DNA contained in various *B. avium* strains, a technique known as polymerase chain reaction (PCR) is utilized. PCR is the process through which a short region of a DNA molecule is selectively and exponentially amplified by a DNA polymerase enzyme. We used a thermostable DNA polymerase, *Taq* polymerase, to prevent its denaturation by heat treatment (see Figures 4, 5, and 6).



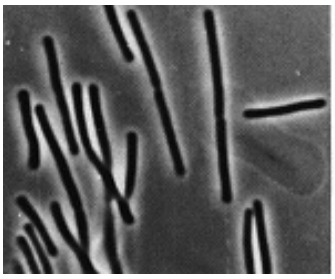


Figure 4 (far left): *Thermus aquaticus* in Hot Springs
Figures 5 and 6 (top and bottom): *Thermus aquaticus*

In order to ensure specificity, oligonucleotides (primers) are added to both ends of the DNA to flank the specific regions of interest. Three steps are involved in PCR – denaturation, in which hydrogen bonds between nucleotides are broken; annealing, during which primers are attached; and synthesis where bases are added to complete the new strands (Figure 7). Reaction mixtures obtained from PCR are analyzed by agarose gel electrophoresis, in which molecules are separated based on size.

PCR is applied in many ways, including clinical diagnosis and DNA profiling. It is used to study minute amounts of DNA, allowing genetic fingerprinting techniques to be done with single hairs and even bloodstains. This is crucial in forensic analysis, adding critical data to criminal cases. A current application of PCR is in the study of cancer and in the detection of cancer on a micrometastatic level. PCR's extreme sensitivity also provides new frontiers for archaeology and paleontology because it enables attainment of nucleotide sequences from traces of DNA found in preserved or fossilized material. Therefore, this process can be used to compare different genomes, which is one of the most important principles behind our research.

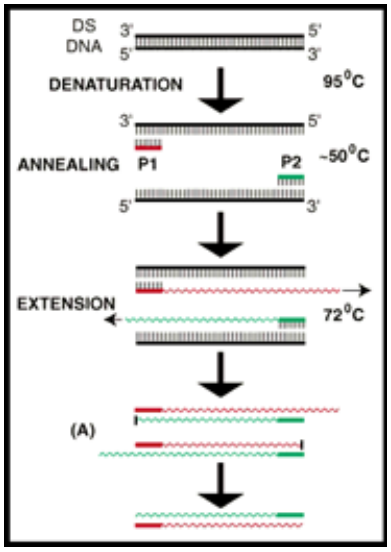


Figure 7: PCR Process—exponential amplification [6]

Before research began at Drew University this summer, two phages were found in *B. avium*: Ba1 and Ba2 [5]. After sending the Ba1 phage for sequencing, researchers discovered that there were actually two different phage chromosomes, now known as Ba1-1 and Ba1-2. Under the scanning electron microscope, the structure of both phages look exactly the same;

however, genomes differ in size and composition. There are large sections of Ba1-1 and Ba1-2 genomes that are very similar to each other, while some regions are completely unrelated. For example, after comparing the DNA from Ba1-1 and Ba1-2, the tail fiber region for both phages are homologous, but the recombinase regions are divergent. This example implies that the structural aspect of both Ba1-1 and Ba1-2 are almost identical, while the viral enzymes are different. The two phages are interesting in that they show so much similarity in structure yet many divergent viral proteins and enzymes.

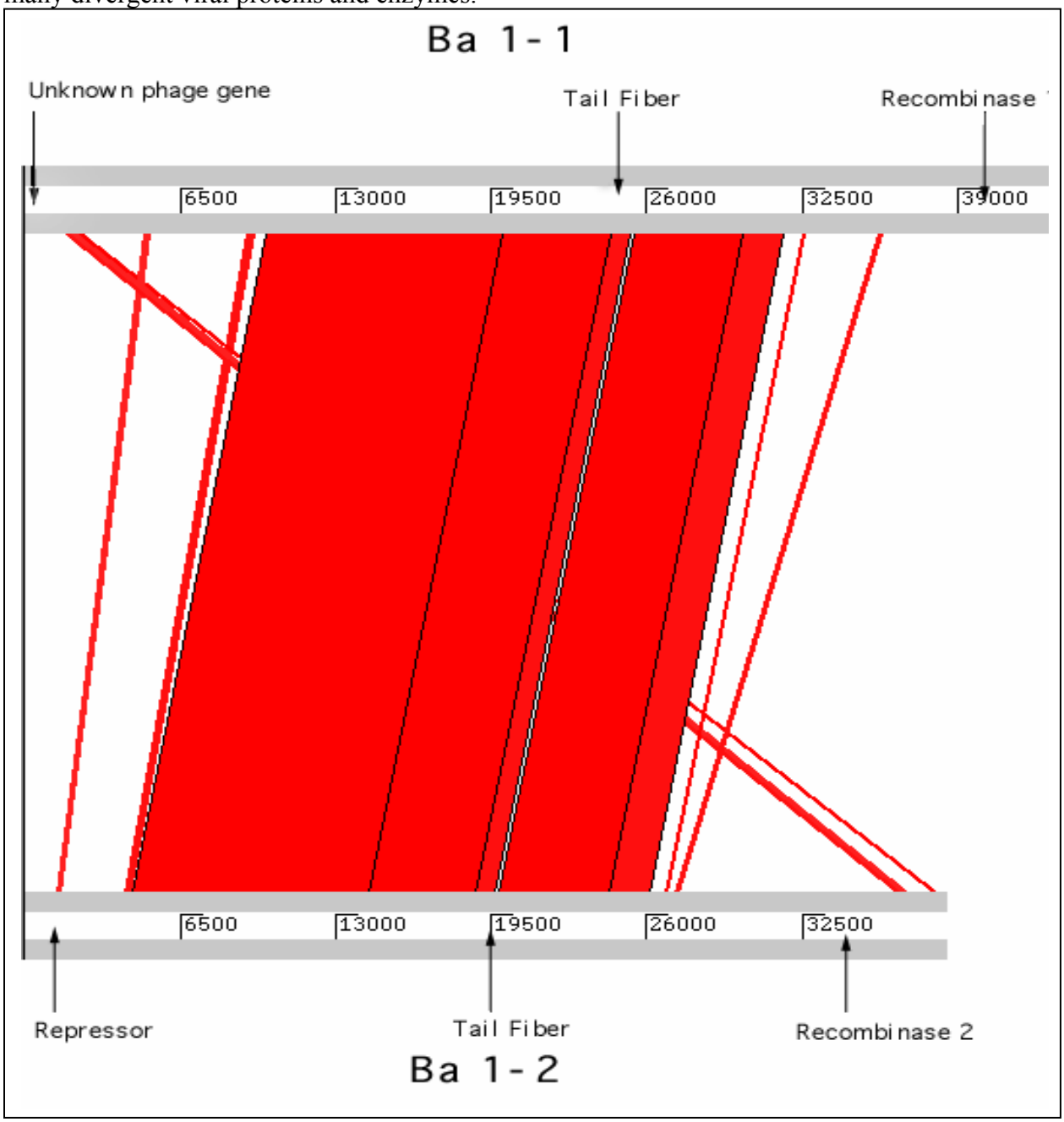


Figure 8: A comparison of the genes between Ba 1-1 and Ba 1-2. The top represents Ba 1-1's DNA, the bottom represents Ba 1-2's DNA, and the red lines represent the homologous regions between the two.

METHODS AND MATERIALS

Twenty eight various bacterial strains that have been isolated from birds such as ducks, geese, and turkeys were tested for the presence of phage (Table 1).

Table 1: Bacterial strains and their sources

Bacterial Strain	*Source of Strain*	*Bacteria*	*References*
Ba002	Isolated from sick turkey	*Bordetella avium*	[7]
Ba011	Isolated from sick turkey	*Bordetella avium*	[7]
Ba169	Isolated from sick turkey	*Bordetella avium*	[7]
Ba177	Isolated from sick turkey	*Bordetella avium*	[7]
Ba198	Isolated from sick turkey	*Bordetella avium*	[7]
F241-95-931	Isolated from sick turkey	*Bordetella avium*	[1]
F242-95-950	Isolated from sick turkey	*Bordetella avium*	[1]
F243-P4485	Isolated from sick turkey	*Bordetella avium*	[1]
F247-P4164	Isolated from sick turkey	*Bordetella avium*	[1]
D4	Isolated from wild duck	*Bordetella avium*	[8]
D10	Isolated from wild duck	*Bordetella avium*	[8]
D27	Isolated from wild duck	*Bordetella avium*	[8]
DBL239	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
254-3	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
DBL260	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
GOBL110	Isolated from well turkeys	*Bordetella hinzii*	[9]
G24	Isolated from Canada goose	*Bordetella avium*	[8]
T4	Isolated from wild turkey	*Bordetella avium*	[8]
Wampler	Isolated from sick turkey	*Bordetella avium*	Gift from G. Luginbuhl
197N	Isolated from domesticated turkey	*Bordetella avium*	[10]
4084	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
4085	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
4087	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
4094	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
4143	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
4480	Isolated from sick turkey	*Bordetella avium*	Gift from K. Register
JBBA	Isolated from sick turkey	*Bordetella avium*	Gift from John Barnes, 1978
ATCC35086	Type strain	*Bordetella avium*	American Type Culture Collection

All bacterial media and chemicals were obtained from Fisher Scientific unless otherwise noted. The bacterial strains were grown from stock on MacConkey plates, and a single colony was added to three ml of Brain Heart Infusion (BHI) broth. The cultures were then kept at 22°C for about 18 hours. To test for spontaneous lysis, 400 µl of the culture was added to a tube containing three ml melted BHI top agar (0.7%). The tube was vortexed for about 10 seconds, and immediately afterwards the contents were poured and spread over a BHI plate. The plate sat stationary for five minutes before being placed upside down in a 30°C incubator for 18-24 hours.

To test the bacterial cell cytoplasm for phage that would infect 197N, two drops of chloroform were added to a culture in order to kill the bacteria. The tube was then vortexed, and the culture was allowed to sit for five minutes. Making sure not to disturb the chloroform at the bottom of the tube, 500 µl of the culture was removed and added to a microfuge tube. The microfuge tube was centrifuged for three minutes and 400 µl of the strain supernatant with a potential for containing phage was added to a different microtube. Two hundred µl of 197N culture (a strain known to be sensitive to Ba1 infection) was added to the strain supernatant, vortexed, and allowed to sit at room temperature for 15 minutes. All 600 µl of culture were then removed and added to three ml of melted BHI top agar. It was then plated in the same way as described above. Both the spontaneous lysis plates and the 197N infection plates were observed for the presence or absence of plaques after 20 hours.

The PCR was run with a GeneAmp® PCR System 2700 machine by Applied Biosystems according to manufacturer's instructions, with reagents supplied by Clonetech. Chromosomal DNA was prepared using a kit from Qiagen, Inc, according to directions. Varying temperatures and cycle durations were used to run the PCR (Table 2).

Table 2: PCR cycle temperatures and durations

First Set (Ba1-1 and Ba1-2)		**Second Set (Recombinase 1 and 2 and Tail Fiber)**	
Annealing Temp. (°C)	*Duration*	*Annealing Temp. (°C)*	*Duration*
94 °C	3 min.	94 °C	3 min.
94 °C	30 sec.	94 °C	15 sec.
64 °C	15 sec.	60 °C	15 sec.
68 °C	15 sec.	68 °C	45 sec.
68 °C	7 min.	68 °C	7 min.

These three steps were run for 30 cycles.

A number of primers were used to amplify the target genetic sequences coding for phage (Table 3).

Table 3: Left and right primers and the genetic sequences they locate.

Name of Primer	***Genetic Sequence***	***Size of Product***
Unk1.358F	tggatggtgggcttcaatcttgc	291
Unk1.649R	tgcatttccacccgcacttcc	291
Rep2.622F	gctaacgcgccttccagtttgc	257
Rep2.879R	ggcaaaccgtccagcaatgg	257
Recombinase1.40889F	attcgaccgtgcaacccatc	189
Recombinase1.41086R	cctccctcgctgacctcaag	189
Recombinase2.33851F	agcaggatttggccgttgac	216
Recombinase2.34066R	acggctgccgaacagtaagg	216
Tail Fiber12.24889F	gaggccatggcaagtctgaaac	729
Tail Fiber12.25617R	ctgattaagctgcgcctgagc	729

All agarose gel preparations followed the same basic guidelines. The agarose was weighed out and the buffer solution of Tris-acetate-EDTA (TAE, 40 mM Tris-acetate, 2 mM EDTA, pH 8.5) with ethidium bromide (10 µl/mL) was measured. The two were combined, then heated and stirred. Once the solution boiled, it was removed from the heat to a water bath. Its temperature was monitored until it fell below 60°C. It was then poured into gel holder that had been prepared with combs; the combs formed wells into which the PCR product was injected once the gel hardened. When the gel hardened the combs were removed and the gel was placed into a gel electrophoresis machine. The gel was covered with the TAE buffer solution to just above its surface. The DNA was then mixed with loading dye (0.25% bromophenol blue, 40% w/v sucrose) and the mixture was injected into the wells. A standard, in this case a one kb ladder, which provides a reference against which the PCR products' results can be compared, was also loaded (Figure 9). The gel was run at approximately 100 volts for one hour. When the gel electrophoresis was finished, the agarose gels were read under fluorescent light using a Hitachi FMBIOII Multi-View scanner so that DNA markers in the form of dark lines could be read across the gel. The size of the DNA samples was determined by judging how far they traveled relative to the standard.

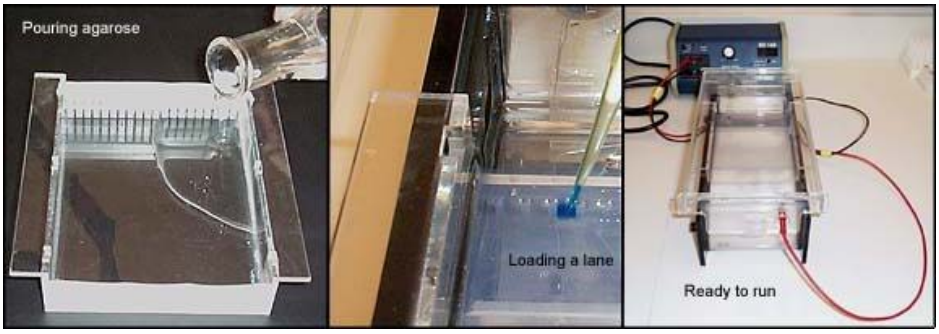


Figure 9: Steps of gel electrophoresis

RESULTS

Spontaneous Lysis and Phage Infection

Strain JBBA was the only strain that exhibited spontaneous lysis in our experiments, showing JBBA producing only one plaque, (Figure 10). No other plates showed evidence of spontaneous lysis. On the test for infecting strain 197N, plaques were seen on plates infected with the supernatant from Wampler, Ba 177, DBL 260, and ATCC. (Table 4)

Table 4: The formation of plaques in spontaneous lysis and infection of 197N

Strain	Spontaneous Lysis	Infection
Ba 002	0	0
Ba 011	0	0*
Ba 169	0	0
Ba 177	0	+*
Ba198	0	0
F241-95-931	0	0
F242-95-950	0	0
F243-P4485	0	0
F247-P4164	0	0
D4	0	0
D10	0	0
D27	0	0
239	0*	0*
254	0	0
DBL260	0	+
GOBL110	0	0
G24	0	0
T4	0*	0*
Wampler	0*	+*
197N	0	0
4084	0	0
4085	0	0
4087	0	0
4094	0	0
4143	0	0
4480	0	0
ATCC 35086	0	+*
JBBA	+	0

* These strains have been previously shown to produce phage
0 indicates negative
+ indicates positive

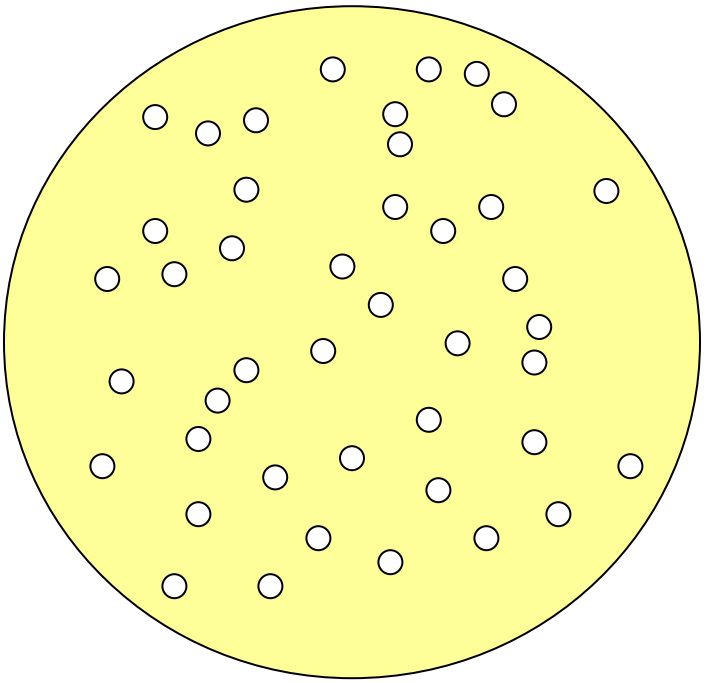


Figure 10: Cartoon of plaques on lawn of bacteria

PCR from Various Primer Sets

Table 5 shows the PCR results from each set of primers used. A positive result was demonstrated by the appearance of a dark band on the agarose gel. Examples of this are shown in Figures 11, 12, and 13. In each case the size of the PCR product corresponded to the expected size (Table 3). The size was estimated using a standard containing DNA fragments of known sizes.

Table 5: Results from visualizing PCR products in agarose gels.

	BA	*1-1*	*Both*	*BA*	*1-2*
Strain	**Unk 1.649 R Unk 1.358 F**	**Recombinase 1.41086 R Recombinase 1.40889 F**	**Tail Fiber 12.25617 R Tail Fiber 12.24889 F**	**Ba 1- 2.879 R Ba 1- 2.622 F**	**Recombinase 2.34066 R Recombinase 2.33851 F**
Ba 002	0	+	0	0	0
Ba 011	0	+	0	0	0
Ba 169	0	+	0	0	0
Ba 177	0	+	0	+	0
Ba198	+	+	0	+	0
F241-95-931	0	+	0	0	0
F242-95-950	0	+	0	0	0
F243-P4485	0	+	+	0	0
F247-P4164	0	+	+	+	0
D4	+	+	0	+	0
D10	+	+	0	+	0
D27	+	+	0	+	0
239	+	+	0	0	0
254	0	+	0	0	0
DBL260	+	+	0	0	0
GOBL110	0	0	0	0	0
G24	0	0	0	0	0
T4	+	+	0	+	+
Wampler	+	+	+	+	0
197N	0	0	+	+	0
4084	*ND*	+	0	0	0
4085	0	+	0	0	0
4087	0	0	0	0	0
4094	+	0	0	0	0
4143	0	0	0	0	0
4480	+	0	+	0	0
ATCC 35086	+	+	0	0	0
JBBA	0	+	0	0	0

0 indicates negative
+ indicates positive
ND indicates not done

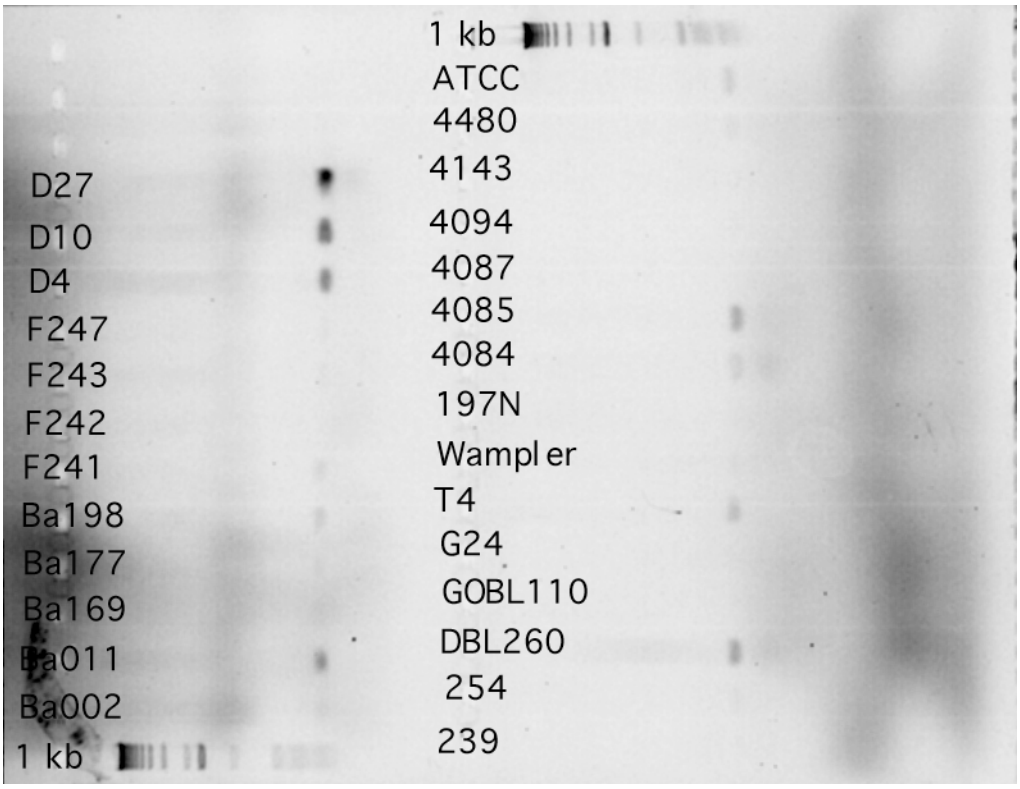


Figure 11: Agarose gel of PCR products using primers Recombinase 1.41086 R & Recombinase 1.40889 F

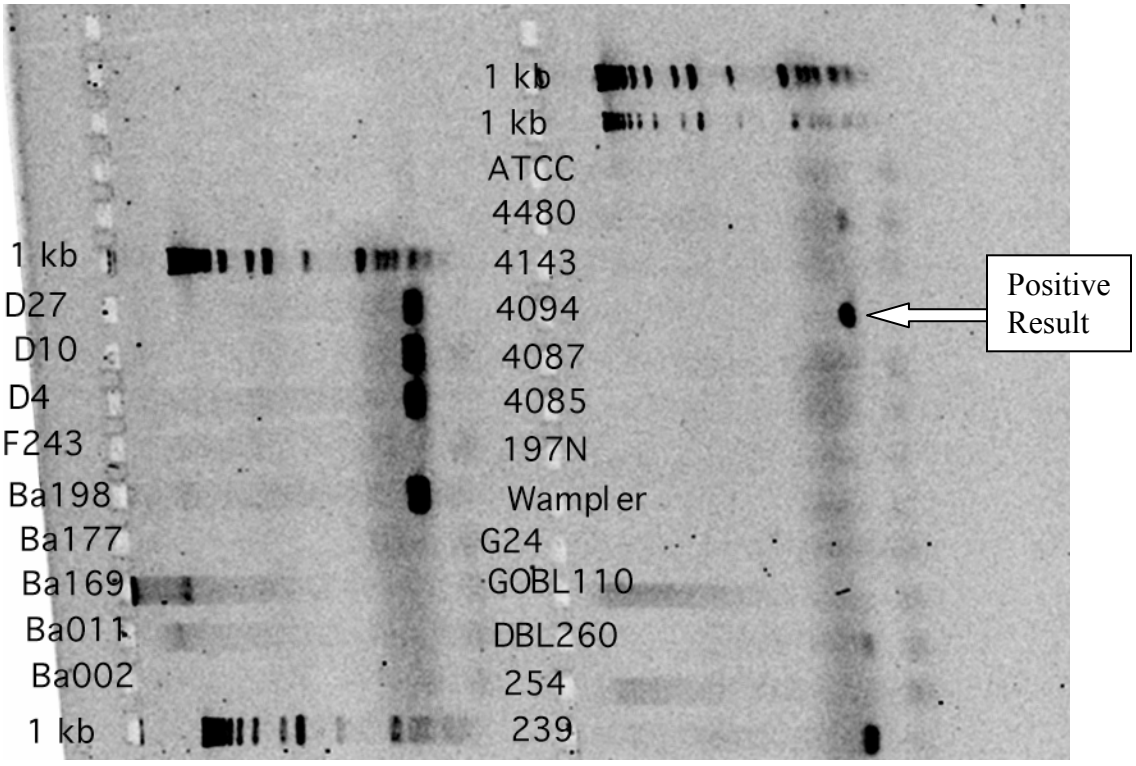


Figure 12: Agarose gel of PCR products using primers Unk 1.649 R & Unk 1.358 F

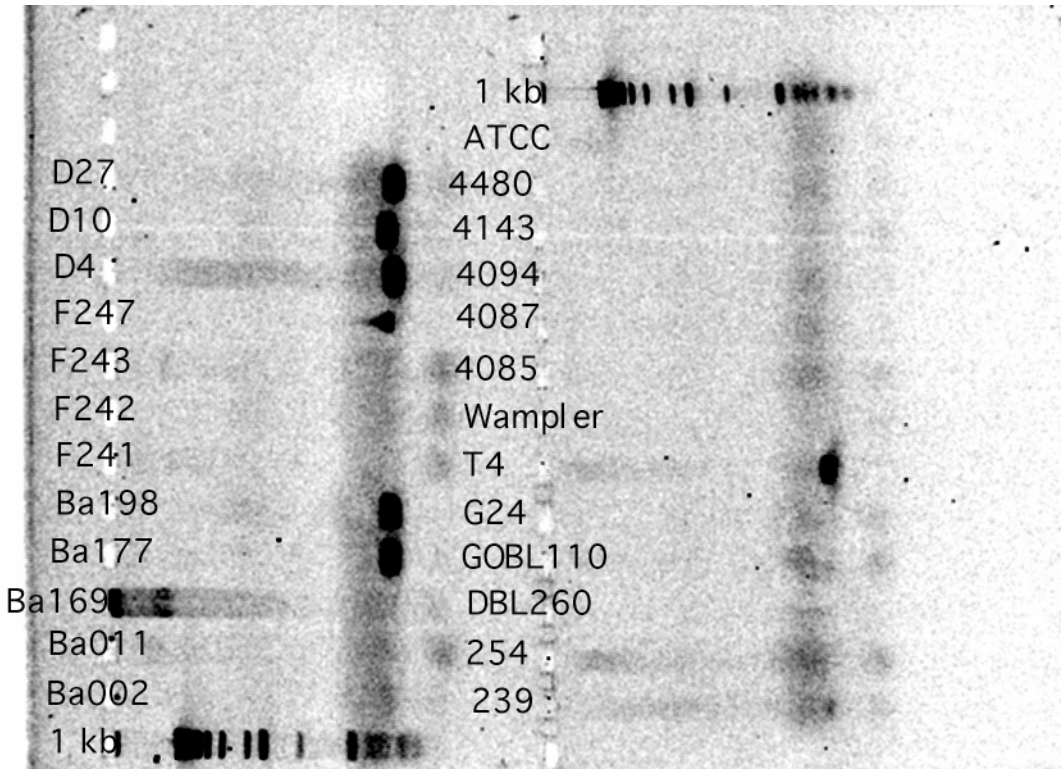


Figure 13: Agarose gel of PCR products using primers Rep 2.879 R & Rep 2.622 F

Major Trends

Several patterns can be seen from the table of results. Strains that test positive for all primers sets included strains Wampler, T4, D2, D10, D27. Strains G24 and strain GBL110 tested negative for all primers tested. All remaining strains had mixed results.

DISCUSSION

In assessing our results, it is important to address the specific relevance our methods, and how their usage influenced the team's course of action. The details of the polymerase chain reaction and gel electrophoresis were discussed earlier in this paper. For our specific purposes, the PCR was used to amplify portions of the genomes of the two bacteriophages we were studying. When observed after agarose gel electrophoresis, the presence or absence, in a given strain, of the gene or fragment we amplified told us if that particular portion of phage DNA was integrated into the chromosomal DNA of that strain. If the project was extended in scope and duration, PCR could be used to amplify many different genes, gene fragments, or chromosomal regions of interest (once sequences were located and appropriate primers could be designed). If subsequent electrophoresis and analysis yielded consistently positive results for these for a particular strain, that would be a good indication of the presence of a complete phage genome in that bacterial strain. In this project, the team studied the presence of five DNA sequences: a Ba1-1 sequence near a gene encoding for phage-related protein, a Ba1-2 sequence near a repressor

protein gene, genes thought to encode for respective recombinase enzymes in each of Ba1-1 and Ba1-2, and a gene thought to encode for a tail fiber protein common to both.

However, based on genetic evidence alone, we can't determine if a strain is lysogenic or simply has fragments of a formerly complete phage. Theoretically, if our experiments could be extended to amplify many sequences unique to phage genomes and a given bacterial strain showed incorporation of those sequences into its DNA, then it is likely that phage would be present, if not active, in that bacterial strain. The only way to tell with certainty how a phage behaves is to observe its activity in plated bacterial culture strains

The two methods used in this project to observe phage behavior were a test for spontaneous lysis of different *B. avium* strains and a test for infection of 197N (another *B. avium* strain previously shown to be sensitive to Ba1-1) by phage from those strains. In the spontaneous lysis test, described earlier, if plaques were observed on a plate where a strain was grown in top agar, then it was concluded that phage was both present and lytically active in that strain. The cloudiness of a plaque was thought to be directly proportional to the relative percentage of bacteria that contained phage in the lysogenic phase. In this test, plaques were only observed for strains 239, Wampler, and T4, so we concluded that these strains are lysogenic for a complete and active phage. If plaques were not observed on a plate of a particular strain in the spontaneous lysis test, it was thought that phage was either absent or lytic at a frequency too low to be observed.

If plaques were observed on a plate where the 197N strain had been mixed with the supernatant of a lysed strain and subsequently grown, then it was also concluded that phage was both present and active in that different strain. For strains DBL260, ATCC, Ba177, Ba011, T4, 239, and Wampler, plaques were observed (in more than one experiment for Ba177, ATCC, and Wampler), and it was concluded that phage had been produced from those strains that could infect 197N. If plaques were not observed in the infection test for a given strain, it was thought that phage were either absent, unable to infect 197N, or present in quantities too small to be observed in this manner.

Strains 239, Wampler, and T4 showed plaques in both tests; we concluded that phage are present, spend a significant part of their life cycle in the lytic phase, and definitely have the capability to infect 197N. Strains DBL260, ATCC, Ba177, and Ba011 only showed plaques in the infection test, so we concluded that phage are present and have the capability to infect 197N, but we were unable to tell with reasonable certainty just how lytic or lysogenic they were.

Since plaques all look the same, we cannot know if it is phage Ba1-1, phage Ba1-2, or both, that is or are active. We found that generally, when a given strain showed plaques in culture, DNA gel electrophoresis also yielded positive results for presence of phage DNA in that strain. However, the reverse was not always true, due to the more sensitive nature of test for phage presence by DNA gel electrophoresis, or the presence of defective rather than complete and active phage.

The results of the PCRs run during the experiment showed a few interesting patterns. Some strains of bacteria, including Wampler, the source of the bacteriophage, and T4, contained

all the genes for which the experimenters tested, and showed positive results in the spontaneous lysis. This result is consistent with data expected from bacteria containing both phages. However, there were other strains, such as D4, D10, and D27, which showed a number of positive results for fragments, but did not have any plaques in the spontaneous lysis or 197N infection. Such a result could show that the phages within these bacteria were highly lysogenic, and the conditions under which the bacteria were kept allowed the bacteria to live in lysogeny and not begin the lytic cycle. Another pattern, which appeared in G24 and GOBL110, was negative for all the amplifications. This pattern shows that G24 and GOBL110 did not contain any of the fragments we were looking for. Therefore, one can conclude that phages are probably not present in these strains. The G24 was the only *B. avium* strain that did not show any evidence of phages. The GOBL110 strain was a control for this experiment—it was a *Bordetella hinzii* species, not *Bordetella avium*. Because the strain did not show any evidence of bacteriophages, it can be inferred that the bacteriophages studied in this experiment are specific to *B. avium* and do not infect *B. hinzii*, a *B. avium*-like species. This observation confirms previous work showing that Ba1 does not infect *B. pertussis* or *B. bronchiseptica*, other species in the genus *Bordetella*. One final common pattern was positive for the Ba1-1 or Ba1-2 fragment, the recombinase 1 or 2 fragment, and a tail fiber fragment. The strains 197N, ATCC, and DBL260 all display results like these. Such a result shows that the bacteria were infected by one of the bacteriophages, but not by the other. Despite all these patterns, some results from the PCR appear to be random. There are a number of phage that appear to only have one or two of the fragments, but do not seem to have the proper fragments for an entire bacteriophage. This could be because there were bacteriophage DNA fragments that were picked up by most of the strains. Another explanation is that the experimenters did not repeat the tests enough to be sure of all the results.

The research performed in this experiment is only the beginning. To confirm the results from the studies, many more experiments must be performed. Sometimes PCR will not give completely correct results, for a number of reasons. The primers could be the incorrect sequence, there could be too much chromosomal DNA or too much primer, or the conditions could not work for the primer. If negative results are obtained repeatedly, it is more likely that the results will be correct, so researchers may perform more PCR using more different primers to distinguish between the Ba1-1 and Ba1-2 genes. Also, the spontaneous lysis and infections may be done again using a higher concentration of the infected bacteria. Often, lysis cannot be identified unless there is a large amount of bacteriophage present. Therefore, researchers might carry out spontaneous lysis on many more plates with a higher concentration of bacteriophage to attempt to confirm its presence in the bacteria.

Even after this experiment, there are still many questions left about bacteriophages in the *Bordetella avium*. One of the most important problems is locating all the genes in the bacteriophage's genome and figuring out the proteins for which they code. After discovering all the proteins in the bacteriophage, the purposes of all the proteins can be discovered. Finding the purposes and genes of all the bacteriophage proteins would pave the way for much more research about bacteriophages. The search for genes is already being undertaken by researchers, and the genes which code for the tail fibers, the recombinase enzyme, and a repressor protein have already been found.

Another important project which may commence is the comparison the phages Ba1-1 and Ba1-2 and location the differences in their genes. By looking at the genomes of these separate phages and using PCR to replicate the differing portions of the bacteriophage DNA, researchers will be able to discover what proteins are created by the genes that differ in the two different strains of phage. Using this, the genes for the physical and behavioral differences between the phages may become apparent. Such a project may also help researchers find the reason that certain phages act more lytic or lysogenic under certain conditions. In addition, researchers may look at the commonalities between Ba1-1 and Ba1-2. By finding those genes that are present in all phages, researchers may find genes that code for the head, tail, and tail fibers of bacteriophages, along with some genes that allow the phages to attach to and enter bacteria. There is still much research on the genes and proteins in Ba1-1 and Ba1-2 left to be done.

Since bacteriophages are very simple creatures, they have often been used in experiments to discover certain basic biological facts, particularly gene regulation. Information can often be extrapolated from phage experiments to shed light on genetic processes that are important in larger organisms, even humans. The sequences, properties, and functions of the proteins found in phages can be useful for the study of human proteins. Additionally, phages can be useful in fighting bacterial diseases. With the current problem of antibiotic resistance, some American companies are beginning to try to use bacteriophages to fight disease. This phage therapy has been used for a number of years in other countries and it is beginning to catch on in the United States.

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