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Isolation and Characterization of Novel Mycobacteriophages From the Central Illinois Region

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Presenter Information
Rachel Aron; Blake Beehler; Rachel Ende; Logan Garthe; Netherland Joiner; Tess Kelley; Lauren Killough; Michal Kozdronkiewicz; Jessica Kraut; Jennifer Lang; Matthew Piotrowiak; Prartana Ramachandran; and Loni Walker, Faculty Advisor

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ISOLATION AND CHARACTERIZATION OF NOVEL MYCOBACTERIOPHAGES FROM THE CENTRAL ILLINOIS REGION

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INTRODUCTION

Bacteriophages are a type of virus that infect bacteria. Most phages contain DNA in a hollow capsid (head) and a tail that binds to a specific host species of bacteria. Here we describe the isolation of 15 new phages capable of infecting *mycobacterium smegmatis* mc^{2}155. One of these phages (Kazan) was sequenced, annotated and genomically characterized.

Phage Life Cycles. When a bacteriophage infects a bacterium (injects its DNA into a bacterium), one of two distinct lifestyles can occur. The *lytic life cycle* involves phage replication and lysis of the bacterium, releasing new phages into the environment (figure 1). In the *lysogenic cycle*, phage DNA is integrated into the bacterial chromosome and forms a lysogen. The bacterium then reproduces as normal, forming new bacterial cells, each with the phage DNA integrated into its chromosome (prophage). Under favorable conditions, the prophage will excise from the bacterial genome and commence with the lytic life cycle (figure 1). Lytic phages do not produce lysogens and produce clear plaques since they lyse all bacteria they infect. Temperate phages may produce turbid plaques since some bacteria will lyse and some will be lysogens (and not lyse).

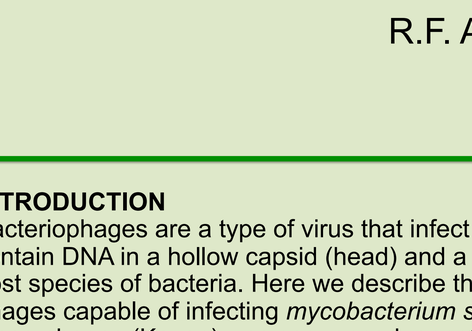

Figure 1. Lytic and Temperate phages with life cycles demonstrated. Figure is from Jordan et al. 2011.

MATERIALS AND METHODS

1. ***Phage Isolation and Characterization***. Each student collected soil and mixed with *Mycobacterium smegmatis* mc^{2}155. Using prescribed protocols phages were identified and subsequently purified (Jordan et al. 2011). Once purified, plaques were characterized, DNA was isolated and digested with restriction enzymes.
2. ***Genomic Characterization of Kazan***. DNA was isolated from Kazan and complete genome sequencing was performed at the University of Pittsburgh. The Genome was annotated for ORFS, tRNA, tmRNA's and other various features. DNA Master, Phamerator, Gepard, Meme, Hhpred and BLAST were used for genomic analysis.

RESULTS AND DISCUSSION

Phage Isolation and Plaque Characterization

1. 15 novel phages were isolated from central Illinois region (figure 2). Fourteen phages were isolated after enrichment and one phage isolated from direct plating (BassSlayer; table 1).

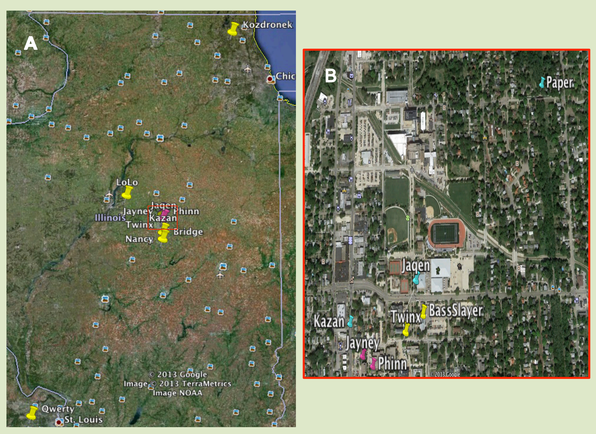

Figure 2. Geographical distribution of isolated phages. The majority of phages were isolated in Bloomington Illinois as indicated by red box in A. Figure B is an enlargement of the area within red box.

Phage	Diameter (mm)	Lytic or Temperate	Student
Nancy	4 - 5	Lytic	Ashlyn Calhoun
JoJo13	3 - 4	Temperate	Jessica Kraut
Kazan	3	Temperate	Tess Kelley
Phinn	3	Temperate	Rachel Aron
Pratna	3	Temperate	Prartana Ramachandran
BassSlayer	2 - 4	Lytic	Jacob Koster
Paper	2	Temperate	Matthew Piotrowiak
Jaqen	1.5 - 2.0	Temperate	Blake Beehler
Twinx	1 - 2	Temperate	Netherland Joiner
LoLo	1	Lytic	Lauren Killough
Qwerty	1 - 2.	Lytic	Logan Garthe
Bridge	0.5 – 1.0	Lytic	Jennifer Lang
Jayney	0.5 – 1.0	Temperate	Rachel Ende
Kozdronek	‹1	Temperate	Michal Kozdronkiewicz
Schwarz	‹1	Lytic	Jessica Diaz

Table 1. Character description of 15 new mycobacteriophages isolated by IWU students.

2. Nine phages produced turbid/cloudy plaques and were classified as temperate. The other six phages produced clear plaques and were classified as lytic (table 1, figure 3).

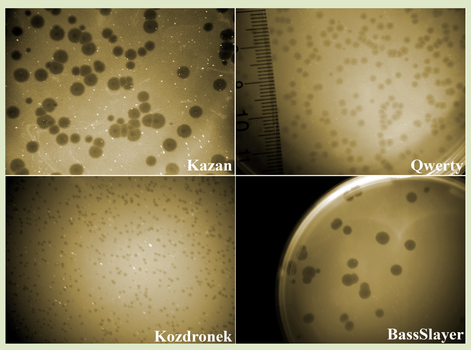

Figure 3: Plaque morphology for Kazan, Qwerty, Kozdronek, and BassSlayer. Kazan produced turbid plaques with while Qwerty, Kozdronek, and BassSlayer produced clear plaques. The BassSlayer plaques are 2.0-3.9 mm and Kozdronek plaques are <1 mm. Note the clear center and cloudy outer ring in Kazan.

3. There was no obvious correlation between plaque size, geographic isolation location and/or life style (table 2).
4. Two temperate phages (Phinn, Kazan) had a bull's-eye plaque morphology (Kazan in figure 3). Based on this plaque morphology, the quality and quantity of DNA, and restriction digest pattern (data not presented), the class chose to sequence Kazan.

Kazan Genome characterization.

1. The Kazan genome encodes 102 potential genes and 3 tRNA's .
2. The first 38 genes are transcribed in the rightwards direction and the rest in the leftwards direction (figures 4 & 6) . The 3 tRNA's occur between genes 7 and 11 on figure 4.
3. Based on DNA sequence similarity, Kazan was determined to belong in the A cluster of mycobacteriophages, specifically as a member of the A6 subcluster.

Genome Comparison in the A6 Subcluster

1. 8 Mycobacteriophages are in the A6 Subcluster. All are siphoviridae morphotypes (double stranded DNA genome and a long, flexible non-contractile tail). All 8 genomes have defined physical ends with a conserved 10 bp 3' overhang (CGGTCGGTAA). The genomes range in size from 48,963 to 52,502 bp and encode 92 - 103 genes (figure 4) and 3 tRNAs.
2. All A6 phage genomes are organized in a similar manner, with the greatest sequence diversity between phages occurring in the right arm (figure 4).
3. Kazan is 99% identical to EricB and DaVinci, but only 90-93% identical to the other A6 phages (Gladiator, Blue7, Hammer, Jeffabunny; figure 4).
4. Nine genes are specific to the A6 subcluster. The putative gene products (called phams) are listed in table 2. Pham 5101 is only present in Kazan, EricB and DaVinci (table 2).

Pham #	Protein Function	Found In
3946	No Known Function	All A6 Phages
5097	RNA Ligase	7 A6 Phages (EricB, DaVinci, Kazan, Blue7, Hammer, Jeffabunny, McFly)
7121	Endo-beta-N-acetylglucosaminidase	4 A6 Phages (EricB, Jeffabunny, Kazan, McFly)
3948	No Known Function	All A6 Phages
3949	DNAB-like Replicative Helicase	All A6 Phages
5101	No Known Function	3 A6 phages (EricB, DaVinci, and Kazan)
3950	No Known Function	All A6 Phages
3951	No Known Function	All A6 Phages
3952	No Known Function	All A6 Phages

Table 2. Phams (proteins) specific to the A6 Subcluster. Protein functions were determined using Phamerator and the open-source software Hhpred (Söding 2005).

5. There are minor genomic differences between Kazan, EricB, and DaVinci. Two examples are provided in figures 5a – b and one is shown on figure 4 (boxed area).

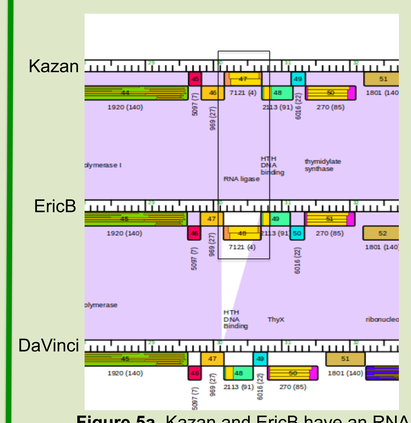

Figure 5a. Kazan and EricB have an RNA ligase (Pham 7121; boxed) and DaVinci does not.

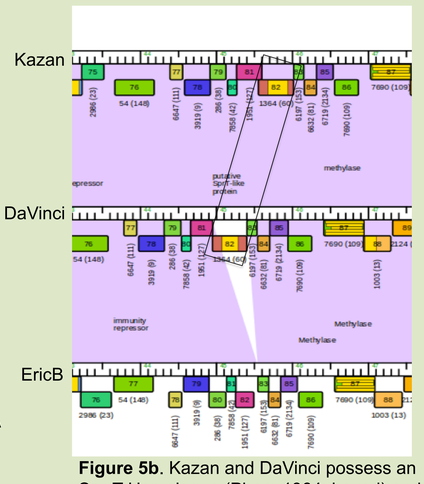

Figure 5b. Kazan and DaVinci possess an Spr-T Homologue (Pham 1364; boxed) and EricB does not.

Immunity Testing

1. Of the eight temperate phages isolated, four produced stable lysogens (Kazan, Paper, phinn, JoJo13; table 3). Immunity testing was performed with each lysogen to determine if the other temperate phages were homoimmune (see table 3 legend for explanation).
2. Kozdronek, Jaqen, and Paper show homoimmunity with Kazan, suggesting they may be closely related to Kazan and also in the A6 subcluster. Interestingly Kozdronek exhibits homoimmunity with three different lysogens (Kazan, phinn, Paper).

Homoimmune to Kazan	Homoimmune to Phinn	Homoimmune to Paper	Homoimmune to JoJo13
Kozdronek	Kozdronek	Kozdronek	None
Jaqen	Jayney	Jaqen	
Paper			

Table 3. Phages with homoimmunity to the lysogens of Kazan, Phinn, Paper, and JoJo13. Phages in cluster A encode unique repressor proteins that bind to their own repressor binding sites (called stoperators) and to repressor binding sites of closely related phages (Pope et al. 2011). This prevents the infecting phage from undergoing the lytic life-cycle (and producing a plaque) once it has injected its DNA into the lysogen. If an infecting phage is temperate and can not produce a plaque on a lysogen, it is homoimmune to the lysogen and probably in the same subcluster. If it produces a plaque on a lysogen, it is heteroimmune and likely to be in a different subcluster (Pope et al 2011). Kozdronek appears to be homoimmune to three of the four immunity groups.

Stoperator Sites in Kazan, EricB, and DaVinci

1. The stoperator consensus sequence for Kazan, EricB, and Davinci was GGTGGGTGTCAAG, the same consensus sequence identified in an A2 subcluster phage (L5).

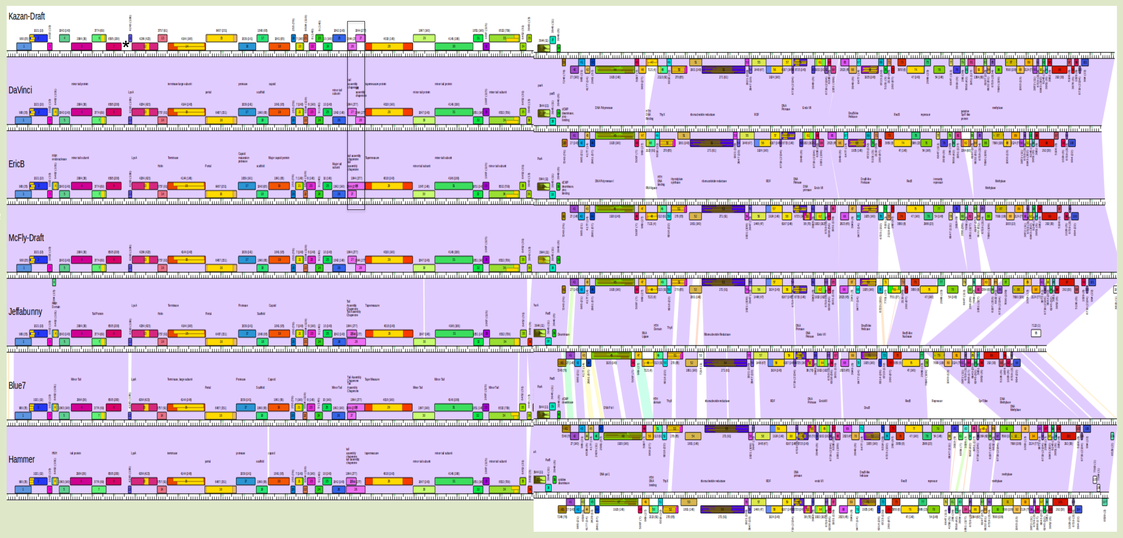

Figure 4. In the left arm, the genes (colored boxes) are transcribed in a rightwards direction (as indicated by the genes being placed on the top of the horizontal bar) and the right arm is transcribed in a leftwards direction (as indicated by the genes being placed on the bottom of the horizontal bar). The greatest genome diversity occurs within the right arm of the genomes. The DNA sequence similarity between genomes is color coded, with purple having the most similarity and white having the no similarity. The * in Kazan between genes 7-11 indicates the position of the 3 tRNA's. A tail assembly chaperone frameshift (boxed area; Pham 1844) occurs in EricB but not Kazan or DaVinci.

2. The majority of stoperator sites in Kazan, EricB, and DaVinci are located between genes (figure 6). Kazan has four stoperator sequences inserted in unique locations from EricB and six unique from DaVinci. In addition, all three phages had five stoperator sequences located within the early lytic promoter (not pictured on figure).

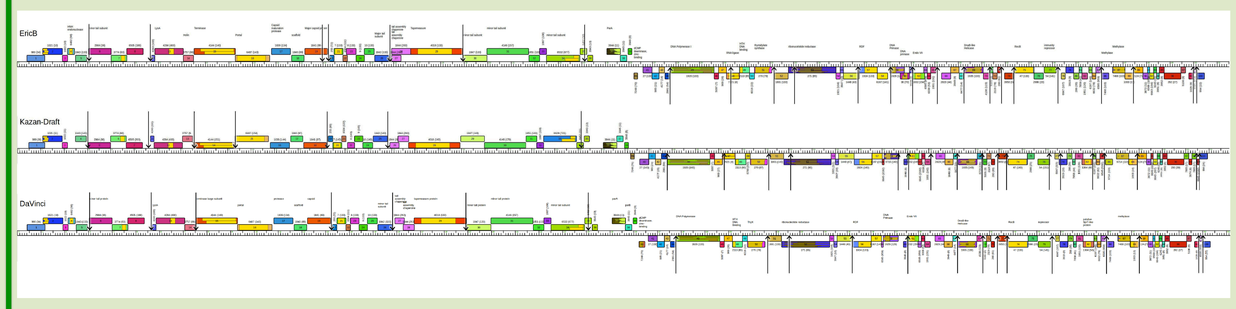

Figure 6 Stoperator sites within Kazan, EricB, and DaVinci. Stoperators are asymmetric segments of DNA, 13 base pairs long, that are bound by repressor proteins and terminate transcription elongation (Brown et al. 1997). They are important in stopping transcription of the lytic genes during the lysogenic portion of a temperate phage's life cycle. Previous studies suggest a connection between the conservation of the repressor protein sequence, stoperator sequence and phage subcluster homoimmunity (Pope et al. 2011). The 5' end of the stoperator sequence is indicated by an arrow. A DNA sequence analysis program (MEME) designed to identify and locate motifs repeated within the genome was used to identify potential stoperator sites (Bailey and Elkan 1994).