Mousing Around with Bacteriophages:

Using Antibodies Raised in BALB/c Mice to Analyze Phages

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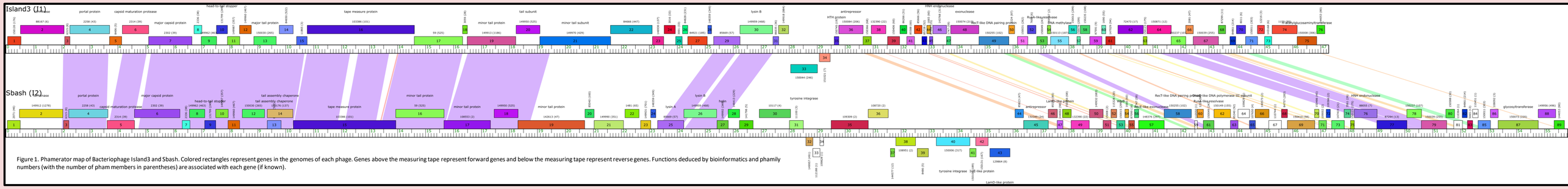

Figure 1. Phamerator map of Bacteriophage Island3 and Sbash. Colored rectangles represent genes in the genomes of each phage. Genes above the measuring tape represent forward genes and below the measuring tape represent reverse genes. Functions deduced by bioinformatics and phamily numbers (with the number of pham members in parentheses) are associated with each gene (if known).

Abstract

Bacteriophages are viruses that infect bacteria and can be used in phage therapy to combat bacterial infections resistant to traditional antibiotics. One current limitation of phage therapy is the lack of understanding about phage genetics and biochemistry. We can learn more about bacteriophages by identifying patterns in their protein structure. In our experiments, we immunized BALB/c mice using whole phage or ~71 kDa proteins extracted from Sbash and Island3. We tail-bled the mice to collect antibodies raised against these phages, then used ELISA assays to detect anti-phage protein antibodies in mouse serum. We are using our antibodies and Western blot analysis to understand biochemical relationships between Island3, Sbash, and several other bacteriophages that infect *Mycobacterium smegmatis*.

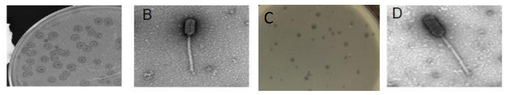

Figure 2. Phage characteristics. A. Plaque assay of phage Island3: 4mm/bulls-eye/24 hours/37°C B. Transmission electron micrograph (TEM) of phage Island3 C. Plaque assay of phage Sbash: 2-7 mm/24 hours/37°C D. TEM of phage Sbash.

Table 1. Characteristics of Island3 and Sbash Genomes

Phage	Genome Size (bp)	Cluster	% GC	Number of Genes	Isolation Location	Isolation Institution
Island3	47287	I1	66.8	76	Oakland, PA	Carnegie Mellon University
Sbash	55832	I2	65.6	89	Durban, South Africa	University of Kwazulu-Natal

Table 2. Clusters and hosts of phages used in this study.

Phage	Abbreviation used in figures	Cluster	Host
Antonia	A	B1	*Mycobacterium smegmatis*
Beelzebub	Bz	S	*Mycobacterium smegmatis*
Butters	B	N	*Mycobacterium smegmatis*
Cborch11	CB	H1	*Mycobacterium smegmatis*
Deadpool	D	Unclustered	*Microbacterium foliorum*
DrLupo	DL	H2	*Mycobacterium smegmatis*
Giles	G	Q	*Mycobacterium smegmatis*
Island3	I3	I1	*Mycobacterium smegmatis*
JacoRen57	JR	AB	*Mycobacterium smegmatis*
Kelly	Ke	Unclustered	*Gordonia terrae*
Knocker	K	B9	*Mycobacterium smegmatis*
Larva	L	K5	*Mycobacterium smegmatis*
Quesadilla	Q	B9	*Mycobacterium smegmatis*
RedRaider77	RR	S	*Mycobacterium smegmatis*
Roots515	R	C1	*Mycobacterium smegmatis*
Sbash	Sb	I2	*Mycobacterium smegmatis*
Sibs6	S	A1	*Mycobacterium smegmatis*
Smoothie	Sm	Unclustered	*Microbacterium foliorum*
Tortellini	T	P2	*Mycobacterium smegmatis*
Wrigley	W	CY	*Gordonia terrae*

Introduction

With antibiotic resistance on the rise, phage therapy presents a new approach to fighting bacterial infections. Phages are viruses that exclusively infect bacteria. When a lytic phage infects a bacterium, the result is lysis of the host bacterial cell; this makes them useful in a clinical setting. Through programs such as SEA Discovery, new phages are constantly being discovered, annotated, and archived.

SBash and Island3 are cluster I phages that infect *Mycobacterium smegmatis* and share considerable genetic identity. SDS-PAGE gels were run to isolate proteins from these phages and determine their molecular weight. We measured protein concentrations with a protein assay and injected the isolated proteins into female BALB/c mice. Another group of mice was immunized with phage high titer lysate (HTL). We collected blood from the tail veins of immunized mice, allowed the blood to clot, then centrifuged it to get serum. We performed ELISA assays to determine if phage and protein-specific antibodies were present in the serum and Western blot to investigate how Island3 and Sbash compare from a biochemical perspective. Learning more about the biochemical relationships between phages is important for advancing the field of phage therapy.

References

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Materials and Methods

Comparing Island3 and Sbash Using a Genetic Screen

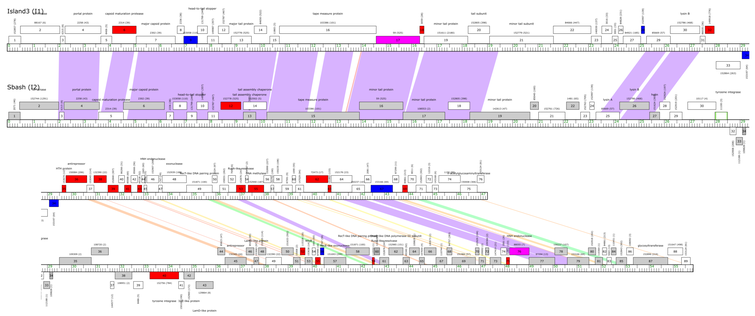

Figure 3. Comparison of Island3 and Sbash cytotoxic and defense genes. Cytotoxic genes are shown in red, defense genes in blue. Genes that are neither cytotoxic nor confer defense are shown in white. Genes that show both cytotoxicity and defense are shown in pink and untested genes are shown in gray. Only three genes that are very similar in both genomes (Island3_6/Sbash_5; Island3_9/Sbash_8; Island3_13/Sbash_12) have contradictory phenotypes. At least one of each of these pairs needs to be sequence verified.

Comparing Island3 and Sbash Biochemically Using Antibodies

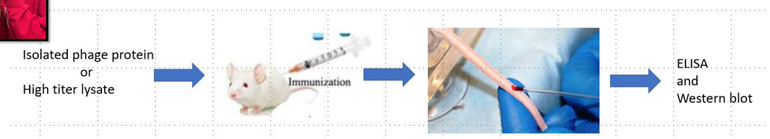

Figure 4. Project strategy. Mice were immunized biweekly with either phage high titer lysate or isolated phage protein. Blood was collected from the tail vein. Serum was prepared and used as primary antibodies in ELISA and Western blot assays.

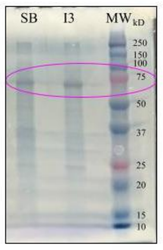

Figure 5. SDS-PAGE analysis of Sbash (SB) and Island3 (I3) high titer lysates (HTLs). The bands (~71 kDa) indicated by the pink oval were excised from preparatory gels and used to immunize Balb/c mice.

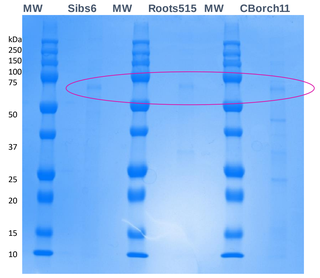

Figure 6. SDS-PAGE analysis of Sibs6, Roots515, and CBorch11 HTLs. Bands indicated by pink oval (~71 kDa) were excised from preparatory gels, eluted, and used to immunize Balb/c mice.

What proteins might we be isolating?

How many amino acids are in a protein that is 71 kDa in size?
71 kDa/0.110 (average MW of an amino acid) = 645 amino acids or 1935 bp

Table 3. Candidate genes encoding ~71 kDa proteins.

Gene	Number of nucleotides	Expected MW (kDa)	Annotated function
Island3_17	1728	63.81	NKF
Island3_19	1734	64.16	Minor tail protein
Island3_21	2532	88.71	Minor tail protein
Sbash_16	1728	64.01	Minor tail protein
Sbash_19	2395	80.81	Minor tail protein
Sibs6_27	2058	74.24	Minor tail protein
Sibs6_29	1803	67.03	Minor tail protein
Sibs6_33	1854	63.89	Minor tail protein
Sibs6_45	1827	67.59	DNA Pol I
Roots515_3	1872	70.37	Nucleotidyl transferase
Roots515_104	1905	72.25	NKF
Roots515_131	2259	78.48	Tapemeasure protein
Roots515_190	1863	70.16	DNA helicase
CBorch11_63	2058	77.39	DNA helicase

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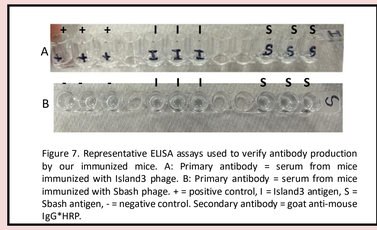

Figure 7. Representative ELISA assays used to verify antibody production by our immunized mice. A: Primary antibody = serum from mice immunized with Island3 phage. B: Primary antibody = serum from mice immunized with Sbash phage. + = positive control, I = Island3 antigen, S = Sbash antigen, - = negative control. Secondary antibody = goat anti-mouse IgG*HRP.

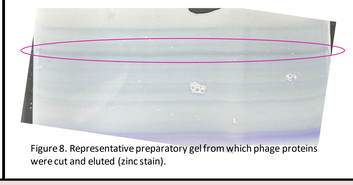

Figure 8. Representative preparatory gel from which phage proteins were cut and eluted (zinc stain).

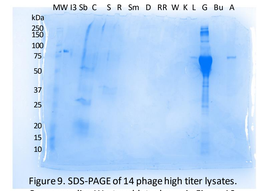

Figure 9. SDS-PAGE of 14 phage high titer lysates. Corresponding Western blots shown in Figure 10.

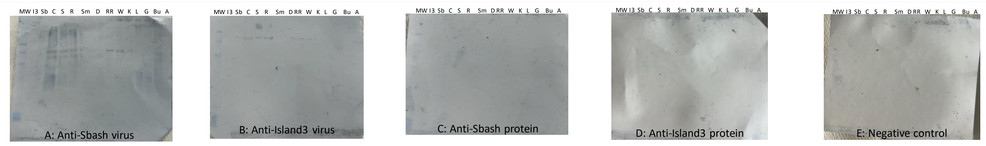

Figure 10. Western blot analysis shows that anti-Sbash antiserum recognizes several proteins in other viruses (A) and anti-Island3 antiserum recognizes a protein in a few other viruses (B). We were unable to detect antibodies in serum from mice immunized with Sbash protein or Island3 protein (C and D). Molecular weight markers of 250, 150, 100, 75, 50, 37, 25, 15, and 10 kDa are in the left-most lane of all blots.

Conclusions and Future Directions

- **Sbash and Island3 are less similar by immunological analysis than expected, based on genome comparisons.**
- **Use mass spectrometry to identify proteins on our gels**
- **See if what we observed is repeatable**
- **Express candidate genes in *E. coli* and use these lysates in Western blots**

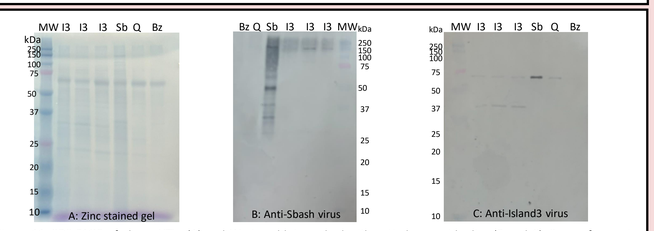

Figure 11. SDS-PAGE of phage HTLs (A) and Western blots probed with anti-phage antibodies (B and C). Serum from mice immunized with Sbash recognize many Sbash proteins (B). Panel B: Anti-Sbash antibodies recognized one or two large MW Island3 proteins but no Quesadilla or Beelzebub proteins. Panel C: Serum from mice immunized with Island3 recognized two Island3 proteins (~71 kDa and ~40 kDa) plus proteins of similar size (~71 kDa) in Sbash and Quesadilla. Secondary antibodies = goat anti-mouse IgG*HRP. Blots with no primary antibody (PBS only) showed no bands (data not shown).

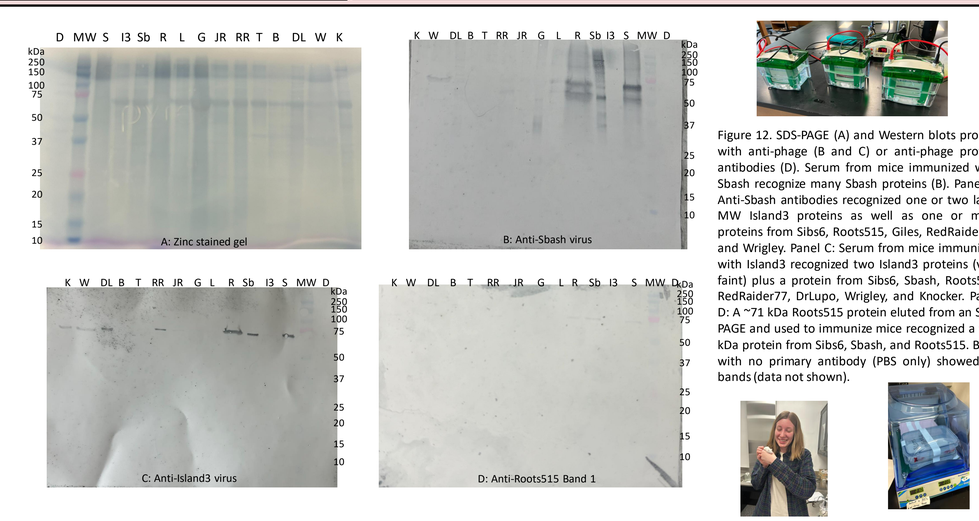

Figure 12. SDS-PAGE (A) and Western blots probed with anti-phage (B and C) or anti-phage protein antibodies (D). Serum from mice immunized with Sbash recognize many Sbash proteins (B). Panel B: Anti-Sbash antibodies recognized one or two large MW Island3 proteins as well as one or more proteins from Sibs6, Roots515, Giles, RedRaider77, and Wrigley. Panel C: Serum from mice immunized with Island3 recognized two Island3 proteins (very faint) plus a protein from Sibs6, Sbash, Roots515, RedRaider77, DrLupo, Wrigley, and Knocker. Panel D: A ~71 kDa Roots515 protein eluted from an SDS-PAGE and used to immunize mice recognized a ~71 kDa protein from Sibs6, Sbash, and Roots515. Blots with no primary antibody (PBS only) showed no bands (data not shown).