Novel *Propionibacterium acnes* Phage Leviosa Demonstrates Broad Infectivity

Connie Giang[1], Samantha Kaufhold[1], Adilene Barajas[1], Andy Vu[1], Rocky Ng[1], Jordan Moberg Parker[1]

[1]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles

Abstract

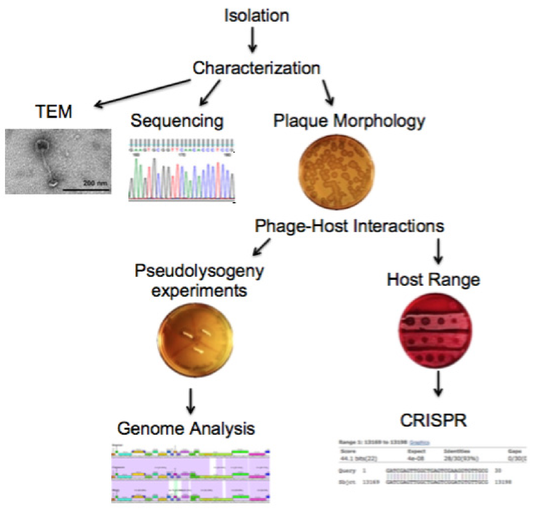
Propionibacterium acnes is an aerotolerant, Gram-positive bacterium that has been implicated as a pathogenic factor in acne vulgaris, a skin disease that affects more than 80% of teenagers and adults. Given increasing rates of antibiotic resistance, phage therapy needs to be explored as an alternative treatment for acne vulgaris. To that end, a novel *P. acnes* bacteriophage, Leviosa, was isolated and characterized to evaluate its potential as a phage therapy candidate. Though no previously isolated *P. acnes* phages contain lysogenic modules, multiple lines of evidence suggest that Leviosa is not a purely lytic phage. Leviosa's ability to produce plaques with a bullseye morphology, turbid mesas, and clearings in subsequent patch tests are suggestive of either pseudolysogenic or lysogenic behavior. Due to this unexpected behavior, Leviosa's host range was assessed by spotting the bacteriophage on various *P. acnes* isolates, including highly resistant strains. The phage was able to lyse all but two *P. acnes* isolates that it encountered. This broad infectivity was further examined using BLASTn to compare Leviosa's genome to CRISPR protospacers from resistant *P. acnes* strains, B101.9 and B66.8. Significant mismatches between Leviosa's genome and bacterial protospacers would suggest that the bacterial defense mechanism is unable to recognize the phage genome as foreign, rendering the bacteria susceptible to infection. Results from BLASTn showed that there were similar levels of mismatches between Leviosa's genome and protospacers from B101.9, a strain susceptible to Leviosa, and B66.8, a strain resistant to Leviosa, indicating that CRISPR defense may partially explain Leviosa's host range. While Leviosa's non-lytic behavior may pose a problem, its unusual ability to lyse highly resistant *P. acnes* strain B101.9 elevates its potential as a phage therapy candidate.

Background

- *Propionibacterium acnes* is an aerotolerant, Gram-positive bacterium that makes up 90% of the commensal bacteria in the pilosebaceous follicles (Fitz-Gibbon et al, 2013)
- *P. acnes* has been implicated as a pathogenic factor in acne vulgaris (Leyden 2001; Bojar and Holland, 2004)
- *P. acnes* have developed antibiotic resistance, leading to ineffective acne vulgaris treatment (Farrar et al, 2007)
- *P. acnes* phages modulate the relative abundances of *P. acnes* strains and may prove useful in acne vulgaris treatment (Liu et al, 2015)
- No sequenced *P. acnes* phage genome contains the genes necessary for true lysogeny (Liu et al, 2015)
- Pseudolysogeny is an alternative life cycle in which the phage genome exists as an episome, which functions like an extrachromosomal plasmid, rather than integrating into the host genome (Ackermann and DuBow, 1987)
- To be an ideal phage therapy candidate for acne vulgaris treatment, a phage should have a broad host range against *P. acnes* and should not be lysogenic
- The purpose of this project is to characterize isolated phage Leviosa in order to assess whether it could be used in phage therapy

Hypothesis and Approach

Since all previously isolated *P. acnes* phages have broad lysing abilities against *P. acnes* and are incapable of true lysogeny, if a novel *P. acnes* phage is isolated and assessed for infectivity, then this novel phage will likewise have a broad host range against *P. acnes* and undergo a lytic or a pseudolysogenic life cycle.



Leviosa Siphoviridae Morphology via TEM

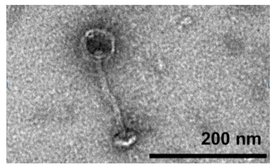


Figure 1. Transmission electron microscopy image of Leviosa magnified 30,000x. A small volume of high titer lysate was diluted 1:2 and a negative uranyl acetate stain was used to visualize. TEM revealed that Leviosa has a 139.55 ± 1.31 nm long, non-contractile tail and an icosahedral head with a 50.78 ± 4.11 nm diameter, which are characteristic of *Siphoviridae*.

Leviosa Bullseye Plaque Morphology

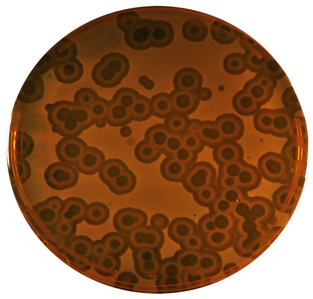


Figure 2. Plaque assay conducted with 10^{-4} dilution of Leviosa lysate on A Media agar and ATCC 6919. The soft agar overlay method was used to plate, and the plate was incubated at 37 °C under anaerobic conditions for three days. A negative control containing only SM-buffer did not yield any plaques. The overgrowth of bacteria in the presence of phage, as shown by the bullseye plaque morphology, is indicative of potential pseudolysogeny.

Leviosa Pseudolysogeny Test

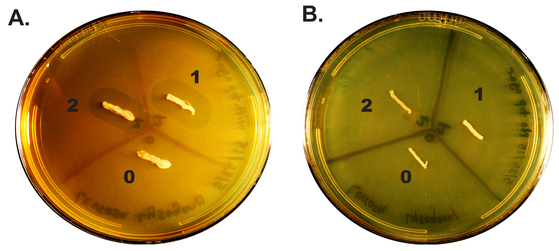


Figure 3. Pseudolysogeny patch test. Potential pseudolysogens from mesas were streak purified and incubated. They were then streaked diagonally onto A media plates (A) with top agar and *P. acnes* ATCC 6919 and (B) without top agar and *P. acnes* ATCC 6919. Clearings were seen around potential pseudolysogens 1 and 2 in the plate with top agar and additional *P. acnes*, suggesting viral release from the streaks. Since these plates did not have additional phage added, viral release indicates that phage was present within the bacteria. This is initial evidence for pseudolysogeny, though further streak purification is necessary.

Leviosa Genome Comparison via Phamerator

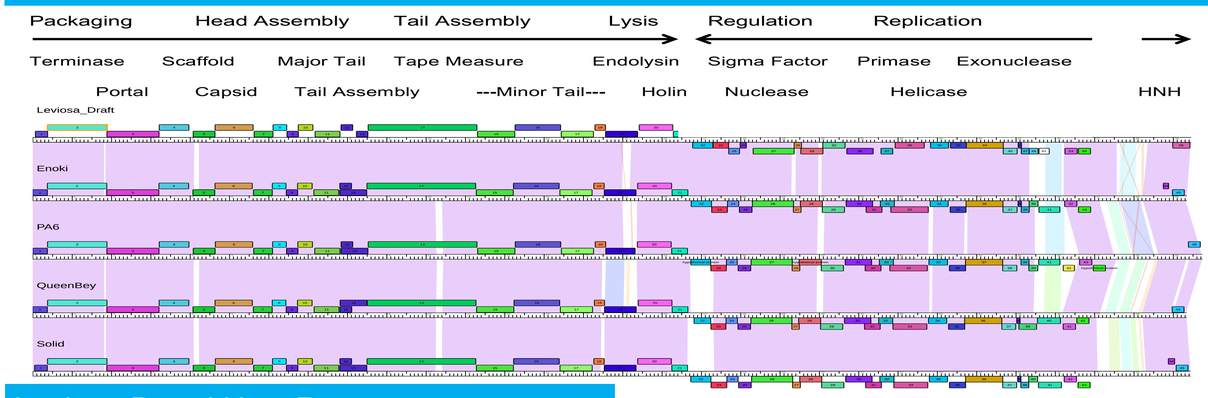


Figure 4. Phamerator comparison of Leviosa's genome to other *P. acnes* phage genomes. The genomes are highly conserved, and none of the phages' genomes contain lysogenic modules. The left half of each genome contains primarily structural genes transcribed in the forward direction, while the right half of each genome contains regulatory and replication-associated genes transcribed in the reverse direction. Leviosa's genome as depicted is a draft version which has since been updated.

Leviosa Broad Host Range

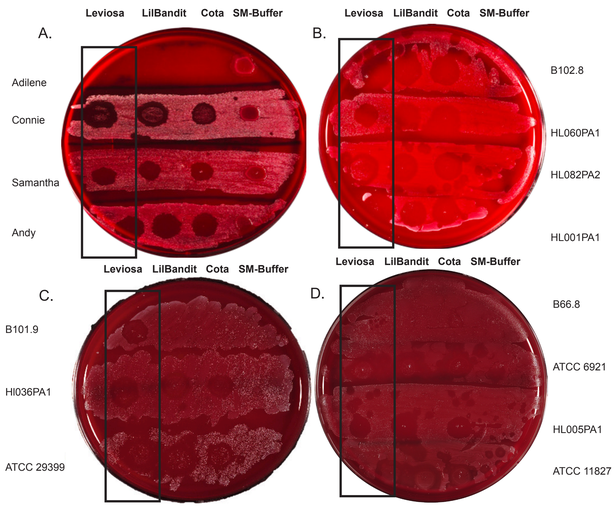


Figure 5. Determination of Leviosa host range. Different *P. acnes* isolates in liquid culture were spread onto Brucella blood agar plates. Different phages or SM-buffer were then allowed to adsorb, and plates incubated under anaerobic conditions at 37°C for seven days. From left to right: Leviosa, LilBandit, Cota, and SM-buffer (A) Top to bottom: putative *P. acnes* isolates from Adilene, Connie, Samantha, and Andy (B) Top to bottom: *P. acnes* clinical isolates B102.8, HL060PA1, HL082PA2, and HL001PA1 (C) Top to bottom: *P. acnes* clinical isolates B101.9, HL036PA1, and ATCC 29399 (D) Top to bottom: *P. acnes* clinical isolates B66.8, ATCC 6921, HL005PA1, and ATCC 11827 (Marinelli et al, 2012).

Leviosa CRISPR Analysis

Strain	Spacer	Spacer Sequence	Mismatches/ Total Sequence Length (nt)	Database Match
B66.8	1	GATCGAGTTGGCTGAGTCGAAGGTGTTGCGGTT	5/33	*PA6 gp16 (13172-13204)*
B66.8	2	CTGCTCATCGCTCAGCTCCTGCGCCTCATCACA	15/33	*PA6 gp2 (944-912)*
B66.8	3	CTGCGCCAACAGCCGCATCTGATCCGAATACGG	11/33	*PA6 gp3 (2770-2738)*
B66.8	4	CGCAGCAATCTCAGAAGGCCACAACAAGTTCGT	7/33	*PA6 gp7 (5931-5899)*
B66.8	5	CAAATCACCCAAGCCCAACACGCCGCCACCACC	13/33	*PA6 gp29§ (20127-20095)*
B66.8	6	TGTCACCGATTCAATGTATCTATGAGTGGTGTA	20/33	None
B66.8	7	TTGGGTGGGTGAGGTCGGGTCGTCAGTCATGAG	23/33	None
B66.8	8	GTCGATGTCGAGATTGGCCTGGGGGTCCATGTC	19/33	*P. acnesmobile element*
B66.8	9	ACGTCGTGAACGTACCCCTTGACGGAGACGGCA	21/33	None

Strain	Spacer	Spacer Sequence	Mismatches/ Total Sequence Length (nt)	Database Match
B101.9	1	GTCTTCTGGGGTGCAGTCGATGTTGGCTGAGCG	11/33	*PA6 gp9 (6511-6543)*
B101.9	2	GTCTTCTGGGGTGCAGTCGATGTTGGCTGAGCG	11/33	*PA6 gp2 (479-447)*
B101.9	3	CTGCGCCAACAGCCGCATCTGATCCGAATACGG	11/33	*PA6 gp3 (2770-2738)*
B101.9	4	CGCAGCAATCTCAGAAGGCCACAACAAGTTCGT	7/33	*PA6 gp7 (5931-5899)*
B101.9	5	CAAATCACCCAAGCCCAACACGCCGCCACCACC	13/33	*PA6 gp29§ (20127-20095)*
B101.9	6	TGTCACCGATTCAATGTATCTATGAGTGGTGTA	21/33	None
B101.9	7	TTGGGTGGGTGAGGTCGGGTCGTCAGTCATGAG	23/33	None
B101.9	8	GTCGATGTCGAGATTGGCCTGGGGGTCCATGTC	19/33	*P. acnesmobile element*
B101.9	9	ACGTCGTGAACGTACCCCTTGACGGAGACGGCA	19/33	None
B101.9	10	ACGTCGTGAACGTACCCCTTGACGGAGACGGCA	19/33	None

Figure 6. BLASTn and local alignment using EMBOSS Water were used to align all known spacer sequences of resistant *P. acnes* strains B66.8 and B101.9 to Leviosa's genome to determine the presence of phage protospacers. More mismatches suggest phage resistance to CRISPR defense. Conversely, little to no mismatches suggest susceptibility to the bacterial immune response. Left: Comparison between *P. acnes* strain B66.8 CRISPR spacers and Leviosa's genome sequence. Right: Comparison between *P. acnes* strain B101.9 CRISPR spacers and Leviosa's genome sequence. Mismatches are indicated in red and counted relative to the total spacer sequence length. Strain B66.8, which is more resistant to infection by Leviosa, had the spacer with the least mismatches (Spacer 1). Of note, all of the mismatches in that spacer are located in the 3' end of the sequence, which is thought to be less important than the 5' end for CRISPR recognition. This is consistent with the phage being more susceptible to the bacterial CRISPR defense and the concomitant resistance of the bacteria to the phage. Strain B101.9 is susceptible to infection by Leviosa, which is consistent with the majority of the spacer mismatches being located in the 5' ends of the spacer sequences.

Conclusions

- Isolated novel *P. acnes* phage of *Siphoviridae* morphology
- Potential pseudolysogenic properties
  - -Bullseye plaque morphology
  - -Mesas with subsequent patch test clearings
  - -No lysogenic genes found in genome annotation
- Fully lysed all but two *P. acnes* strains
- Genome contained no identical CRISPR protospacers to known spacers in *P. acnes* strains
  - -B101.9 (susceptible to Leviosa)
  - -B66.8 (partially resistant to Leviosa)
- CRISPR defense cannot fully explain Leviosa's infectivity pattern

Future Directions

- Further host range analysis
  - -Pathogenic and commensal *P. acnes* ribotypes
  - -Other commensal bacteria found on human skin
- Pseudolysogeny experiments
  - -Multiple streak purification assays of potential pseudolysogens between mesa and patch test steps
  - -Induction with UV and other agents

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Acknowledgements

We want to give thanks to our TA, Rocky Ng, Dr. Reddi, Dr. Marinelli, and Dr. Parker for their guidance and support.