Fusobacterium necrophorum phages as a potential tool for bovine liver abscess management

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Abstract

In the beef cattle industry, prophylactic treatment with antibiotics targeting putative etiologic agent of liver abscess formation, *Fusobacterium necrophorum*, has been shown to be inadequate, resulting in notable product losses ($60m per year) - a situation that would considerably worsen if antibiotics were curtailed. Public sentiment and stringent regulations are increasing against antibiotic feed additives, given their perceived role in the proliferation of resistant bacterial strains. To address this, our work has looked to phage therapy as a potential alternative, or adjunct, to antibiotics. Here, we present the isolation and characterization of six phages with the ability to infect *F. necrophorum* and document the efficacious application of four of isolates as part of high-dose, sequential treatment protocol, utilizing two distinct rotating cocktails to suppress a challenge strain in the rumen of nine cannulated calves. These findings underscore the potential of lysogenic phages as therapeutic agents for sustained bacterial population control when lytic phages prove challenging to acquire.

Methodology

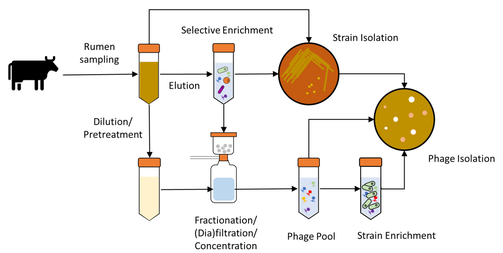


Fig. 1. Overview of phage isolation workflow. Phage pools were prepared from bovine rumen fluid, sequentially filtered, and PEG precipitated. Aliquots were subjected to both host and substrate enrichment. Prophages were also induced using both heat shock and mitomycin C.

Table 1. Animal trial design.

Animal ID	Treatment	Sampling Schedule	Frequency	Duration	Washout
1	Control	3X daily during treatment, 1X daily all other days	2X Daily	3 Days	4 Days
3					
6					
4	Rotated Low Dose Cocktail				
5					
9					
7	Rotated High Dose Cocktail				
8					
10					

Results: Isolation and Characterization

Table 2. Summary of host ranges of six phage isolates.
†phage obtained through mitomycin induction, ‡phage obtained from ruminal enrichment

Subspecies	Strain	Obtained from:	ϕFN37†	ϕHugo‡	ϕPaco‡	ϕRTG5†	ϕKSUM‡	ϕBB
necrophorum	25286	ATCC	+	+	-	-	+	+
necrophorum	A	KSU	+	-	-	-	+	+
necrophorum	C	KSU	+	+	-	-	+	+
necrophorum	FN38	KSU	-	+	-	-	+	-
necrophorum	8L1	KSU	+	+	-	-	+	+
funduliforme	FF16	KSU	-	-	-	+	-	-
funduliforme	FF34	KSU	-	-	-	+	-	+
funduliforme	KL3	laboratory	-	-	+	+	+	-
funduliforme	RTG5	laboratory	-	-	-	-	-	-

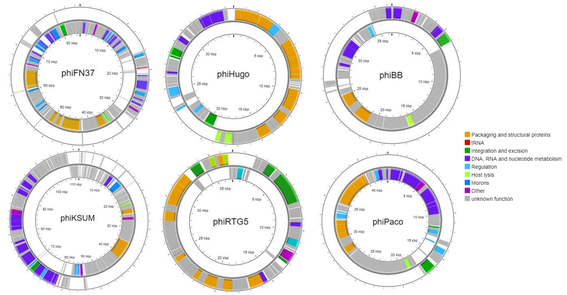
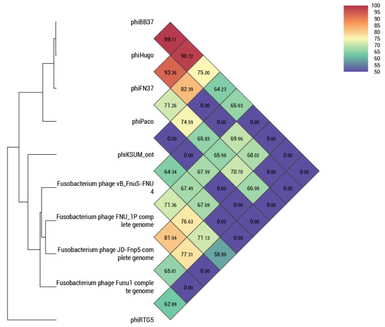


Fig. 2. Genome sequencing and annotation revealed a collection of diverse phages ranging from 111kbp to 35kbp. All contain genes associated with lysogeny. None contained known virulence factors or resistance genes of concern, though the two larger genomes (ϕFN37 and ϕKSUM) each contained several morons and all phages contained at least one DNA methyltransferase (1).

Figure 3. OrthoANI Analysis (2) indicated that three of the six phages isolated were very similar though they were not closely related to other phages capable of infecting other members of the *Fusobacterium* genus. ϕKSUM clustered most closely with genomes which were returned by discontiguous megablast. ϕRTG5 was found to be the most dissimilar of the six phages isolated.



Results: Cocktail Design

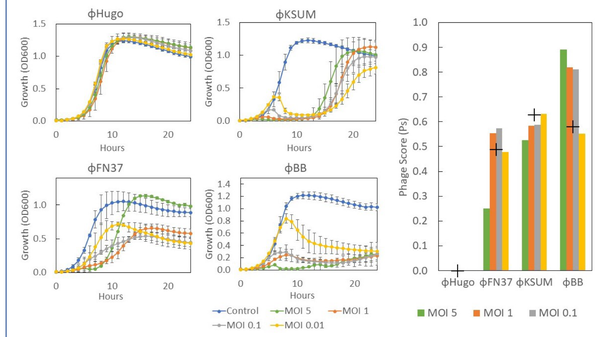


Fig. 4. Targeting test strain 8L1 (highlighted in table 2), killing efficiency (as measured by Phage Score and Phage Total Score (3)) was measured at MOIs of 5, 1, 0.1 and 0.01 over 24hours. ϕHugo had no effect on this strain, while ϕBB and ϕKSUM demonstrated similar behavior to that observed in their production strains. Most notably, ϕFN37 showed better inhibition of the test strain than its native strain (data not shown), achieving a higher Phage Score at MOIs of 0.1 and 0.01.

Table 4. Four Fusobacterium necrophorum subsp. necrophorum phages were checked for cross resistance. A, always resistant; S, sometimes resistant, N, never resistant

Prior phage exposure	φFN37	φHugo	φKSUM	φBB
φFN37	A	S	S	N
φHugo	S	A	N	N
φKSUM	A	A	A	N
φBB	N	N	N	N

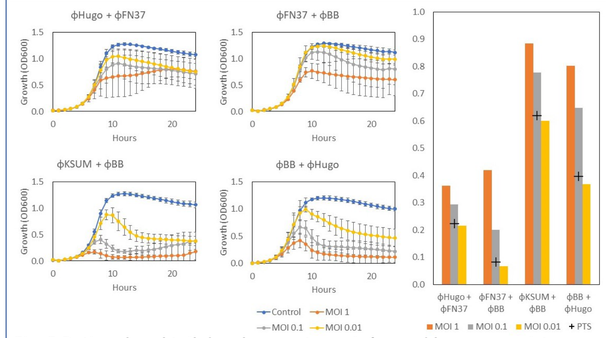


Fig. 5 Rational cocktail development was initiated by cross resistance. Phage pairs added in a 50:50 ratio were monitored at MOIs of 1, 0.1, and 0.01 over 24 hours, with ϕKSUM + ϕBB pair showing the highest Pts, leading to the selection of the other cocktail: ϕHugo+ ϕFN37.

Results: Animal Trial

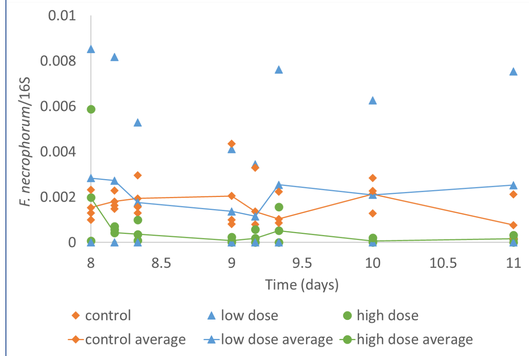


Fig. 6. Relative abundance of ruminal *F. necrophorum* after phage treatment. Cannulated calves were administered phosphate buffered saline (controls), 10^9 PFU, or 10^{11} PFU of two rotated, phage cocktails (2 phages each) twice daily for three days (day 7 – 9). Each calf was challenged with *F. necrophorum* 8L1 (10^{10} CFU) on day 7, and monitored according to the trial schedule (Table 1), followed by sample analysis by qPCR (4). High dose, rotated phage cocktails inhibited *F. necrophorum* growth in cattle rumen by 86% relative to untreated controls (AUC). Phage treatments were demonstrated to be safe with no adverse effects.

Discussion and Conclusions

- ❑ Phages isolated bear little similarity to other complete phage sequences within current databases, only returning results using discontiguous megablast.
- ❑ Lysogenic capacity as detected by genetic sequencing does not necessarily predict performance.
- ❑ Rational cocktail development is predicted by phage score and cross resistance capacity.
- ❑ Lysogenic phages can provide alternative avenues for enhancing phage therapy applications where lytic phages are challenging to acquire.
- ❑ Phage-mediated control of ruminal *F. necrophorum* is possible and safe over short durations.

Ongoing Research

- ❑ Analysis of animal trial data is ongoing to monitor phage retention within the rumen and to reveal any community shifts in response to treatment.
- ❑ Additional studies to assess efficacy over longer periods are warranted.

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