234

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BACTERIAL VIRUSES IN THE FEMALE URINARY MICROBIOME

Hypothesis / aims of study

Prior research identified many bacterial components of the bladder microbiota. The viral fraction, however, is largely unknown. Viruses in the human microbiome far outnumber bacteria; the most abundant viruses are those that infect bacteria (bacteriophages). Bacteriophages (phages) play key roles shaping bacterial metabolism and community structure. Thus, previously observed links between urinary bacteria, clinical symptoms and outcomes may be due, in part, to phage activities. The study's aim was to isolate and characterize phages from the urinary microbiome of females with and without symptoms of urgency urinary incontinence (UUI).

Study design, materials and methods

We sequenced genomes from bacteria isolated previously from urines obtained from women enrolled in IRB-approved female urinary microbiome studies focused on UUI. By analyzing these sequenced bacterial genomes, we identified numerous phage species that had integrated their genomes into their host ("latent" phage). Latent phage genome sequences were identified using the tools VirSorter [1] and Phaster [2] and results were manually curated. These phage sequences were next compared to publicly available phage genome sequences in NCBI's GenBank through BLAST queries.

Bacterial isolates harboring such latent phage genomes were cultured under conditions that induced latent phages to reproduce and release mature virions into the culture medium ("active" phage). To assess the active phage's host-range, each isolated phage was plated onto diverse bacterial strains, including lab strains and clinical isolates. Phage growth characteristics were evaluated in culture with each of their identified hosts. Morphology of the isolated phages were assessed using transmission electron microscopy (TEM).

Results

From >100 urinary bacterial genomes isolated from unique women with and without UUI symptoms, we detected upwards of 5 latent phage sequences per genome. There is a rich diversity of novel active phage species in the bladder microbiota; latent phage genome sequences identified within the bacterial urinary isolates exhibit little to no similarity to sequenced phage species in extant databases.

From urinary bacteria, 9 active phages able to infect *Escherichia coli* were isolated. These isolated phages were tailed phages, as determined by TEM (**Fig. 1**). Although morphologically similar, they differ with respect to their genome sequences and genome size ([3] and unpublished results). Furthermore, while these phages could infect standard laboratory strains of *E. coli*, their ability to infect urinary *E. coli* isolates varied (**Table 1**), signifying specificity and thus diverse mechanisms of host infection. The burst (number of progeny produced per infection) and generation time (time from infection to the release of mature virions) varied between strains.

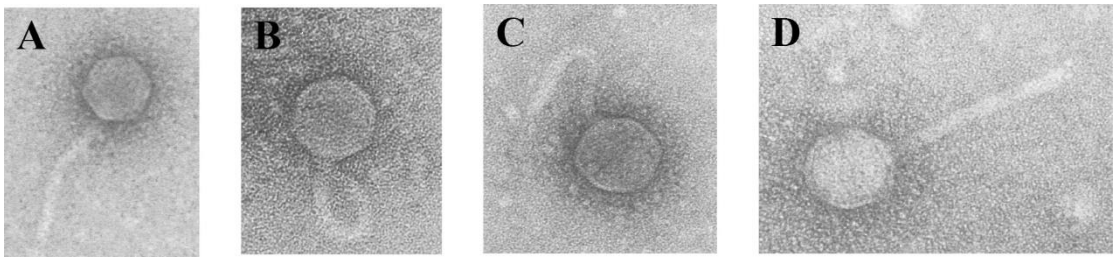

Fig. 1. TEM micrograph of phage Envy isolated from unique women with UUI **(A)** Gluttony, **(B)** Lust, **(C)** Greed, and **(D)**.

Phage	Lab. strains		Urinary Isolates					
			Control	ABU	UTI		Cystitis	Pyelonephritis
Species	C	K12	UMB789	UMB901	UMB900	UTI89	NU14	CFT073
Lust	✓	✓	✓		✓			
Greed	✓	✓	✓		✓			✓
phiA	✓	✓						
phiJ	✓	✓						

Table 1. Host-range of 4 of the *E. coli*-infecting phages isolated from the urinary microbiome.

Interpretation of results

Latent and active phages are abundant in the female urinary microbiome. Active phages can be collected from bacterial cultures for subsequent analyses. These phages are unique to the urinary microbiome; nevertheless, it is worth noting that phage species within the human microbiome are largely uncharacterized to date.

Concluding message

Discovery of phage species able to selectively infect urinary bacteria and shape bacterial communities in the bladder could inform treatment, as phage could be used as an alternative to antibiotics and/or to augment efficacy of current treatment regimes for urinary disorders.

References
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Disclosures
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