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ABSTRACT Bacterial viruses (bacteriophages) play a significant role in microbial community dynamics. Within the human gastrointestinal tract, for instance, associations among bacteriophages (phages), microbiota stability, and human health have been discovered. In contrast to the gastrointestinal tract, the phages associated with the urinary microbiota are largely unknown. Preliminary metagenomic surveys of the urinary virome indicate a rich diversity of novel lytic phage sequences at an abundance far outnumbering that of eukaryotic viruses. These surveys, however, exclude the lysogenic phages residing within the bacteria of the bladder. To characterize this phage population, we examined 181 genomes representative of the phylogenetic diversity of bacterial species within the female urinary microbiota and found 457 phage sequences, 226 of which were predicted with high confidence. Phages were prevalent within the bladder bacteria: 86% of the genomes examined contained at least one phage sequence. Most of these phages are novel, exhibiting no discernible sequence homology to sequences in public data repositories. The presence of phages with substantial sequence similarity within the microbiota of different women supports the existence of a core community of phages within the bladder. Furthermore, the observed variation between the phage populations of women with and without overactive bladder symptoms suggests that phages may contribute to urinary health. To complement our bioinformatic analyses, viable phages were cultivated from the bacterial isolates for characterization; a novel coliphage was isolated, which is obligately lytic in the laboratory strain *Escherichia coli* C. Sequencing of bacterial genomes facilitates a comprehensive cataloguing of the urinary virome and reveals phage-host interactions.

IMPORTANCE Bacteriophages are abundant within the human body. However, while some niches have been well surveyed, the phage population within the urinary microbiome is largely unknown. Our study is the first survey of the lysogenic phage population within the urinary microbiota. Most notably, the abundance of prophage exceeds that of the bacteria. Furthermore, many of the prophage sequences identified exhibited no recognizable sequence homology to sequences in data repositories. This suggests a rich diversity of uncharacterized phage species present in the bladder. Additionally, we observed a variation in the abundances of phages between bacteria isolated from asymptomatic "healthy" individuals and those with urinary symptoms, thus suggesting that, like phages within the gut, phages within the bladder may contribute to urinary health.

KEYWORDS bacteria, bacteriophages, microbiome, prophage, urinary tract, virus

Surveys of the bacteria inhabiting the human body have led to the discovery of new taxa (1), as well as a greater diversity among "healthy" individuals than originally hypothesized (for a review, see reference 2). Studies have identified associations

between the presence, absence, and/or prevalence of bacterial taxa and an individual's health and response to treatment (for reviews, see references 3–5). For instance, *Lactobacillus gasseri* within the bladder is strongly associated with urinary urgency incontinence (UUI) in women; this contrasts with *Lactobacillus crispatus*, which is associated with asymptomatic controls (6). An imbalance of the microbiota (dysbiosis) also can be connected with an individual's health, as reported within the gastrointestinal tract (7). In addition to bacteria, the microbial communities that colonize the human body include other cellular organisms (archaea and fungi) and viruses, all of which are involved in complex interactions with each other and surrounding human cells. Of particular note are the interactions between bacteria and their natural predators: bacteriophages (viruses that infect bacteria). Bacteriophages (phages) are crucial members of microbial communities, shaping bacterial metabolism and community structure (8, 9). Within the gut, for instance, this influence extends from infancy through adulthood (10, 11). Similar observations have been made within the microbiota of the oral cavity (see a review in reference 12).

Human microbiome surveys have identified phages within every community examined (13). In fact, they are the most abundant members of the human virome, far exceeding eukaryotic viruses (13). Despite their prevalence, phages are significantly understudied in comparison to their hosts. As such, phages detected within the human microbiome are often novel, with their sequences exhibiting no recognizable similarity to those of characterized species (13, 14). While phages can exploit a variety of infection strategies, lytic and lysogenic phages are most frequently found. Lytic phages are exclusively predatory, killing their host cell. Lysogenic phages have two lifestyle choices: they can be lytic immediately, or they can incorporate their genome into the host's bacterial genome or remain in the cytoplasm as a plasmid. The phage genome (now called a prophage) generally replicates in synchrony with the host chromosome until some signal induces the phage to enter the lytic path. Within the gut microbiota, this shift from lysogeny to the lytic path has been correlated with disease (14).

In comparison to the sites of the body investigated by the Human Microbiome Project (HMP), the urinary bladder is understudied. While new urinary microbiome research has primarily focused on the bacterial constituents of this community (5), a few studies have shown that the urinary microbiota also includes viruses. First, viruses have been isolated from urine, including several eukaryotic viruses (15–21) and phages (22, 23). Second, two metagenomic sequencing studies of the urinary virome (eukaryotic viruses and phages in the lytic cycle) have been conducted (24, 25). Although these virome studies found that phages vastly outnumber (>99%) eukaryotic viruses, they have sampled but a fraction of the phage community; they did not capture the diversity of prophages present.

While prophages can account for up to 20% of a bacterial genome (26), they are not simply passengers along for the ride; they can be selectively advantageous or disadvantageous to the host cell (26). For example, harboring a prophage can provide the bacterial host with protection from superinfection (27, 28). The presence of a prophage also can increase bacterial fitness through phage genes; for instance, the λ phage genes *bor* and *lom* increase the *Escherichia coli* host's survival in macrophages (29). Furthermore, a prophage can encode a critical virulence factor. For example, the botulinum, Shiga, and cholera toxins, among others, are encoded by prophages (30). In the event that a prophage reduces its host's fitness, selection should lead to inactivation and/or deletion (31). In fact, the Shiga toxin in *Shigella dysenteriae* 1 is the functional relic of a once-functional prophage (32).

Based upon prior sequencing of bacterial isolates from the bladder and the abundance of prophage sequences detected (33, 34), we hypothesize that the prophage population in the urinary microbiota is large. This is a reasonable assumption, as lysogenic phages have been found to outnumber obligately lytic phages within the microbiota of the gut, particularly in the microbiota of healthy individuals (14). Because the urinary microbiota (35–37) exists at a substantially lower biomass than the gut (38) and prior research has found that the frequency of lysogeny is inversely related to

TABLE 1 Number of phage sequences identified within the 181 genomes for each confidence category

Phage type	Category	No. of phage sequences	No. of bacterial isolates
Unintegrated	1	7	3
	2	41	28
	3	67	47
Integrated	4	8	8
	5	170	105
	6	164	95

susceptible host density (39–42), we anticipate that the abundance of lysogenic phages may exceed that of the gut. To explore the lysogenic phage population of the urinary microbiome, we sequenced 181 genomes that represent the phylogenetic diversity of bacterial species isolated from the bladder (K. Thomas-White, S. C. Forster, N. Kumar, M. Van Kuiken, C. Putonti, M. Stares, E. E. Hilt, T. K. Price, A. J. Wolfe, and T. D. Lawley, unpublished data). Within these sequenced genomes, which represent 51 bacterial genera and 102 species, phage sequences were identified and characterized. Whereas seeking phage sequences within bacterial genomes would preclude the detection of most lytic phages, culture-dependent methods, such as those employed here, provide a key piece of information about the phages identified: each phage's native host. The result is the first catalogue of the rich diversity of uncharacterized phage species present in the bladder microbiota.

RESULTS

The genome assemblies of 181 bacterial isolates from the bladder were examined for the presence of phage sequences. These bacterial isolates were collected from women both with and without lower urinary tract symptoms (LUTS) and were selected to represent the phylogenetic diversity of bacteria within the urinary microbiota (see Data Set S1 in the supplemental material) (Thomas-White et al., unpublished). The taxonomy of each isolate was determined by 16S rRNA gene analysis (Data Set S2). Clustered regularly interspaced short palindromic repeat (CRISPR) arrays were identified within 109 of the 181 strains sequenced (Data Set S1). The tool VirSorter (43) was used to detect the phage sequences of both prophages that are integrated into the bacterial chromosome (categories 4 to 6) and phages that are not, including lytic phages and prophages existing as an extrachromosomal plasmid (categories 1 to 3). Given that this tool can detect both lytic and lysogenic phages, we refer to categories 1 to 3 as "unintegrated phages" and categories 4 to 6 as "integrated prophages." Category designations indicate the confidence of the prediction defined by VirSorter (43). Categories 1 and 4 are the most confident predictions, while 3 and 6 are the least. Here, we define categories 1, 2, 4, and 5 as "high-confidence" predictions and categories 3 and 6 as "lower-confidence" predictions. In total, 226 high-confidence phage sequences and 231 lower-confidence phage sequences were identified (Table 1 and Data Set S3). The nucleotide sequences for these phages are included in Data Set S4. Each phage sequence was annotated; the presence/absence of a terminase-coding region was used as a marker to assess if the phage sequence identified by VirSorter was representative of a complete phage genome. The majority of sequences in the high-confidence group (85%) contained a recognizable terminase-coding region. In contrast, many of the phage sequences predicted with lower confidence lacked an identifiable terminase-coding region. Upon further investigation, most (~71%) of the phage sequences within this lower-confidence group are partial phage genome sequences, which is either a residual of the assemblies themselves (as genomes are in scaffolds) or defunct integrated phages.

Phage sequences were abundant among the bacterial isolates from the bladder; 86% of these isolates had one or more phages associated with their genome sequence (Fig. 1). The number of phage sequences found within a genome had a low correlation
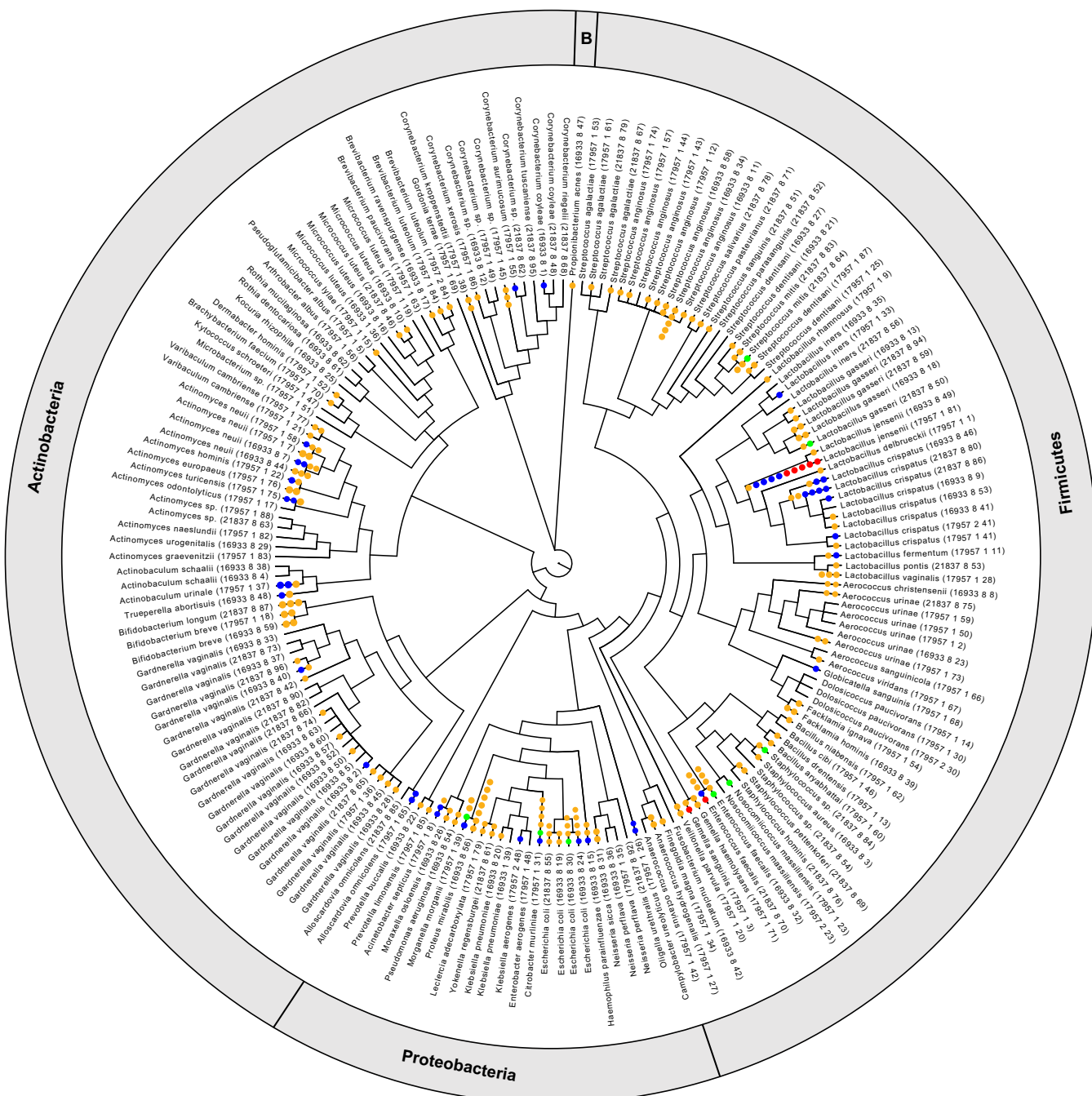
FIG 1 Phage presence within bacteria of the bladder. Each phage sequence identified at a high confidence is represented by a dot on the isolate's branch in the phylogenetic tree, which was built using the 16S rRNA gene sequences of the isolates. The phyla for the isolates in the phylogenetic tree are indicated in the outer circle, with "B" indicating the single isolate from the phylum *Bacteroidetes*. Red and blue circles represent unintegrated phages of categories 1 and 2, respectively. Green and yellow circles represent integrated phages of categories 4 and 5, respectively. It is important to note that branches without dots may include lower-quality predictions (categories 3 and 6).

with the genome's size (Spearman's $\rho = 0.09$ for high-confidence predictions and $\rho = 0.21$ for all predicted phages, with P values of 0.26 and 0.005, respectively). Additionally, no significant difference was found between the average length of the genomes of bacterial isolates containing phage and those lacking phage (with P values of 0.43 and 0.13 for high-confidence and lower-confidence predictions, respectively).

In characterizing the phage population within the bladder, we first focused on the 226 phage sequences predicted with high confidence, as these were found to resemble
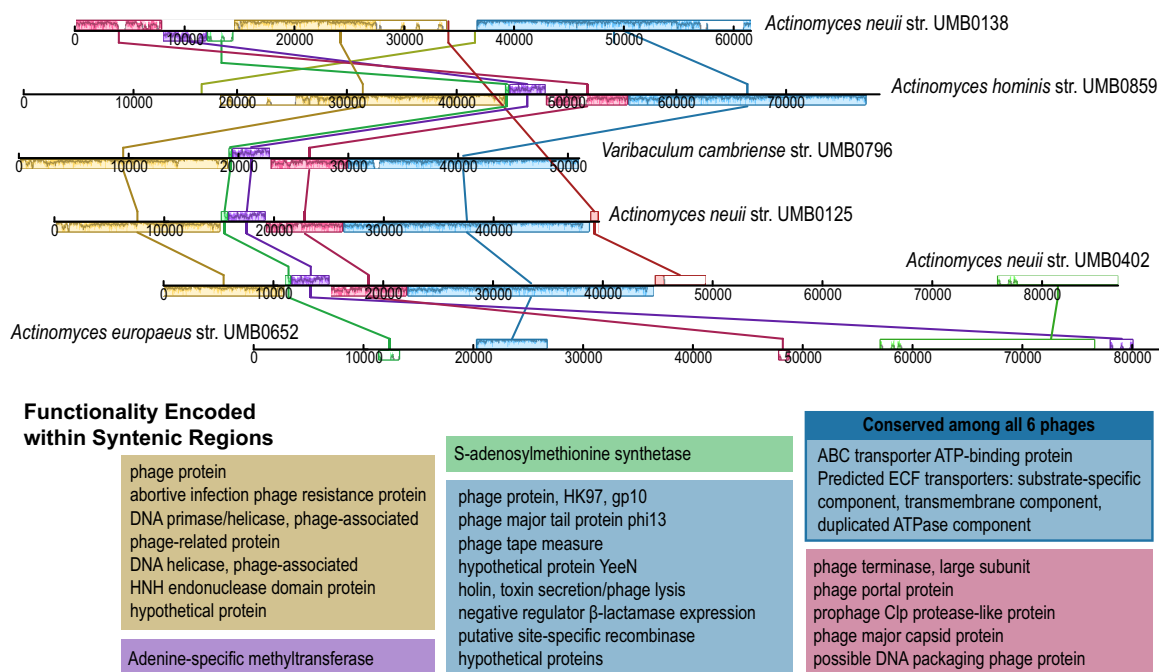
FIG 2 Phage conserved among *Actinomycetaceae* strains isolated from different patients. Syntenic regions are shown between the strains. The annotations for coding regions within each syntenic block are listed in the corresponding colors. ECF, energy-coupling factor.

intact viable phages. These sequences were compared to all bacterial sequences in public repositories, as well as all publicly available viral genomic sequences. Eighty-five of the 226 high-confidence phages exhibited sequence similarity to prophage sequences within previously sequenced bacterial genomes (Data Set S5; see Materials and Methods for further details), many from the sequencing efforts that established the human microbiome reference set. In contrast, only six phages resembled previously characterized isolated phages at a strict threshold (>50% phage genome query coverage), while an additional six phages were identified at a more relaxed threshold (>25% phage genome query coverage). The majority of high-confidence phage sequences (129/226 [57%]), however, exhibited no sequence similarity to any known phages. Thus, we have uncovered 129 novel phages from the urinary microbiota. A similar examination of the lower-confidence phage sequences (Data Set S6) revealed 91 hits to previously sequenced bacterial genomes. Again, the majority of sequences in this second lower-confidence set (140/231 [61%]) appear to be novel.

The phage sequences identified within the bladder's bacterial isolates, including both the high- and lower-confidence sets, were next compared to each other. Significant sequence homology was detected within the phages found in isolates of the bacterial family *Actinomycetaceae*, including five *Actinomyces* strains and one *Varibaculum cambriense* strain (Fig. 2), each isolated from a different patient. The first five phages in Fig. 2 were predicted within the higher-confidence set; in contrast, the prophage from *Actinomyces europaeus* strain UMB0652 was predicted to be in category 6 and thus in the lower-confidence set. Next, these six phage sequences were annotated via RAST (44), revealing several phage and phage-associated coding regions (Fig. 2). The ~60-kbp region conserved among five of the six *Actinomycetaceae* phages discovered in this study showed greatest similarity to a prophage within the genome of *Corynebacterium atypicum* strain R2070 (family *Corynebacteriaceae*) with 65% coverage and 90% nucleotide identity (megablast). Homologous regions between the *Corynebacterium* prophage and these *Actinomycetaceae* phages included "hallmark" viral gene sequences. We thus turned our attention to the hallmark viral genes of the capsid and terminase large subunit. blastx analysis of these genes to viral genomes in GenBank identified sequence homology (E value, 0) to *Streptococcus* phages, indicative
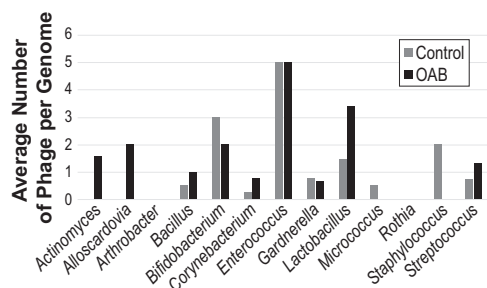
FIG 3 Average number of high-confidence phage sequences per genome isolated from control and OAB patients. Genera with representatives from both patient populations are shown.

of a common, albeit distant, ecological or evolutionary history between the *Strepto-coccus* and *Actinomycetaceae* phages. In addition to these *Actinomycetaceae* phages, several other phage sequences were found to include syntenic blocks across strains of bacteria from the bladder, including species from the genera *Gardnerella*, *Aerococcus*, *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, and *Escherichia* (Table S1).

The *Actinomycetaceae* phages shown in Fig. 2 were all found in strains isolated from women with overactive bladder (OAB), whereas the *V. cambriense* strain was obtained from a woman with stress urinary incontinence (SUI). In contrast, these *Actinomyceta-ceae* phages were not identified within any of the *Actinomyces* strains isolated from asymptomatic controls. While the purpose of this study was to explore phage diversity and prevalence within the bacteria of the bladder, the *Actinomycetaceae* phages motivated us to examine the phage populations relative to the patient condition. Looking more broadly at the data as "condition−" versus "condition+," a subset of the data was selected in which the patient had not received any treatment for urinary conditions. Thus, the condition− group included 69 genomes, and the condition+ group included 73 genomes (Data Set S1). The probability distributions of the number of high-confidence phage sequences between the two groups were not found to be statistically different, as assessed by calculating the generalized likelihood ratio test for comparing two negative binomial distribution parameters. Next, the phage populations were examined between the two cohorts by genus. A Wilcoxon test was performed; although there appears to be variation in the mean number of phage sequences within several genera, a marginally statistically significant difference was only detected within the genus *Lactobacillus* ($P = 0.06647$). It is important to note that not all *Lactobacillus* species chosen to be sequenced were collected from both populations; for example, the *Lactobacillus iners* genomes examined here were only from women in the control population, although *L. iners* is commonly found in the urinary microbiome of both condition− and condition+ women (6, 36). Figure 3 illustrates the number of high-confidence phages per isolate found within the condition− (control) population and the largest subset of condition+ patients, individuals with OAB symptoms ($n = 63$). (Similar analysis of the lower-confidence phages can be found in Fig. S1.) The mean count between the control and OAB populations was not statistically different for either category (high-confidence phages, $P = 0.2057$; lower-confidence phages, $P = 0.2084$).

Whereas we identified with high confidence an abundance of phage sequences within the bladder bacteria, some prophages integrated into the bacterial chromosome (categories 4 to 6) may be unable to enter the lytic cycle (i.e., defunct or defective prophages). For instance, eight phages were identified within the genome of *E. coli* strain UMB0901, including four predicted with high confidence. All four of these phage sequences are integrated prophages (categories 4 and 5) and exhibit the greatest sequence similarity to annotated prophages within *E. coli* genomes from the human microbiota (45) (Table 2). To determine if these prophages can enter the lytic cycle, *E. coli* UMB0901 was selected for further analysis in the lab. One phage was isolated (see Materials and Methods). While lysogenic within its UMB0901 host, this phage was capable of lysing laboratory strains of *E. coli* C and *E. coli* K-12 under both aerobic and

TABLE 2 Sequence examination of high-confidence phages within the genome of *E. coli* UMB0901

Category	Length (bp)	Best blast hit	Accession no.	Identity (%)	Query cover (%)
4	38,132	*Escherichia coli* K-15W01	CP016358	98	84
		Salmonella phage STYP2	KX833213	97	58
5	10,493	*E. coli* ECONIH2	CP014667	99	100
		E. coli K-15KW01	CP016358	99	99
5	63,683	*E. coli* 536	CP000247	99	100
		E. coli ECONIH2	CP014667	99	100
5	69,863	*E. coli* 542	CP018970	99	99
		E. coli ECONIH2	CP014667	99	88

anaerobic conditions; infection of the laboratory strain *E. coli* B was observed on several occasions, although not consistently. We also tested the phage's lytic activity on the other *E. coli* isolates from this study and several other *E. coli* bladder isolates in our collection (see Materials and Methods); lysis was not observed for any of these clinical isolates (data not shown). High-titer stocks of the purified phage were used to examine the phage's morphology via transmission electron microscopy (TEM) (Fig. 4). The presence of a contractile tail indicates that this phage belongs to the family *Myoviridae* of the order *Caudovirales*.

DISCUSSION

The bacteria isolated from the female urinary bladder include an abundance of bacteriophages. Considering just those phage sequences predicted with high confidence, the number of phage sequences exceeded that of the number of bacterial genomes examined. The abundance of prophage sequences within the urinary bacterial strains may be the result of the bladder itself; lysogeny may serve as a survival strategy when the bacterial community density is low or in the presence of other environmental parameters, e.g., temperature and metabolic community composition (39–42). Nevertheless, not all genomes examined in this study were found to contain phage sequences; in fact, 13.8% of the bacterial isolates from the bladder did not include any detectable phage sequence, and an additional 22% only contained phage sequences predicted at the lower-confidence level (see Data Set S3 in the supplemental material). Taxa that did not show any evidence of phage infection included all three *Dolosicoccus paucivorans* strains examined and several, but not all, isolates of *Gardner-*
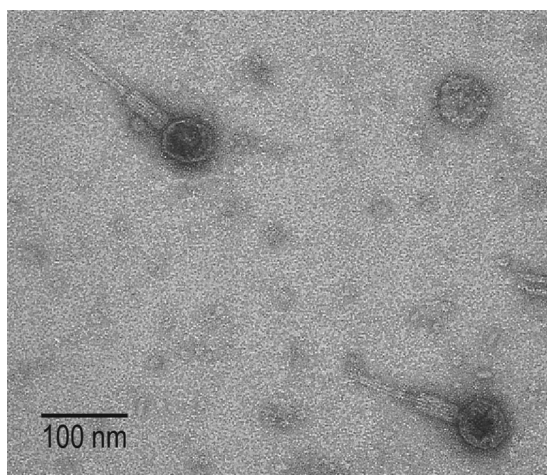
FIG 4 TEM of phage isolated from bladder strain *E. coli* UMB0901.

ella vaginalis and *Micrococcus luteus*. At present, a lytic phage from either *D. paucivorans* or *G. vaginalis* has yet to be characterized, although prophage sequences within *Gardnerella* spp. have previously been described (33). In contrast, a few phages capable of lysing *Micrococcus* strains were isolated and studied in the 1960s and 70s (see, e.g., references 46 to 48). The 226 phages predicted with high confidence were exclusive to just 116 of the genomes examined. No correspondence between the presence/absence of CRISPR arrays and prophage was observed (Data Set S1). While prior studies of the microbiomes of other niches have found a correlation between bacterial genome size and the number of prophages in genomes (49, 50), the strains from the bladder did not fit this expectation.

Whereas the number of phage sequences per genome varied, *Lactobacillus* strains from the bladder frequently harbored more than one phage. For example, *Lactobacillus jensenii* strain UMB0077 was the most phage-rich genome examined, with 10 phages predicted at high confidence and another three phages at lower confidence (Data Set S3). Surveys of the urinary microbiome have routinely found *Lactobacillus* species and provided evidence of their association with urinary symptoms (6, 35, 51–54). Lactobacilli also are dominant organisms within other niches, most notably the vagina (4, 55), and some species have direct correlations to health outcomes (56–58). Microbiome studies, particularly of the vaginal community, have discovered lytic phages of human-associated *Lactobacillus* species (see, e.g., references 59 to 61). While some of the *Lactobacillus* phages found in our study showed sequence homology to the previously characterized *Lactobacillus* phage Lv1 (60), 15 *Lactobacillus* phage sequences predicted with high confidence exhibited no sequence similarity to any known lytic or lysogenic phage. The fact that these urinary *Lactobacillus* genomes include prophages that have yet to be found within vaginal lactobacilli leads us to posit that a subset of the microbiota of these two niches may be isolated.

In addition to these novel *Lactobacillus* phages, novel phage genomes were also identified within other bacteria common to the urinary microbiome (5, 6, 36, 51, 62), including species of *Actinomyces* and *Varibaculum* (Fig. 2), *Bifidobacterium*, *Gardnerella*, and *Streptococcus*, as well as the uropathogen *Proteus mirabilis* (Data Set S3). This suggests a rich diversity of uncharacterized phage species present in the bladder, confirming prior conclusions of viral metagenomic analyses of urine samples (24, 25). This observation is not unique to the bladder virome, as approximately 50% of the phages within the gut virome cannot be classified (14). The phages isolated in prior studies (22, 25) and the single phage reported here (Fig. 4) are limited to two of the 51 bacterial genera found within the bladder. For many of the species within the bladder, there are no characterized lytic phages, and the ability for prophages to enter the lytic cycle is unknown. Thus, to understand intracommunity interactions, it is imperative to isolate and characterize phages from the bladder.

Further investigation into the diversity of phages within the bladder could provide critical information concerning shifts in bladder community structure. Recent studies have found that changes in the gut's phage population may be associated with dysbiosis and disease (11, 14, 63–65). Similar observations have been made from studies of the vaginal microbiota (4). Furthermore, prior studies have suggested that *Lactobacillus* phages could play a role in the shift in the vaginal community structure and contribute to bacterial vaginosis (for a review, see reference 66). The bacterial communities within the gut, vagina, and other areas targeted in the HMP are, however, significantly better characterized than those within the urinary bladder. The abundance of lysogenic phages found in the bladder suggests that phages also may contribute to urinary microbiota stability. Furthermore, the variation observed between the phage populations within the strains selected of individuals with and without OAB symptoms (Fig. 3), although not found to be statistically significant, suggests that phages may contribute to urinary health, thus warranting further study, including sequencing of multiple strains and species of genera found within both populations.

While metagenomic studies can comprehensively sample microbial communities, culture-dependent studies provide a key piece of information when investigating

phages, namely, information regarding the bacterial host. For example, in this study, we can associate each phage with at least one of its native host species. This includes the 129 novel and 140 putative phages that exhibit no sequence homology to any known annotated (pro)phage sequence in public sequence repositories (Data Sets S5 and S6). For those phages that do resemble a previously sequenced phage, the bacterial and/or phage sequences recognized are typically annotated as the same bacterial host, e.g., the prophages of *E. coli* strain UMB0901 most closely resemble prophages within other *E. coli* strains (Table 2). However, the sequence homology observed for the *Actinomycetaceae* phages (Fig. 2) suggests that their host range might extend across genera and even across taxonomic families. Similarly, analysis of two prophage sequences from *Actinomyces turicensis* strain UMB0250 and *Corynebacterium* sp. strain UMB0763 suggested that these phages may also be capable of infecting bacterial species of different families (Data Set S5). These findings suggest that some phages within the microbiota of the bladder may have a broad host range and should guide subsequent experimental work to test this hypothesis. While the notion of broad-host-range phages is controversial within the phage research community (67), bioinformatic and experimental studies have identified such phages (68–70). Host range itself is likely transient, as ecological factors, including drug treatment, may enable a phage's ability to infect a new bacterial host (71).

Our survey of lysogenic phages provides the first step in the process of unraveling the complex dynamics of phage-bacterium interactions within the urinary microbiota. Additional sequencing of the bacterial population within the bladder, coupled with further metagenomic sequencing of the urinary virome, is needed to both determine if a core "bladder phageome" exists and whether phages play a role in urinary health and disease.

MATERIALS AND METHODS

Bacterial isolates. The genome sequences examined in this study were of bacterial isolates isolated from urine collected during the conduct of several institutional review board (IRB)-approved studies. In the majority of cases, urine was collected aseptically via transurethral catheter. In a few cases, urine was collected by midstream void. In either case, the urine sample was placed in a BD Vacutainer Plus C&S preservative tube for culturing. Expanded quantitative urine culture (EQUC) was performed as described previously (36). Matrix-assisted laser desorption ionization–time of flight mass spectrophotometry (MALDI-TOF MS) with the MALDI Biotyper 3.0 software program (Bruker Daltonics, Billerica, MA) was used to identify the bacterial strains selected for sequencing, as described elsewhere (36).

Isolates were collected from individuals with or without lower urinary tract symptoms (LUTS). LUTS included overactive bladder (OAB; $n = 101$), interstitial cystitis/painful bladder syndrome (IC/PBS; $n = 1$), depression with diabetes (DD; $n = 1$), stress urinary incontinence (SUI; $n = 2$), or urinary tract infection (UTI; $n = 6$). One isolate was from a patient presenting with a kidney stone. Individuals without LUTS ($n = 69$) included a small cohort of pregnant women ($n = 16$). Thirty-nine of the samples collected were from individuals after LUTS treatment. The patient symptom status using these codes is indicated for each isolate in Data Set S1 in the supplemental material.

Genome sequencing and assembly. Genome sequencing and assembly are described in full by Thomas-White et al. (unpublished). Briefly, the bacterial isolates were grown in their preferred medium and pelleted. Genomic DNA was extracted using a phenol-chloroform method (72). Genome sequencing was completed at the Wellcome Trust Genome Campus (Hinxton, UK). The Illumina Nextera kit was used for whole-genome library preparation with fragment sizes of 200 to 300 bp. Sequencing was conducted on the Illumina HiSeq 2000 platform, producing paired-end 2×100-bp reads, and annotated assemblies were produced using the pipeline described previously (73). Sequence reads were used to create multiple assemblies using VelvetOptimiser version 2.2.5 (74) and Velvet version 1.2 (75). The assembly with the best N_{50} score was selected, and an assembly improvement step was performed using the tools SSPACE (76) and GapFiller (77). Additionally, we computed the genome coverage using BBMap (78). All reads and assemblies are publicly available via EBI and GenBank; accession numbers are listed in Data Set S1.

Phylogenetic analysis. Taxonomic classification of isolates was performed based upon the genome's 16S rRNA gene sequence. 16S rRNA gene sequences were detected through blastn queries to a local copy of the 16S rRNA gene sequences from the RNAmmer server (79). The bacterial isolates' sequences were aligned via CLUSTALW through Geneious version 9.1.4 (Auckland, New Zealand). A phylogenetic tree was derived using FastTree (80) through Geneious using the Jukes-Cantor model. The 16S rRNA gene sequences used for construction of this tree are included in Data Set S2. The tree was visualized using PhyloWidget (81).

CRISPR analysis of bacterial genomes. CRISPR spacer arrays were detected using the software minCED (82). Arrays containing three or more spacers were identified.

Phage identification and characterization. Contigs were examined using the tool VirSorter (43). Briefly, VirSorter examines sequences, looking for hallmark viral genes (e.g., capsid protein, terminase large subunit, and tail) and enrichment in virus-like genes. VirSorter predictions include confidence categories. Viral sequences, either prophages that remain in the cytoplasm in plasmid form or as lytic phages, are identified as category 1, 2, and 3 phages, corresponding to predictions that are "most confident," "likely," and "possible," respectively. Categories 4, 5, and 6 are reserved for prophage sequences detected within a cellular contig. Category 4 refers to the most confident predictions, whereas categories 5 and 6 correspond to likely and possible predictions, respectively. Further details regarding these categories can be found online (https://pods.iplantcollaborative.org/wiki/display/DEapps/VIRSorter+1.0.2).

All VirSorter-predicted sequences were compared via blastn to all bacterial nucleotide sequences (query: "bacteria[filter] AND ddbj_embl_genbank[filter]") and viral RefSeq (83) genomic sequences, collected in June 2017, and run locally. Phage sequences were also compared locally via blastx to annotated terminase protein sequences within the RefSeq viral collection. Predicted phage sequences of interest were further examined using the RAST annotation server (44), with blast queries of the complete nr/nt database via NCBI and by manual inspection. Upon manual curation, no sequences were found to be obvious misclassifications of viral sequences. VirSorter-predicted sequences were compared using reciprocal blastn queries locally to identify syntenic regions between the phage sequences. Phage sequences identified with a query coverage of ≥50% were clustered and examined further in Geneious via sequence alignment using Mauve (84).

Statistical analysis. Two negative binomial distributions with different parameters were used to model the probability mass functions of the number of phage sequences observed in the two samples: patients with and without LUTS. Then, a generalized likelihood ratio test was performed to test the hypothesis that their centrality parameters are equal, assuming they have the same dispersion (85). This means that we were testing whether the overall probability distribution of the number of phage sequences is the same in the two groups. A Wilcoxon test was performed to test for differences in the mean number of phage sequence counts between the control and disorder groups for each bacterial genus.

Phage isolation and characterization. *E. coli* isolates from the bladder were retrieved from −80°C storage, streaked on 1.7% LB agar plates, and incubated overnight at 37°C. Individual colonies were selected to inoculate flasks of LB medium and grown overnight with shaking at 120 rpm and 37°C. Cultures were then centrifuged, and the supernatant was filtered using 0.2-μm-pore-size cellulose acetate filters. Pour plates of the supernatant were made using laboratory strains of *E. coli*, including *E. coli* C (obtained from C. Burch, University of North Carolina), *E. coli* B (Ward's, VWR), and *E. coli* K-12 (ATCC 25404). These plates were prepared as follows: 3 ml of LB soft agar (0.7% agar), 500 μl of the respective *E. coli* culture (C, B, or K-12), and 100 μl of supernatant were poured atop a 1.7% LB agar plate. Plating was performed with *E. coli* cultures suspended during exponential growth or stationary phase.

Plaques were identified from the filtrate of the *E. coli* strain UMB0901 plated on *E. coli* C plates. The LB soft agar overlay was harvested and resuspended in LB. Phage stock was grown in high titer and triple filtered (as described above) for microscopy. Purified viral lysate was applied to Pioloform-coated copper grids and left to dry at room temperature. Samples were positively stained with 2% (wt/vol) uranyl acetate (86) and observed at 80 kV using a Hitachi H-600 transmission electron microscope (TEM).

Accession number(s). Raw sequencing reads are available through the NCBI under BioProject no. PRJNA316969 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA316969) and EMBL-EBI under accession no. PRJEB8104 (http://www.ebi.ac.uk/ena/data/view/PRJEB8104). Individual accession numbers for each isolate sequenced are listed in Data Set S1. Assembled genomes can be accessed via the PRJNA316969 BioProject link. The accession numbers for each individual assembled genome are also listed in Data Set S1.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JB.00738-17.

SUPPLEMENTAL FILE 1, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 2, CSV file, 18.9 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 4, CSV file, 19.1 MB.
SUPPLEMENTAL FILE 5, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 6, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 7, PDF file, 0.1 MB.

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