Harnessing the diversity of *Burkholderia spp*. prophages for therapeutic potential

by

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University of Pittsburgh, 2022

Burkholderia is a genus of gram-negative bacteria that naturally reside in the environment. Several species within this genus constitute a major health threat to humans, specifically for people with compromised immune systems or chronic lung diseases such as cystic fibrosis (CF) and chronic granulomatous disease (CGD). Members of *Burkholderia* are often resistant to antibiotics and infections with these organisms are difficult to treat, particularly those caused by bacteria in the *Burkholderia cepacia* complex (Bcc). A potential alternative treatment for these resistant infections is bacteriophage (phage) therapy: the therapeutic use of viruses that target bacteria. However, phage therapy is hindered by difficulty in locating bacteriophages that target members of the *Burkholderia* genus. Bacteriophages incorporated into a cell's genome, also known as prophages, have been identified within *Burkholderia*, and may represent a rich source of phages for antimicrobial therapy. The goal of this study was to investigate whether prophages within the genomes of *Burkholderia* clinical isolates can be harvested and used to kill conspecific and heterospecific species in the genus. Thirty-two *Burkholderia* isolates were screened for prophage release, and harvested prophages were tested for lytic activity using a plaque assay-based screen against the same 32 *Burkholderia* isolates. Through all-by-all screening and subsequent whole genome sequencing of phages and host bacteria isolates, we identified and characterized 4 unique bacteriophages of prophage origin. These newly isolated phages were characterized by host-range analysis, whole genome sequencing, and electron microscopy. Several phages showed a range of lytic activity on both conspecific and heterospecific species of *Burkholderia*. We also analyzed

prophage content of 35 *Burkholderia* clinical isolates and found that each isolate genome encoded between 0-4 different prophages. Some prophages were found to be present in the genomes of multiple isolates of the same species. Finally, while prophage abundance was not associated with phage susceptibility, we did observe that members of *B. cenocepacia* were more phage susceptible compared to members of *B. multivorans*. Overall, the data gathered in this study suggest that prophages present within *Burkholderia spp*. genomes are a potentially useful starting point for the isolation and development of novel bacteriophages for use in phage therapy.

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1.0 Introduction

1.1 *Burkholderia* Bacteria

Burkholderia is a genus of gram-negative bacteria, encompassing a large number of diverse species (1). These organisms are abundant in the environment, are found readily in both soil and water, and are associated with the rhizospheres of several species of plants (2),(3). *Burkholderia* are impressive habitat generalists, meaning that they show broad environmental tolerance and metabolic flexibility. These species are capable of using a wide range of energy sources for growth and metabolism (2). Some bacteria within this genus have been shown to possess the ability to break down environmental pollutants and even use the antibiotic Penicillin G as a sole carbon source (2). Members of this genus have particularly large genomes (6-9 Mb), likely aiding in their flexible lifestyle (2).

Burkholderia bacteria are also opportunistic pathogens of humans, particularly members of the notorious *Burkholderia* cepacia complex (Bcc), a group of at least twenty species which possess high levels of intrinsic resistance to multiple classes of antibiotics (4). Opportunistic pathogens of humans are organisms that do not normally cause harm, but are capable of causing disease in those who's defenses or resistance are low. While infections caused by *Burkholderia* are relatively rare in healthy people, these bacteria are a particular risk for patients with compromised immune systems, or chronic conditions such as cystic fibrosis (CF) and chronic granulomatous disease (CGD) (5–7). Epidemiological studies have identified highly transmissible Bcc lineages associated with both nosocomial and community outbreaks (4). Bcc bacteria are recognized as a clinically important pathogen in CF patient communities, as they are linked with

rapid community transmission and aggressive pathogenesis in these patient groups (6). Chronic colonization with *Burkholderia* in CF patients is associated with increased morbidity and mortality, decreased lung function, as well as shorter life expectancy, with some patients progressing to "cepacia syndrome," a dangerous condition characterized by necrotizing pneumonia, septicemia, and high mortality (6, 8, 9).

Treatment of Bcc infections is further complicated by exceptional intrinsic resistance to many classes of antibiotics, including beta-lactams, aminoglycosides, quinolones, polymyxins, tetracyclines, chloramphenicol and trimethoprim (10, 11). Resistance of Bcc species to antibiotics has been attributed to multiple different cellular mechanisms, including efflux pumps to extrude antibiotic compounds, decreased membrane permeability, production of antimicrobial altering enzymes, and alteration of drug targets within the cell structure (12). Multiple members of the Bcc species group have also been shown to be resistant to benzalkonium chloride, a compound used as the active ingredient in many commercial disinfectants and sanitizers used in hospital settings (13). This characteristic makes Bcc members some of the most challenging bacteria to eradicate, thereby increasing the risk of transmission to vulnerable patients. Two species in particular within the Bcc group make up an overwhelming majority of clinical isolates: *Burkholderia multivorans* and *Burkholderia cenocepacia* (12, 14). Current treatment protocols for managing *Burkholderia* infections typically include long term courses of multiple antibiotics, and treatment failure rates are high (15). Concern over these clinically challenging pathogens has led to an earnest interest in alternative treatment strategies, which include the therapeutic application of bacteriophages.

There are currently more than 30 known species of *Burkholderia*, about two thirds of which belong to the *Burkholderia* cepacia complex (Bcc) (16). The Bcc is made up of 22 phenotypically similar, but genotypically distinct species (Figure 1) (17). This grouping of species, particularly *B.*

multivorans and *B. cenocepacia*, are strongly associated with respiratory tract infections in individuals with compromised immune systems, particularly those with cystic fibrosis or chronic granulomatous disease (18). Once infections with Bcc bacteria gain a foothold, these pathogens are often associated with quick community spread and particularly aggressive infections in these patient populations (12).

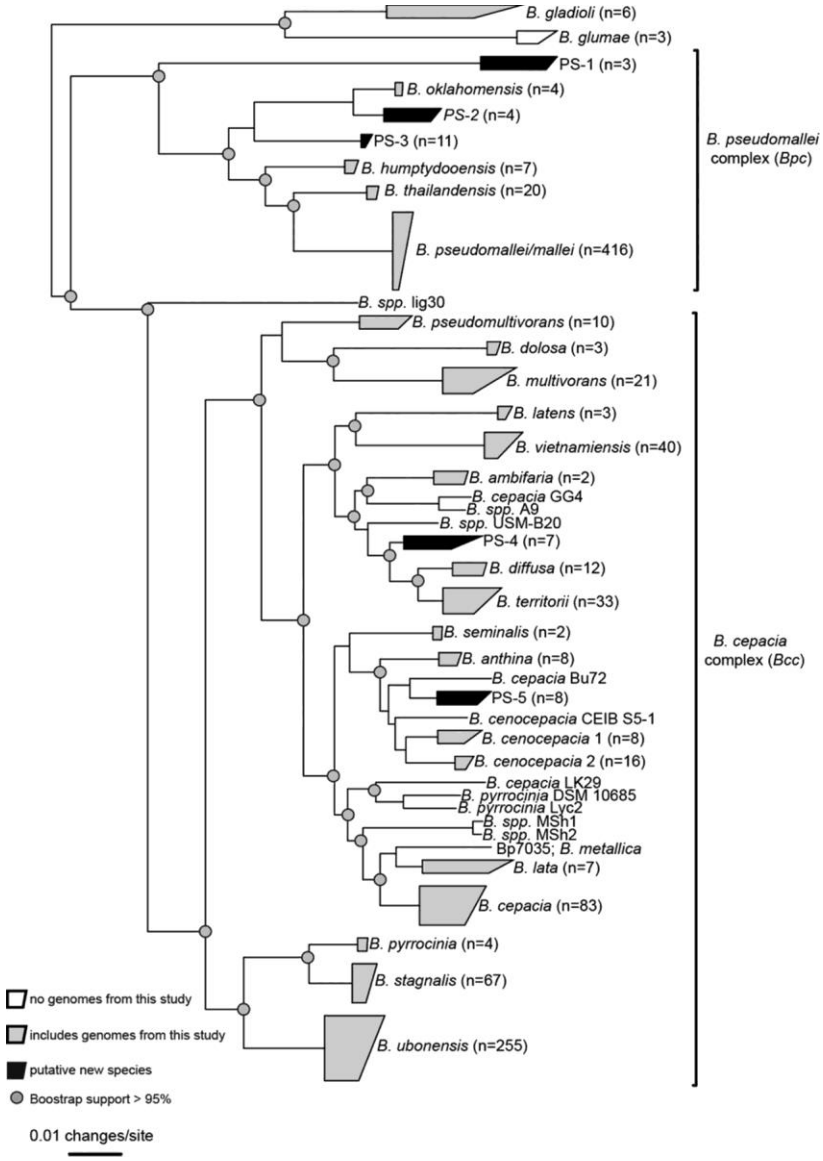

Figure 1. Phylogenetic relationship of *Burkholderia* bacteria species. *Phylogenetic tree depicting the genetic relationship of Burkholderia spp. bacteria. Image is reproduced with permission from J. W. Sahl, et al., The effects of signal erosion and core genome reduction on the identification of diagnostic markers. MBio 7 (2016).*

1.2 Bacteriophage Biology

Bacteriophages (phages) are viruses that infect bacteria. As the most abundant biological entities on the planet, phages are readily available anywhere bacteria can be found (19). As the natural predators of bacteria, they enforce an important ecological balance on bacterial populations. Phages exhibit two primary lifestyle types in relation to their bacterial hosts, which are controlled by environmental and host conditions: the lytic and lysogenic cycles (12). In the lytic lifecycle, phages attach to their target bacteria, inject their genetic material into the host cell, hijack the cell's replication machinery to produce many more copies of themselves, and finally lyse and kill the cell, releasing many more copies of the original phage (20) (Figure 2). These progeny phages then go on to repeat this cycle in new host cells. In the lysogenic lifecycle, genes driving immediate phage replication are repressed, and instead of reprogramming the host cell into a phage factory, the phage genome instead integrates into the bacterial genome and is replicated along with the bacterial genes during normal replication (20) (Figure 2). This type of phage is referred to as a temperate phage, and the quiescent form of the bacteriophage genome, now residing in the bacterial genome, is known as a prophage (20). Prophages can remain in the bacterial genome indefinitely, but intact prophages can excise themselves from the bacterial genome and resume a lytic infection cycle . This switch can be spontaneous, or triggered by external stressors, including nutrient availability, changes in pH or temperature, exposure to antibiotics, or DNA damaging agents (21).

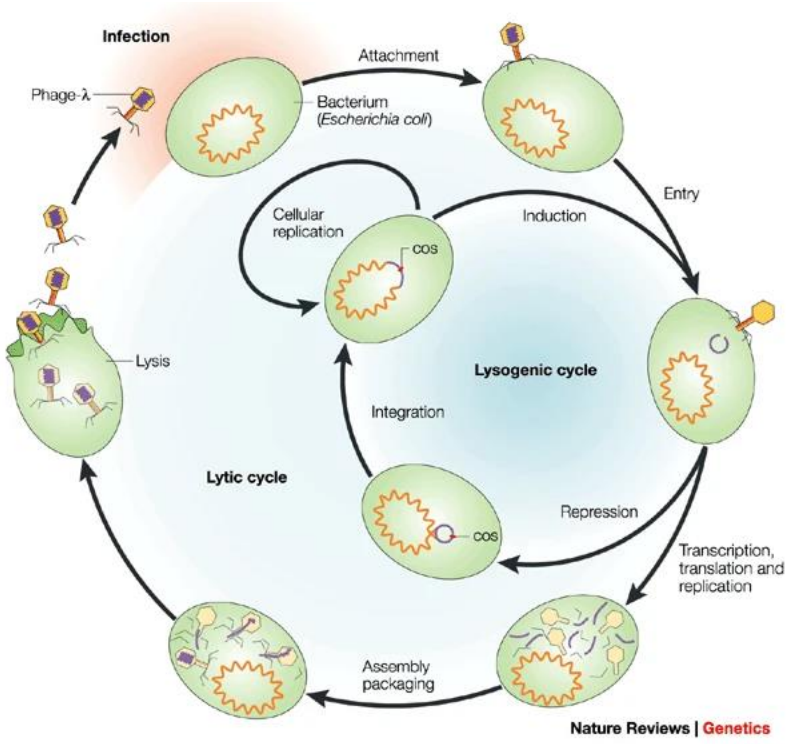

Figure 2. Illustration of the bacteriophage lytic and lysogenic life cycles. *Graphic depiction of the two main lifestyle stratigies of bacteriophages in relation to their bacterial hosts. Larger circle depicts the lytic lifecycle and smaller circle depicts the lysogenic lifecyle. Image is reproduced with permission from A. Campbell, The future of bacteriophage biology. Nat. Rev. Genet. 4, 471–477 (2003).*

1.3 Bacteriophage Therapy

Bacteriophage (phage) therapy is the strategic use of naturally occurring viruses that infect bacteria to treat infections. Although bacteriophage therapy has a rich history, reaching back as early as the late 19th century, it fell out of favor with the advent of broad-spectrum antibiotics in the 1940s (12). However, as bacterial resistance to existing antibiotics has rapidly increased and development of new antibiotic compounds has failed to keep pace, a renewed interest in bacteriophage therapy has emerged. Phage therapy has been undergoing a renaissance in western

medicine since the 1990s (12). Compassionate use cases of phage-based therapy have risen dramatically in the last decade (22). Phages can be used to treat respiratory infections, infections of indwelling medical devices, UTI's, osteoarticular infections, gastrointestinal and endovascular infections, among other ailments (22). Numerous success cases have been reported, including landmark cases of life saving treatment for an abdominal abcess (23) and a lung infection of a patient with cystic fibrosis (24). Modern laboratory techniques, including rapid bacterial species identification and the increasing availability of whole genome sequencing, have added to our understanding of these bacteria-killing viruses, further increasing their potential for successful therapeutic use.

Phage therapy boasts several advantages over traditional antibiotics, including a lack of cytotoxicity, precise target specificity, advantageous pharmacokinetics, and limitless environmental availability (22). Additionally, since phage therapy utilizes an entirely different mode of action than chemical antibiotics, it is a potentially powerful tool against antibiotic-resistant pathogens and a valuable addition to mainstream treatment protocols (12). Since bacteriophages are generally specific to a particular species or even strain of host, they do not disrupt the commensal microbiome, and have been shown in many studies to lack significant side effects (25, 26). Since bacteriophages replicate in their target hosts at the site of infection, then dwindle as the target bacterial population decreases, phage therapy also has the advantage of being both a self-amplifying and self-limiting treatment. Finally, because bacteriophages are considered to be the most abundant biological entities on planet, phages represent a virtually limitless reservoir of potential antimicrobial tools. This is in stark contrast to the rapidly dwindling discovery of new antibiotic compounds, with only a handful of new antibiotics being approved in the last five years, and many of these having only limited clinical benefit (27).

1.4 Isolation of Bacteriophages for Clinical Use

With a clear and urgent need for new treatments against multidrug-resistant pathogens, bacteriophages present a resource with untapped therapeutic potential. Many researchers have focused their attention on isolating a sufficient diversity of bacteriophages to target pathogens of clinical interest. While bacteriophage treatment for infections caused by *Burkholderia* species would be advantageous, to date, only one compassionate use case of *Burkholderia*-targeting phages has been reported (28). However, studies investigating the use of phages against *Burkholderia* infection in *in-vivo* models have shown efficacy both in *Galleria mellonella* (greater waxworm) (29)(30) and murine (mouse) lung infection models (31)(32). Part of the limitation on compassionate clinical usage of these types of phages is that relatively few *Burkholderia*-targeting phages have been isolated (12). Before advantageous phage therapy can be employed for infections caused by Bcc bacteria, more phages that can target these pathogens need to be identified and characterized.

While bacteriophages targeting many species of human pathogenic bacteria have been readily isolated from environmental sources such as water and soil, *Burkholderia*-targeting phages discovered from these sources appears to be somewhat limited. One alternative source of novel bacteriophages is the bacterial genome itself (33)(34). Bacteriophages which have lysogenized their host and integrated into the bacterial genome, called prophages, can be induced to release themselves and then be propagated on an alternative bacterial isolate which the bacteriophage is able to infect and lyse. This alternative isolation technique could prove to be useful in finding new phages for targeting pathogens like *Burkholderia*.

A relatively large percentage (estimated 10%) of *Burkholderia* genomes exist as genomic islands, that is, part of a genome that shows evidence of origins from another organism. Many of

these genomic islands are potentially of prophage origin (2, 35, 36). The diversity of prophages present within the genomes of *Burkholderia* bacteria themselves may therefore represent a rich source of bacteriophages for potential therapeutic use. In this study, we investigated a technique for inducing the release of intact prophages from Bcc clinical isolates using the mutagen mytomycin C, and subsequently propagating phages that could lyse alternative strains of bacteria. We show that phages capable of lytic activity against conspecific and heterospecific bacteria can be isolated in this manner, and identified four novel Bcc-targeting phages with this approach. Additionally, we characterized the prophage content of thirty-five Bcc clinical isolates, and explored associations between species, prophage content, and phage susceptibility. Taken together, this project represents a step toward addressing the limited availability of *Burkholderia*-targeting phages for therapeutic use, presents an alternative strategy for future phage discovery methods, and uncovers valuable insights about prophage carriage in Bcc bacteria.

2.0 Statement of Project and Specific Aims

The goal of this project was to investigate the viability of isolating bacteriophages of prophage origin from the genomes of bacteria within the *Burkholderia* genus, specifically within the *Burkholderia cepacia* complex, that show potential for therapeutic use. Therapeutic potential was defined as evidence of lytic activity against heterologous bacterial isolates. A second point of focus was to genomically characterize prophage diversity in a panel of *Burkholderia* clinical isolates. The following specific aims were proposed to accomplish these goals:

2.1 Aim 1

Isolate prophages from *Burkholderia* spp. clinical isolates that have lytic activity against conspecific and heterospecific isolates. Prophages are known to excise from the genomes of their host bacteria when growth stress or DNA damage is sensed. The DNA cross-linking Mitomycin C has been shown to be effective at inducing excision of prophages in bacterial cultures (33). I hypothesize that applying this method to a panel of clinical *Burkholderia* isolates will generate lysates containing bacteriophages of prophage origin. Screening these lysates against the same panel of *Burkholderia* isolates would allow us to isolate phages with lytic activity against alternative hosts.

2.2 Aim 2

Determine if isolated prophages can lyse *Burkholderia* spp. clinical isolates of the same or different species and test host range of these and other *Burkholderia*-targeting phages. Bacteriophages that can target multiple different species of the same bacterial genus are advantageous for therapeutic applications. To characterize the host range of bacteriophages isolated in Aim 1, I will perform an all-by-all host range plaque screening of each isolated phage and two previously isolated *Burkholderia*-targeting bacteriophages of environmental origin against the same panel of clinical *Burkholderia* isolates used in the induction of prophage release. I hypothesize that phages induced in Aim 1 will show lytic activity on alternate host isolates. Lytic phage activity and viral titers for each pairing will be recorded, and variability in host range between the different phages and host susceptibility between different species will be assessed.

2.3 Aim 3

Characterize prophage diversity among *Burkholderia* spp. clinical isolates and prophages isolated in Aim 1. *Burkholderia* are known to harbor a relatively large number of prophages within their genomes (35). In order to describe the genetic landscape of our host bacteria panel as it pertains to prophage carriage, I will analyze the genomes of the bacterial isolates used in this project as well as the prophages they encode (Aim3B). Phylogenetic relationships of all isolates will be determined by making a core genome phylogeny, and the genomic analysis tool PHASTER (37) will be used to identify regions of each genome that are likely to encode intact or partial prophages. Prophage sequences will be compared to one another using nucleotide BLAST,

and a cluster analysis of all prophage sequences will be performed using Cytoscape (38) (Aim3C). I hypothesize that many *Burkholderia* clinical isolates will contain prophages, and some prophages will be present in multiple isolates. Isolated bacteriophage genomes will also be sequenced to characterize them through comparisons with each other and with other phages deposited in the NCBI database (Aim3A).

3.0 Materials and Methods

3.1 Bacterial Isolates

All *Burkholderia* bacteria isolates used in this study were collected from patients at the University of Pittsburgh Medical Center (UPMC) as part of routine clinical care. Many of the isolates came from lung infections of patients with cystic fibrosis. Most isolates were collected as part of the Enhanced Detection System for Healthcare Associated Transmission (39), and others were collected from patients being evaluated for phage therapy. Isolate collection was approved by the University of Pittsburgh Institutional Review Board under protocols PRO07060222 and STUDY19110005.

3.2 Induction of Prophage Release

To induce prophage release, 10µL of stationary phage liquid culture of each bacteria isolate was inoculated into 5mL of Luria Broth (LB) containing 2.5µg/mL mytomycin C (CAS: 50-07-7 Sigma M4287-2mg). Cultures were grown overnight shaking at 37ºC. The next day, bacterial cells were pelleted, and liquid lysates were filtered through a .22µm syringe filter to remove bacteria. The remaining lysates were presumed to contain any prophages that were released due to growth under stress.

3.3 Isolation and Passaging of Bacteriophages

Lytic bacteriophage activity was identified with a soft agar overlay assay. Briefly, square bottom agar petri plates were prepared containing LB media with 1.5% agar, 1mM $CaCl_2$ and 1mM $MgCl_2$. Bacterial isolates were inoculated into LB media and incubated overnight at 37°C. The following day, bacterial soft agar lawns were created by mixing 50µL of liquid bacterial culture with 5mL of top agarose (LB media with 0.5% agarose, 1mM $CaCl_2$, and 1mM $MgCl_2$) cooled to 55°C, plated on top of bottom agar plates and allowed to solidify. After top agar bacterial lawns had solidified, 5µL of each potential bacteriophage-containing lysate prepared previously was spotted on top of the lawn. Plates were incubated upright at 37ºC overnight. The next day, plates were examined for evidence of lytic bacteriophage activity in the form of plaques. Individual lytic (clear) plaques were "picked" with a pipette tip and transferred into 100µL of suspension media (SM) buffer (50mM TrisCl pH 7.5, 100mM NaCl, 8mM $MgSO_4$) and incubated overnight at 37ºC. Areas of full clearance where individual plaques were not visible were noted and bacteriophage-mediated killing was tested by plating 10-fold serial dilutions of phage lysate to observe and pick individual plaques. To propagate and isolate individual phages, individual plaques were picked and prepared in 100µL of SM buffer as described above. Serial 10-fold dilutions were made in SM buffer, and 5µL of each dilution was spotted onto a plate containing 5mL of LB top agarose mixed with 50µL of bacterial culture of the propagating isolate and layered on top of a bottom agar plate. After overnight incubation at 37ºC, an individual plaque was picked and passaged again. Each phage was passaged four times before the generation of high-titer stocks.

3.4 Preparation of High-Titer Phage Lysates

To prepare high-titer liquid lysates of each phage, individual plaques picked after four serial passages were transferred to 100µL of SM buffer and were then mixed with 100µL of overnight LB culture of the propagating bacterial isolate. The mixture was incubated at room temperature for 15 minutes, then mixed with 10mL of LB top agar and overlaid onto large (15 cm) bottom agar plates and allowed to set. Plates were incubated overnight at 37ºC. Plates showing semi-confluent lysis were flooded with 10mL of SM buffer and incubated at 37ºC for 2 hours. The SM lysate was then collected and centrifuged at 4,000g for 10 minutes to pellet bacteria. Supernatants were then filter sterilized through a 0.22µm membrane syringe filter.

3.5 DNA Extraction and Whole Genome Sequencing

Bacterial genomic DNA was extracted from 1mL overnight cultures grown in LB media using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) following the manufacturer's protocol. Phage genomic DNA was extracted using phenol chloroform extraction from 500µL of concentrated phage lysate, followed by ethanol precipitation. Briefly, 500µL phenol:chloroform:isoamyl alcohol (25:24:1) was added to 500µL of each lysate, samples were vortexed and then centrifuged at 16,000g for 1 minute. The upper aqueous phase was transferred to a new tube and 500µL of chloroform was added. Samples were vortexed and centrifuged again at 16,000g for 1 minute, and the upper aqueous phase was again transferred to a new tube. Then 1µL glycogen, 0.1x volume 3M sodium acetate, and 2.5x volume 100% ethanol were added and samples were incubated overnight at -20ºC. The next day samples were centrifuged at 16,000g for

30 minutes at 4ºC, then the supernatant was removed, and the DNA pellet was washed with 150µL 70% ethanol. DNA pellets were resuspended in 100µL nuclease-free water. All DNAs were quantified with a Qubit fluorimeter (Thermo Fisher Scientific, Waltham, MA). Bacterial and phage genomes were sequenced on the Illumina platform at the Microbial Genome Sequencing (MiGS) Center (Pittsburgh, PA). Illumina library construction and sequencing were conducted using an Illumina Nextera DNA Sample Prep Kit with 150bp paired-end reads, and libraries were sequenced on the NextSeq 550 sequencing platform (Illumina, San Diego, CA).

3.6 Genomic Analysis

Genomes were assembled with SPAdes v3.11 to generate contigs with a 200bp minimum length cut-off (40). Phage contigs were extracted from each assembly and separated from contaminating host bacterial sequences by examining the differential read coverage of each contig, and with BLASTN. Assembled genomes were annotated with RAST (41). A core genome phylogenetic tree of all bacterial isolate genomes was generated using RAxML with the GTRCAT substitution model and 1000 iterations (42). Bacterial species were assigned by average nucleotide identity (ANI) comparisons with previously sequenced *Burkholderia* species (43). Prophages were identified in each bacterial genome using PHASTER (37). Prophages of any length that were predicted to be intact or questionable by PHASTER were included. Prophage sequences were compared to one another with BLASTN, and clusters of similar prophage sequences were identified as those sharing >90% sequence coverage and >90% sequence identity. A cluster analysis of all prophage sequences was performed and visualized using Cytoscape (38). Presence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas loci in each bacteria

isolate was analyzed using CRISPRCasFinder (44). Predicted family and genus of each bacteriophage was determined by closest BLAST match in the NCBI nr database.

3.7 Bacteriophage Host Range Screening

An all-by-all screen of each of 9 isolated bacteriophage against a panel of 35 *Burkholderia* isolates was performed using the soft agar overlay spot screening method described above. Briefly, 10-fold serial dilutions of each phage lysate were spotted in 5µL volumes onto top agar lawns of each bacteria and incubated at 37ºC overnight. The following day, each phage-bacteria pairing was assessed for visibility of plaques. For pairings where lysis was noted, bacteriophage titer in plaque-forming units (p.f.u.)/mL was calculated.

3.8 EM Imaging

1-5ul of bacteriophage BCC02 suspension was added to a copper grid and negatively stained with 1% uranyl acetate. Phage suspension was imaged by transmission electron microscopy on a JEW 1400 Flash Transmission Electron Microscope. Imaging was performed by the University of Pittsburgh Center for Biologic Imaging.

3.9 Statistical Analysis

All statistical analyses for this study were performed in GraphPad Prism version 8.0.0, GraphPad Software, San Diego, California USA, www.graphpad.com. Linear Regression and/or two-tailed t-tests were performed to assess significance of associations.

4.0 Results

4.1 AIM 1: Induction and Isolation of *Burkholderia*-targeting Phages

To collect and isolate prophages present within *Burkholderia* bacteria genomes, a library of potential prophage-containing lysates was created. To do this, we inoculated 32 *Burkholderia* clinical isolates in liquid culture with the mutagen mitomycin C (Figure 3). Mitomycin C induces growth stress in the dividing bacterial population via DNA damage, prompting intact prophages to shift back to a lytic life cycle and excise themselves from the host genome (45, 46). The panel of isolates included seven different species, but a majority of isolates belonged to the two most clinically relevant species within the *Burkholderia* cepacia complex: *B. multivorans* and *B. cenocepacia*. Following overnight culture, lysates were filter sterilized to create a screening library of 32 prophage-containing lysates. We then performed a screen which paired each lysate (32 phage-containing lysates) against each bacterial isolate (32 *Burkholderia* isolates), referred to as an "all-by-all" screen, using a spot-plaque screening method (Figure 3). In total, 1,024 pairwise combinations that were tested and scored for evidence of lytic phage activity yielded 11 positive pairings (about 1% hit rate). Each positive pairing was then taken through a secondary screening process to confirm that inhibitory activity was due to the presence of phage. Each lysate was plated in 10-fold serial dilution on the corresponding bacterial host in order to visualize individual plaques indicating phage activity. One pairing did not yield individual plaques, suggesting that the growth inhibition noted in the primary screen may have been due to something other than phage activity. This pairing was dropped from further study. The remaining ten host-lysate pairings were carried on to serial passaging steps to isolate individual phages. Three candidate pairings did not

maintain lytic activity through serial passage, but 7 host-lysate pairs yielded viable isolates bacteriophages, which were made into high-titer lysates and designated as BCC02 through BCC08 (Table 1).

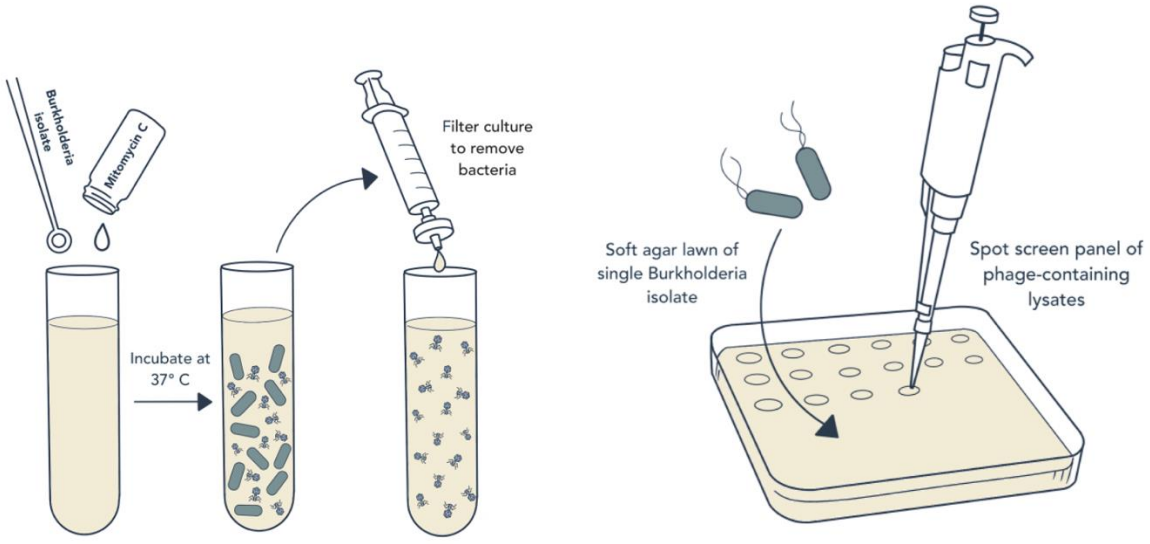

Figure 3. Illustration of prophage release induction methods. Graphical representation of culture and screening methods used to induce release of prophages from bacterial genome. Full description in methods.

4.2 AIM 2: Host Range Screen

To determine the infectivity profile of each isolated phage as well as the phage susceptibility of each *Burkholderia* spp. isolate, we performed an all-by-all lytic activity screen. For this screen, two additional *Burkholderia*-targeting bacteriophages already present in the lab's collection, BCh7 and DSMZ107315, were added to the screening panel for comparison purposes. Both phages were isolated from environmental sources. Three additional *Burkholderia* clinical

isolates, which had been recently collected from patients being evaluated for phage therapy, were also added, bringing our host-range screening panel to a total of 35 bacteria isolates versus 9 bacteriophage lysates (Figure 4). Ten-fold serial dilutions of each phage were spotted onto top agar lawns of each bacterial isolate. Titers were calculated for each pairing that showed lytic activity by counting of individual plaques. Screening results revealed a variation in phage host-ranges, with phages able to lyse between 2 (BCC06) and 12 (BCC02) out of 35 isolates. All phages were able to infect and lyse multiple isolates, and in most cases, phages were able to lyse bacteria belonging to multiple different species (Figure 4). We observed similar host-ranges for two groups of phages: [BCC02, BCC03, and BCC04] and [BCC05 and BCC06]. This finding, along with the fact that these groups contained phages that were each derived from the same "donor" bacterial isolate (Table 1), suggested that these might be duplicate isolations of the same phage. Small differences in host range within each group of phages could be attributed to differences in titer of the lysate stocks. Notably, with the exception of BCC05 and BCC06, lytic host-range of bacteriophages of prophage origin were comparable to those of environmental origin, both within our study (comparing BCC02-BCC08 with BCh7 and DSMZ107315) and in the literature (16, 47). Eight *Burkholderia* isolates were resistant to all phages tested. However, the other 27 isolates (77.14% of all isolates tested) were susceptible to at least one phage.

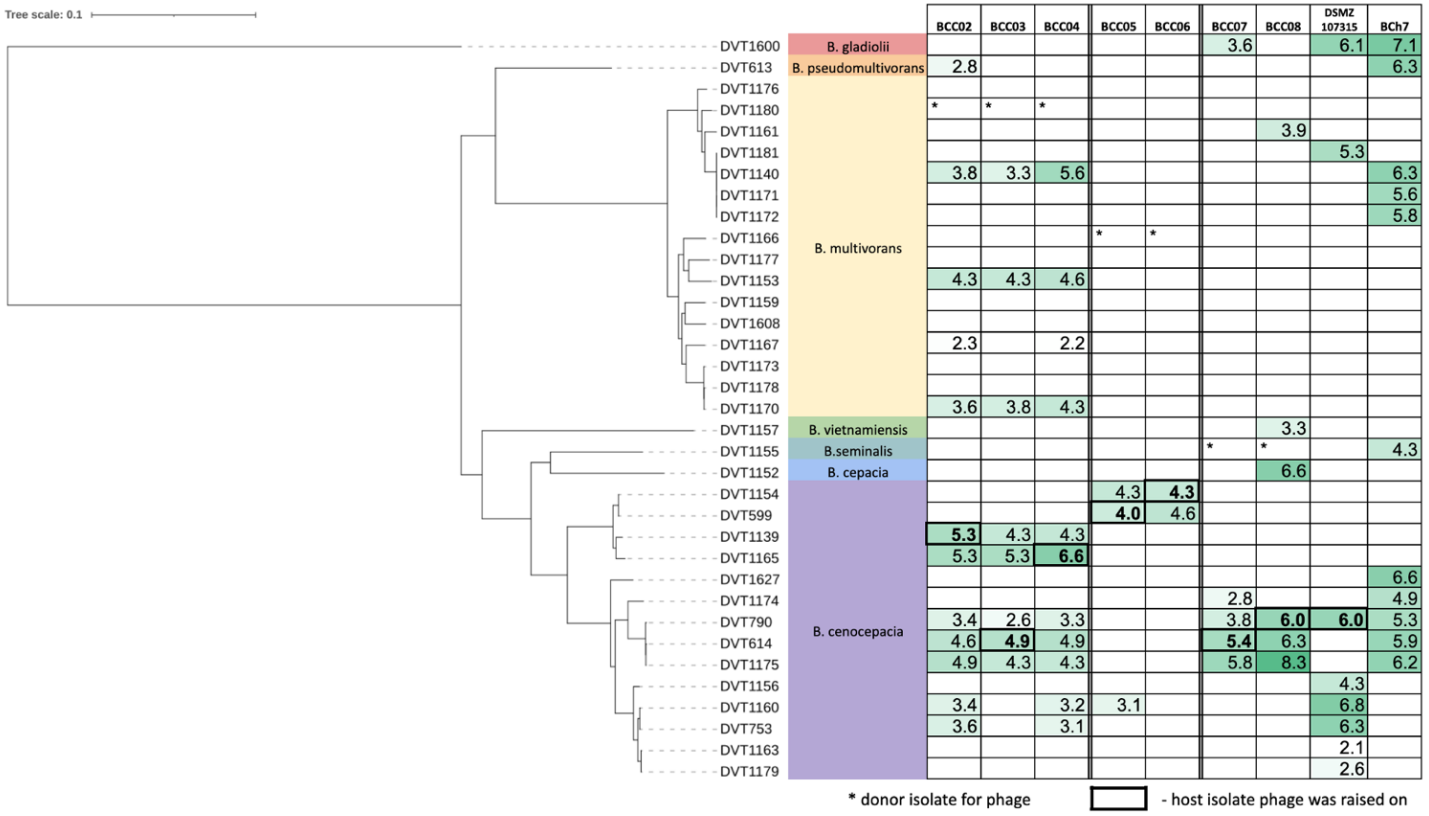

Figure 4 Infectivity of isolated phages against generically diverse clinical *Burkholderia* spp. isolates. Bacteria isolates are ordered according to core genome phylogeny and broken into groupings by species. Infectivity is shown as the log10 titer (PFU/mL) of each phage against each isolate. Bolded values indicate the *Burkholderia* isolate that each phage was isolated and propagated on. Green shading corresponds to phage activity titer, with darker shading indicating a higher titer. Empty cells indicate no phage activity. Asterisks mark donor bacteria isolates for isolated phages of prophage origin

Table 1. Summary of extracted *Burkholderia* proprophages showing lytic activity

Phage ID	Donor	Donor Species	Propagation Host	Host Species
BCC02	DVT1180	*B. multivorans*	DVT 1139	*B. cenocepacia*
BCC03	DVT1180	*B. multivorans*	DVT 614	*B. cenocepacia*
BCC04	DVT1180	*B. multivorans*	DVT 1165	*B. cenocepacia*
BCC05	DVT1166	*B. multivorans*	DVT 599	*B. cenocepacia*
BCC06	DVT1166	*B. multivorans*	DVT1154	*B. cenocepacia*
BCC07	DVT1155	*B. seminalis*	DVT 614	*B. cenocepacia*
BCC08	DVT1155	*B. seminalis*	DVT 790	*B. cenocepacia*

4.3 AIM 3 (A): Genomic Characterization of Isolated Phages

To explore the genetic diversity of phages isolated in this study, phage genomes were sequenced on the Illumina platform and were compared to publicly available genomes in the NCBI nr database. The genome of BCC08 appeared to be only a partial genome, however the remaining eight genomes appeared to be fully resolved. Phage genomes ranged in size from approximately 22-68 kb (Table 2). Based on direct sequence comparisons via sequence alignment, BCC02, BCC03 and BCC04 were shown to be genetically identical. BCC02 was chosen as a representative of this phage for susceptibility calculations. Likewise, BCC05 and BCC06 were found to be identical except for a single nucleotide difference within a gene that was identified via BLAST analysis as coding for a tail fiber protein. However, as we saw no significant differences in host range between the two lysates, for the purposes of our analysis we also considered these two phages to be identical and chose BCC05 as a representative of this phage. The family and genus

of each phage were predicted based on sequence comparisons with previously described phages deposited in NCBI using nucleotide BLAST (Table 2). All phages were predicted to belong to the order Caudovirales and family Myoviridae. These phages are known to have contractile tails and icosahedral heads. Transmission electron microscopy (TEM) imaging of phage BCC02 confirmed this phenotype visually (Figure 5). Phage BCC02, which had the broadest host range in this study, was most similar to bacteriophage KS5, which Seed et al. also noted to have the broadest host range in their screening experiments (18). Overall, genomic analyses revealed that our study yielded 4 novel *Burkholderia*-targeting bacteriophages (BCC02, BCC05, BCC07, and BCC08) that were distinct from other bacteriophages whose genomes have been deposited into GenBank to date.

Table 2. Summary of unique isolated *Burkholderia*-targeting bacteriophages

Phage ID	Donor/Source	Donor species	Genome Size (bp)	%GC	Predicted Family	Predicted Genus	NCBI Similar Phage	% Identity
BCC**02**/03/04	DVT 1180	*B. multivorans*	26,947	67.04	*Myoviridae*	*Peduovirinae; Kisquinquevirus*	*Burkholderia* Phage KS5 (GU911303.1)	98.35
BCC**05**/06	DVT 1166	*B. multivorans*	28,659	67.09	*Myoviridae*	*Peduovirinae; Tigrvirus*	*Burkholderia* Phage phiE12-2 (NC_009236.1)	83.05
BCC07	DVT 1155	*B. seminalis*	38,077	66.66	*Myoviridae*	*Peduovirinae; Kisquinquevirus*	*Burkholderia* Phage Mana (NC_055863.1)	94.89
BCC08 (partial)	DVT 1155	*B. seminalis*	12,322	66.27	*Myoviridae*	*Peduovirinae; Tigrvirus*	*Burkholderia* Phage KL3 (GU911304.1)	96.31
BCh7	N/A	N/A	68,166	54.7	*Myoviridae*	*Bcepfunavirus;*	*Burkholderia* Phage Maja (MT708549.1)	73.73
DSMZ107315	N/A	N/A	22,967	61.5	*Myoviridae*	*Peduovirinae; Kisquattuordecimvirus*	*Burkholderia* Phage FLC5 (NC_055722.1)	89.07

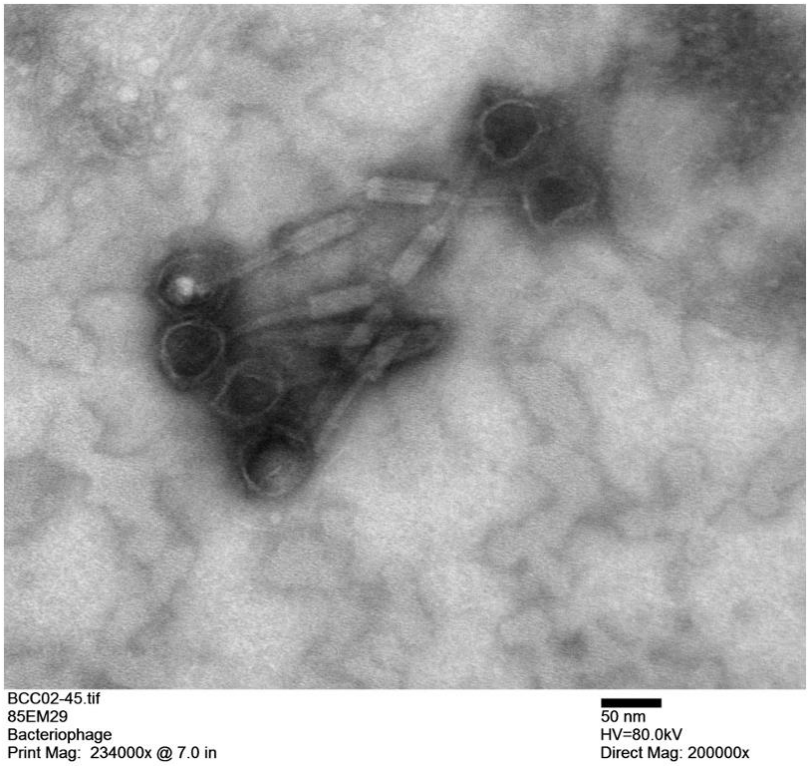

Figure 5. Electron micrograph of bacteriophage BCC02. Transmission electron micrograph showing phage morphology of *Burkholderia* phage BCC02. Image taken at 200,000-fold magnification. Icosahedral head, tail, and contractile tail sheath are visible for 6 virions

4.4 AIM 3 (B): Genomic Characterization of Bacteria and their Prophages

To understand the genetic diversity of the *Burkholderia* clinical isolates we studied, we constructed a core genome phylogeny of all 35 bacteria isolates using RAxML (42), and compared each genome to previously sequenced *Burkholderia* genomes with fastANI. A total of 16 isolates belonged to *B. multivorans*, 14 belonged to *B. cenocepacia*, and one each belonged to *B. cepacia*, *B. seminalis*, *B. vietnamiensis*, *B. pseudomultivorans*, and *B. gladioli*. All isolates except DVT1600 (B. gladioli) fall into the infamous *Burkholderia cepacia* complex grouping. We next

assessed the abundance and diversity of prophage sequences within the 35 *Burkholderia* spp. clinical isolates we used for screening. We searched each isolate genome for sequences of likely prophage origin using the online tool PHASTER (37). A total of 59 prophage sequences (Supplemental Table 1) were extracted, and between 0 and 4 prophages were found within the genomes of each isolate (Figure 6A). We then tested whether prophage abundance was associated with phage susceptibility by counting the number of phages that each bacterial isolate was susceptible to. Susceptibility to BCC02/03/04 and BCC05/06 were each only counted once. We found that prophage abundance was not significantly associated with phage susceptibility (linear regression P-value = 0.42) (Figure 6B).

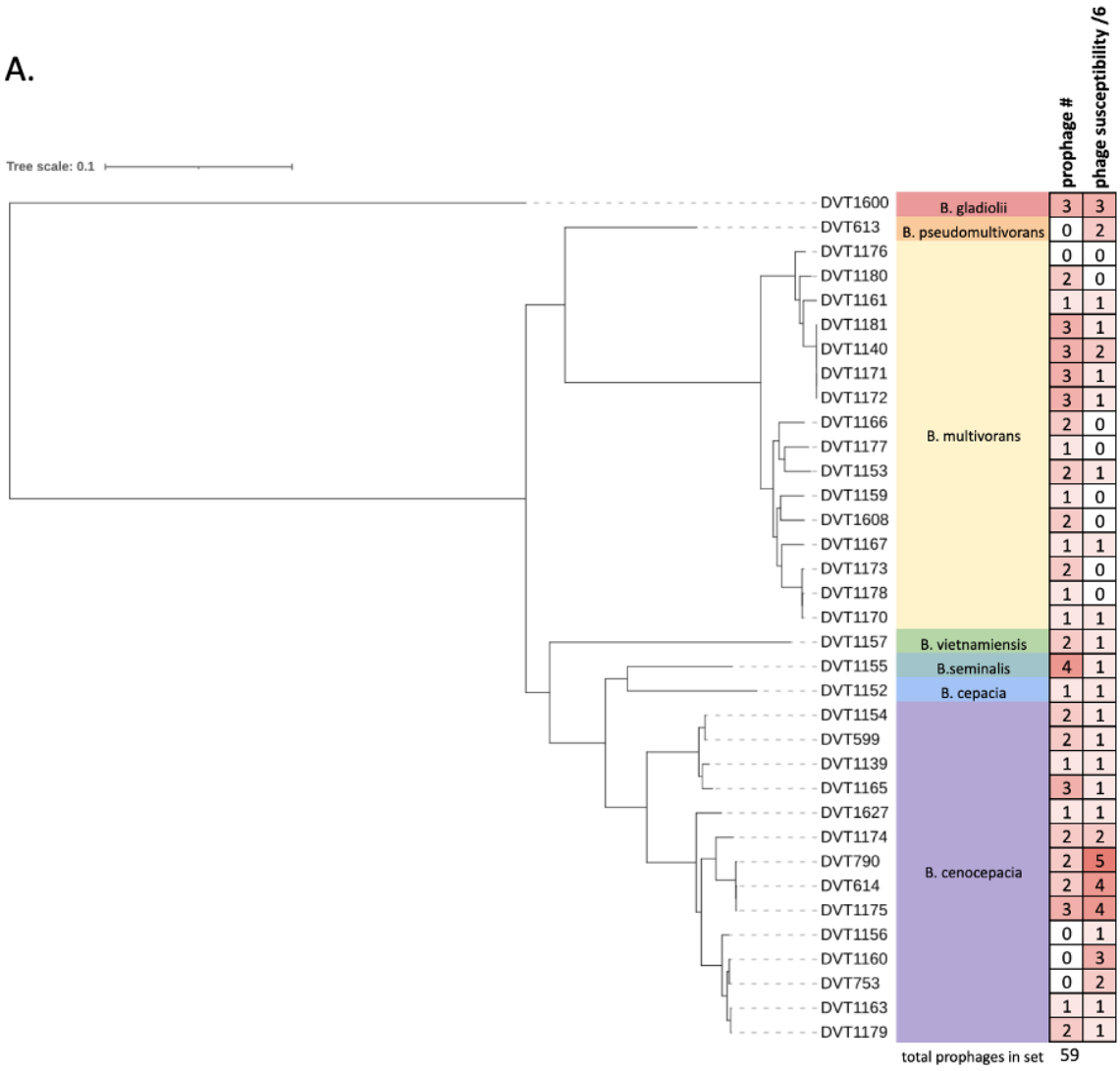

Figure 6(A). Number of prophages and bacteriophage susceptibility of diverse panel of *Burkholderia* spp. Isolates. Core genome phylogeny of 35 clinical *Burkholderia* spp. isolates used for phage isolation. Isolate genomes were mined for prophage abundance using PHASTER and all isolates were screened for susceptibility to 6 genetically distinct phages (BCC02/03/04, BCC05/06, BCC07, BCC08, BCh7, DSMZ107315).

B.

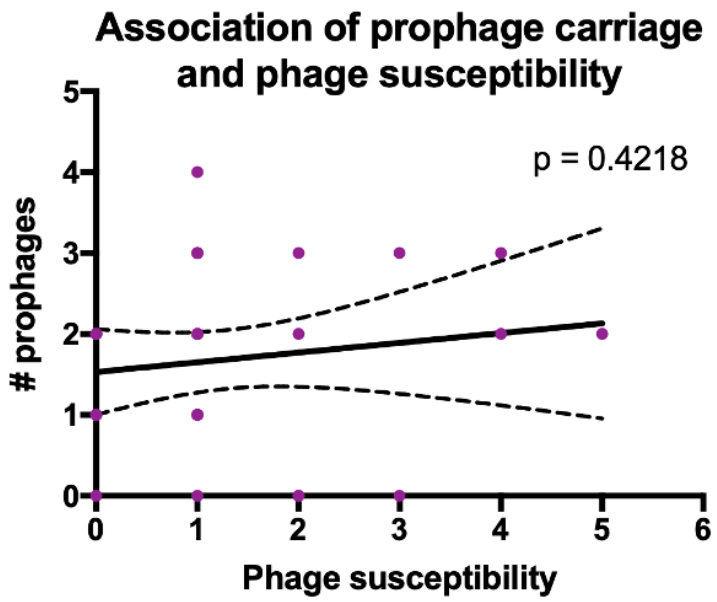

Figure 6(B). Number of prophages and bacteriophage susceptibility of diverse panel of *Burkholderia* spp. Isolates. Linear regression showing no association between susceptibility to phage lysis versus number of prophages. P-value determined by t-test.

We compared the sequences of the phages we isolated to their prophage sources by mapping the sequence of each phage to prophage sequences identified in each donor bacterial isolate genome (DVT1155, DVT1166, and DVT1180). We also identified mutations present in the isolated phages compared to their prophage "ancestors." All isolated phage genomes matched to the original prophage sequences found in the donor bacterial genomes with no more than one nucleotide difference. This finding shows that our approach successfully isolated prophages with lytic activity and confirms that prophages within the genome of one bacterial isolate can lyse other genetically distinct bacteria.

To determine whether any of the prophages encoded by our *Burkholderia* isolate genomes were similar to one another, we compared all extracted prophage sequences to one another using nucleotide BLAST (48). We assessed both nucleotide coverage and identity across all pairwise comparisons. Prophages that shared >90% sequence coverage and >90% sequence identity were considered as similar to one another, and clusters of similar prophages were visualized using Cytoscape (38) (Figure 7). Twelve clusters of similar prophages were identified, which ranged in size from 2-4 isolates. Overall, prophages clustered within closely related isolates and within the species (Figure 7B). Our screening panel contained two sets of isolates that were gathered at different time points from the same patient: DVT1140, DVT1171, DVT1172, and DVT1181 (*B. multivorans*) were collected from one patient, and DVT599 and DVT1154 (*B. cenocepacia*) were collected from another patient. The prophages present in these isolates were shown to cluster together, as expected (Figure 7). Isolated phages BCC02, BCC05, BCC07 and BCC08 did not fall into any of these clusters, confirming that they were present in only a single isolate. Finally, one cluster (Cluster 1) was associated with host resistance to lysis by other phages. All four isolates containing a prophage in this cluster were resistant to killing by any of the isolated phages (Figure 4). This finding suggests that this particular prophage may provide protection to phage-mediated lysis in *B. multivorans.*

A.

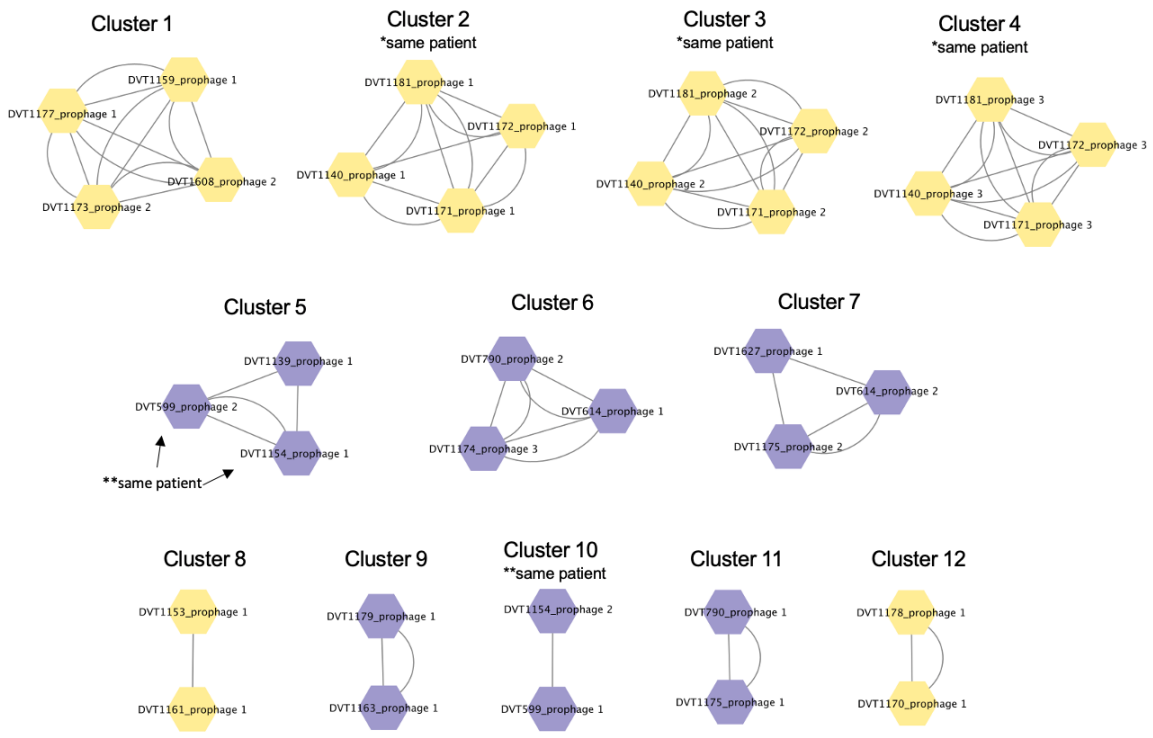

Figure 7(A). Clusters of prophages found in the genomes of *Burkholderia* spp. Isolates. Bacterial isolate names and prophage number are listed inside the nodes of each cluster, and lines connect prophages that share >90% sequence coverage and >90% sequence identity. Yellow nodes indicate prophages from *B. multivorans* isolates and purple nodes indicate prophages from *B. cenocepacia* isolates. Isolates from the same patient (two separate patients) are labeled.

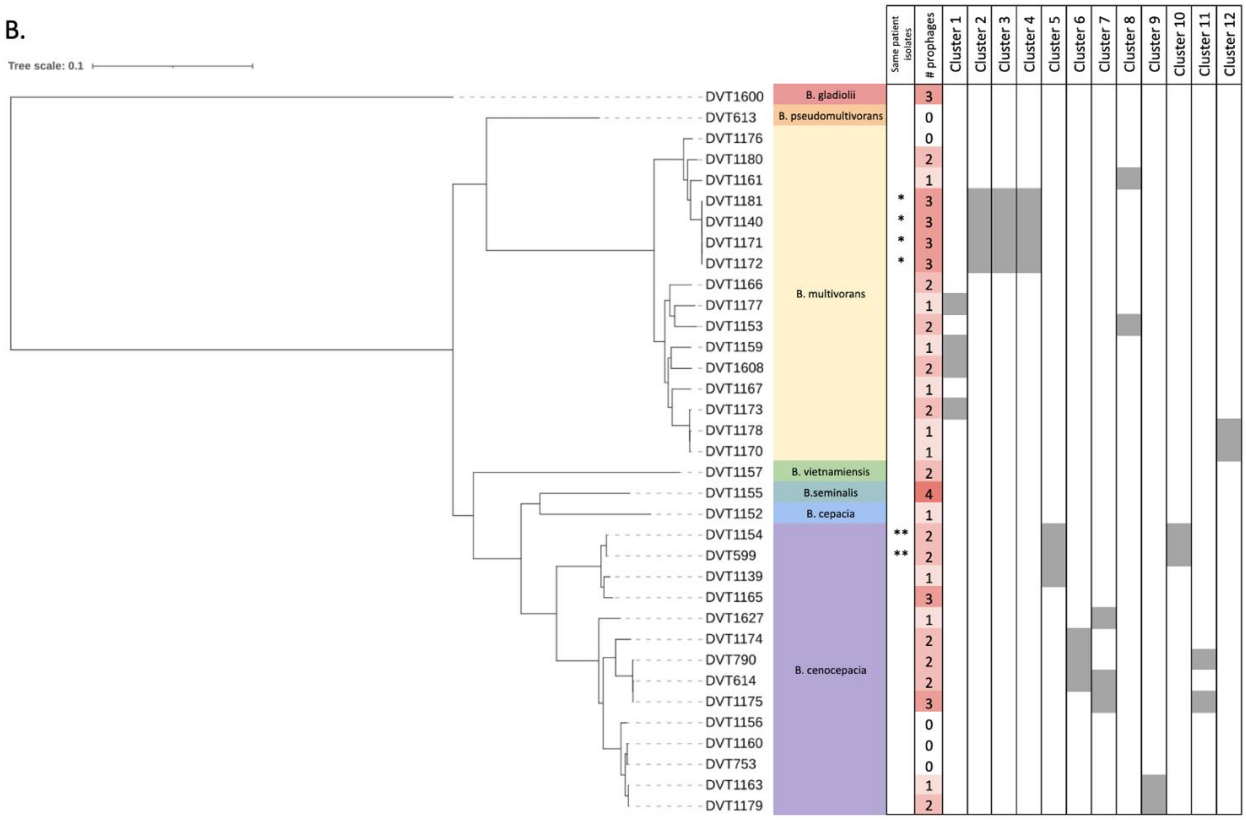

Figure 7(B). Clusters of prophages found in the genomes of *Burkholderia* spp. Isolates. Core genome phylogeny of 35 clinical *Burkholderia* spp. isolates used for phage isolation showing total number of prophages identified in each isolate and appearance of clustered prophages as shown in A. Asterisks indicate isolates from the same patient.

4.5 AIM 3 (C): Association Between Prophages and Phage Susceptibility

Prior studies have shown that endogenous prophages can provide protection against phage-mediated killing by other similar phages (49)(50). To assess a possible relationship between the number of prophage sequences present in each bacterial isolate genome and susceptibility to phage lysis, we quantified the number of prophages in isolates that were susceptible or not susceptible to each individual phage tested (Figure 8A). We found no significant differences except in the case of phage BCh7, where isolates having a greater number of prophages were more likely to be

susceptible to lytic phage killing. We also assessed differences in overall prophage carriage and phage susceptibility between the two major species in our dataset: *B. multivorans* and *B. cenocepacia* (Figure 8B). While there was no overall difference in the number of prophages present in each of these species, we did find that the *B. cenocepacia* isolates in our dataset were significantly more susceptible to phage lysis compared to *B. multivorans* isolates (P=0.001) (Figure 8B).

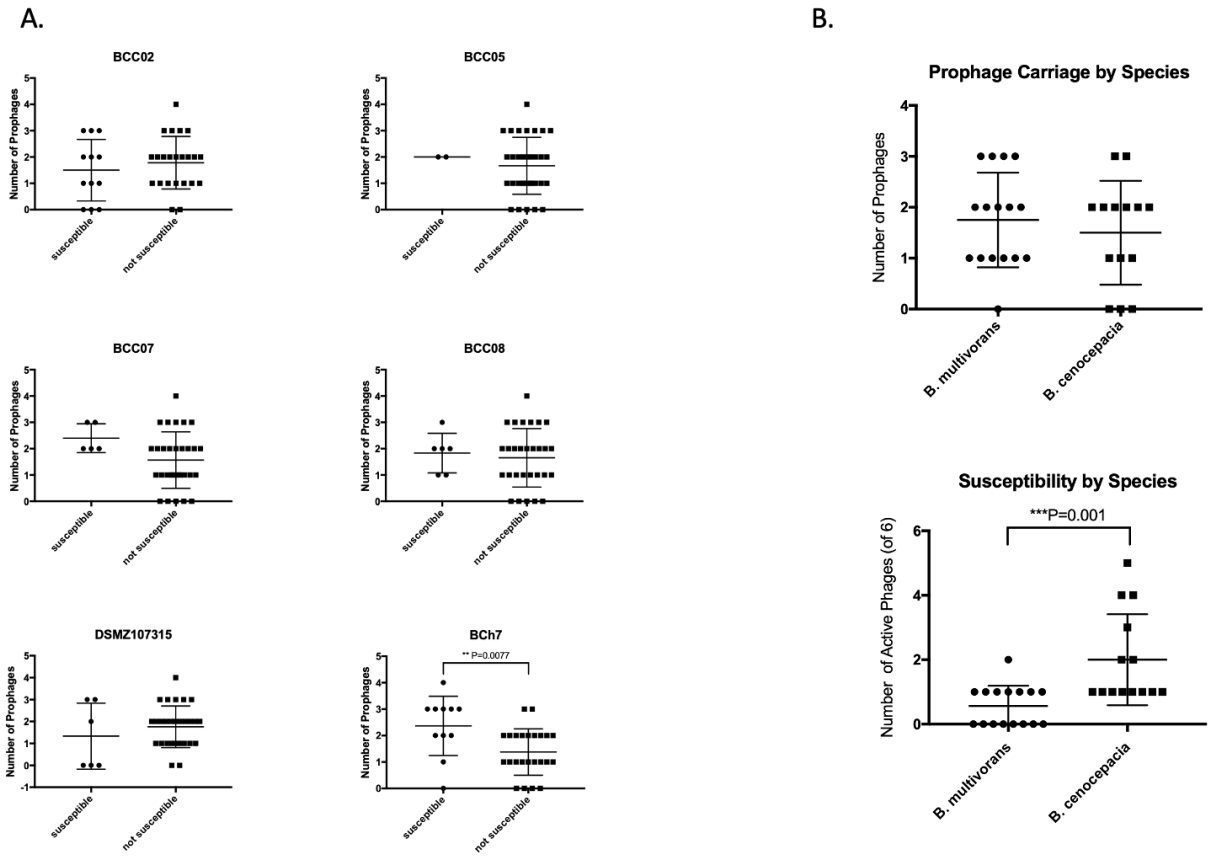

Figure 8. Relationship of prophage abundance and phage susceptibility (A) Prophage abundance in isolates that were and were not susceptible to each unique bacteriophage. (B) Prophage carriage and phage susceptibility by species for two major species in the dataset: *B. multivorans* and *B. cenocepacia*. P-values determined by two-tailed t-test.

5.0 Discussion

The establishment and maintenance of lysogeny in bacterial hosts by temperate phages is a widespread phenomenon that involves a complex interplay of elements both on a cellular and ecological level. These interactions are influenced by many factors, including genetics, cellular development, community dynamics, and environmental conditions (21). In this study, we show that in some cases, the relationship between bacteria and their prophages can be exploited to isolate, characterize, and bank bacteriophages for potential therapeutic use, and that induction with Mitomycin C is an effective method to accomplish this in *Burkholderia* isolates.

The six phages we studied showed a combined host range encompassing more than 75% of the patient isolates that were tested. The isolated prophage BCC02 in particular had a relatively broad host range, with the ability to lyse 34% of the clinical isolates used in our study. This phage is genetically similar to previously described *Burkholderia* phage KS5 isolated by Seed et al, which also demonstrated broad lytic activity (18). The four novel phages isolated and described in this study have potential for further development toward therapeutic application. Exploration of the use of temperate bacteriophages in phage therapy will considerably expand the pool of useful tools against the escalating threat of multi-drug resistant bacteria. Several strategies for their use have already been identified (51). Additionally, identification of phages with varying host ranges and infection dynamics allows for the treatment of infections with multi-phage cocktails in order to reduce the probability of development of phage resistance (22). Phages can also be administered concurrently with traditional antibiotics. While we did not test for synergy with antibiotics in this study, this beneficial relationship has been noted in multiple studies (52)(53)(54). Therapeutic

deployment of phages may be able to shift the bacterial population toward one that is re-sensitized to antibiotics and/or less virulent (12).

Triggering the bacterial DNA damage response using a mutagen like mitomycin C is a simple way to activate the lysogenic-lytic switch in some isolates, and induced phages did, in fact, show a range of lytic activity against conspecific and heterospecific bacterial isolates. Polylysogeny in *Burkholderia* species appears to be common (36), and the prophages present in *Burkholderia* genomes represent a rich hunting ground for clinically useful bacteriophages. However, further characterization and purposeful engineering of temperate phages would likely be required before they could be used clinically. It is known that lysogenic conversion and transduction of virally encoded genes can be an important driver of bacterial virulence (55) (51), and necessary caution should be taken before introducing potentially lysogenic phages into an environment where mixed infections are frequent and bacterial populations can reach high densities, such as the CF airway. However, successful conversion of temperate phages to obligately lytic mutants has been shown (56), (24). This would be a logical future direction to explore for this project.

Our findings demonstrate that clinically significant *Burkholderia* isolates are host to a variety of prophage elements, in agreement with previous studies showing that lysogeny is relatively common in this genus (2), (47), (16). We found that a higher overall abundance of prophages did not correlate with phage susceptibility in our dataset. Our study also found greater phage resistance overall in *B. multivorans* isolates than *B. cenocepacia* isolates. At least one other study has noted the relative phage resistance of *B. multivorans* isolates (47). The presence of the Cluster 1 prophage was associated with complete resistance to phage lysis in 4 *B. multivorans* isolates carrying this prophage, which may indicate some evolutionary or ecological relevance,

possibly enabling a form of superinfection immunity. The occurrence of this prophage could indicate ancestral integration maintained through a fitness advantage, or direct phage transmission between hospital-associated strains in close contact. However, the nature of these types of interactions is highly complex and resolving the details of the effects of this prophage on the infection dynamics of other phages would require further study.

The prophage isolation method that we employed here could be further refined and expanded to identify and characterize more phages that target Bcc bacteria. Currently, very little is known about the entry receptors used by *Burkholderia*-targeting bacteriophages (12). In future work, we plan to focus on identification of potential entry receptors and genes responsible for lysogeny, in order to gain a better understanding of infection dynamics. We would also like to test the activity of these novel phages against a larger and more diverse panel of *Burkholderia* isolates.

This study had several limitations. The small volume spot-screening method used to isolate induced phages likely missed phages that were present in lysates at low concentration. Additionally, slow-growing clinical isolates may have led to lower indication of lytic activity, since some temperate phages are known to employ the lytic lifestyle only when the density of available hosts is high (21). In our genomic characterization, only a partial genome of phage BCC08 was able to be resolved and all genomes used for our analysis were draft genomes that were not able to be fully closed within the scope of this project. This could potentially skew our analysis of the number of prophages in each isolate, as some prophages may have spanned multiple contigs. Finally, all work in this study was performed *in-vitro,* thus we are not able to conclude that these newly characterized phages would be useful for clinical therapy without further testing in a relevant *in-vivo* model of infection.

In this project, we isolated 4 novel bacteriophages with lytic activity against a variety of Bcc isolates. A recent review by Lauman and Dennis notes that as of 2021, only 34 Bcc-targeting bacteriophages have been described in the literature (12), highlighting the significant contribution of the findings of this study. The data generated in this study represents a valuable addition to the literature characterizing *Burkholderia*-targeting bacteriophages, as well as prophage abundance and diversity in clinically relevant *Burkholderia* species. Isolated phages of prophage origin may prove to have clinical use, and a greater understanding of prophage biology will further the utility of bacteriophage therapy in general.

Appendix A

Appendix Table 1. Prophages Identified in *Burkholderia* Bacterial Isolate Genomes

Supplemental Table 1. Prophages identified in *Burkholderia* bacterial isolate genomes

Prophage ID	Host Isolate	Host Species	Length (bp)	% GC	Cluster	Most Common Phage (# genes that match)
DVT 1139_prophage 1	DVT 1139	*B. cenocepacia*	22,693	69.1	5	PHAGE_Burkho_KL3_NC_015266(24)
DVT 1140_prophage 1	DVT 1140	*B. multivorans*	39,836	63.8	2	PHAGE_Burkho_KS10_NC_011216(43)
DVT 1140_prophage 2	DVT 1140	*B. multivorans*	32,331	62.2	3	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(8)
DVT 1140_prophage 3	DVT 1140	*B. multivorans*	32,989	65.2	4	PHAGE_Burkho_KS5_NC_015265(37)
DVT 1152_prophage 1	DVT 1152	*B. cepacia*	36,096	63.3	-	PHAGE_Mannhe_vB_MhM_3927AP2_NC_028766(14)
DVT 1153_prophage 1	DVT 1153	*B. multivorans*	47,703	62.2	8	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(8)
DVT 1153_prophage 2	DVT 1153	*B. multivorans*	34,871	62.5	-	PHAGE_Salmon_SEN34_NC_028699(21)
DVT 1154_prophage 1	DVT 1154	*B. cenocepacia*	29,199	68.1	5	PHAGE_Burkho_phiE202_NC_009234(24)
DVT 1154_prophage 2	DVT 1154	*B. cenocepacia*	49,434	65	10	PHAGE_Salmon_SEN34_NC_028699(20)
DVT 1155_prophage 1	DVT 1155	*B. seminalis*	44,391	62.8	-	PHAGE_Rhodof_P26218_NC_029061(7)
DVT 1155_prophage 2	DVT 1155	*B. seminalis*	37,802	63.6	-	PHAGE_Burkho_KL3_NC_015266(39)
DVT 1155_prophage 3	DVT 1155	*B. seminalis*	26,712	65.2	-	PHAGE_Burkho_AP3_NC_047752(27)
DVT 1155_prophage 6	DVT 1155	*B. seminalis*	37,166	61.8	-	PHAGE_Burkho_ST79_NC_021343(46)
DVT 1157_prophage 1	DVT 1157	*B. vietnamensis*	47,046	65.5	-	PHAGE_Burkho_BcepC6B_NC_005887(37)
DVT 1157_prophage 2	DVT 1157	*B. vietnamensis*	41,263	61.5	-	PHAGE_Burkho_Bcep176_NC_007497(24)
DVT 1159_prophage 1	DVT 1159	*B. multivorans*	37,188	63.4	1	PHAGE_Burkho_KS5_NC_015265(35)
DVT 1161_prophage 1	DVT 1161	*B. multivorans*	38,405	62.3	8	PHAGE_Escher_vB_EcoM_ECO1230_10_NC_027995(8)
DVT 1163_prophage 1	DVT 1163	*B. cenocepacia*	44,021	65	9	PHAGE_Salmon_SEN34_NC_028699(14)
DVT 1165_prophage 1	DVT 1165	*B. cenocepacia*	34,706	63.3	-	PHAGE_Burkho_BcepMu_NC_005882(46)
DVT 1165_prophage 2	DVT 1165	*B. cenocepacia*	30,623	65.5	-	PHAGE_Myxoco_Mx8_NC_003085(7)
DVT 1165_prophage 3	DVT 1165	*B. cenocepacia*	40,032	63.5	-	PHAGE_Burkho_KS10_NC_011216(43)
DVT 1166_prophage 2	DVT 1166	*B. multivorans*	23,631	65.3	-	PHAGE_Salmon_SEN34_NC_028699(23)
DVT 1166_prophage 4	DVT 1166	*B. multivorans*	29,994	63	-	PHAGE_Burkho_phiE12_2_NC_009236(31)
DVT 1167_prophage 1	DVT 1167	*B. multivorans*	62,320	62.7	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(9)
DVT 1170_prophage 1	DVT 1170	*B. multivorans*	41,030	64.4	1	PHAGE_Burkho_KS5_NC_015265(35)
DVT 1171_prophage 1	DVT 1171	*B. multivorans*	39,836	63.8	2	PHAGE_Burkho_KS10_NC_011216(43)
DVT 1171_prophage 2	DVT 1171	*B. multivorans*	32,331	62.2	3	PHAGE_Escher_vB_EcoM_ECO1230_10_NC_027995(7)
DVT 1171_prophage 3	DVT 1171	*B. multivorans*	33,430	65.2	4	PHAGE_Burkho_KS5_NC_015265(37)
DVT 1172_prophage 1	DVT 1172	*B. multivorans*	39,836	63.8	2	PHAGE_Burkho_KS10_NC_011216(43)
DVT 1172_prophage 2	DVT 1172	*B. multivorans*	32,331	62.2	3	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(7)
DVT 1172_prophage 2	DVT 1172	*B. multivorans*	32,970	65.2	4	PHAGE_Burkho_KS5_NC_015265(38)
DVT 1173_prophage 1	DVT 1173	*B. multivorans*	17,089	62.4	-	PHAGE_Entero_SfV_NC_003444(4)
DVT 1173_prophage 2	DVT 1173	*B. multivorans*	37,187	63.3	1	PHAGE_Burkho_KS5_NC_015265(35)
DVT 1174_prophage 1	DVT 1174	*B. cenocepacia*	40,429	62.4	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(9)
DVT 1174_prophage 2	DVT 1174	*B. cenocepacia*	16,336	62.1	6	PHAGE_Entero_fiAA91_ss_NC_022750(2)
DVT 1175_prophage 1	DVT 1175	*B. cenocepacia*	8,307	63.1	11	PHAGE_Stx2_c_1717_NC_011357(3)
DVT 1175_prophage 2	DVT 1175	*B. cenocepacia*	16,371	62.3	7	PHAGE_Burkho_KS9_NC_013055(21)
DVT 1175_prophage 3	DVT 1175	*B. cenocepacia*	18,522	62.4	-	PHAGE_Burkho_KS9_NC_013055(14)
DVT 1177_prophage 1	DVT 1177	*B. multivorans*	37,188	63.4	1	PHAGE_Burkho_KS5_NC_015265(35)
DVT 1178_prophage 1	DVT 1178	*B. multivorans*	39,737	64	1	PHAGE_Burkho_KS5_NC_015265(35)
DVT 1179_prophage 1	DVT 1179	*B. cenocepacia*	44,021	65	9	PHAGE_Salmon_SEN34_NC_028699(14)
DVT 1179_prophage 2	DVT 1179	*B. cenocepacia*	7,338	59.8	-	PHAGE_Stx2_c_1717_NC_011357(3)
DVT 1180_prophage 3	DVT 1180	*B. multivorans*	34,935	64.2	-	PHAGE_Pseudo_NP1_NC_031058(5)
DVT1180_prophage 6	DVT 1180	*B. multivorans*	36,515	60.7	-	PHAGE_Salmon_118970_sal3_NC_031940(7)
DVT 1181_prophage 1	DVT 1181	*B. multivorans*	39,836	63.8	2	PHAGE_Burkho_KS10_NC_011216(43)
DVT 1181_prophage 2	DVT 1181	*B. multivorans*	32,331	62.2	3	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(7)
DVT 1181_prophage 3	DVT 1181	*B. multivorans*	32,989	65.2	4	PHAGE_Burkho_KS5_NC_015265(37)
DVT 1600_prophage 1	DVT 1600	*B. gladiolii*	38,172	61.2	-	PHAGE_Burkho_KS9_NC_013055(22)
DVT 1600_prophage 2	DVT 1600	*B. gladiolii*	34,696	61.3	-	PHAGE_Sphing_Lacusarx_NC_041927(4)
DVT 1600_prophage 3	DVT 1600	*B. gladiolii*	39,615	64.3	-	PHAGE_Burkho_AP3_NC_047752(35)
DVT 1608_prophage 1	DVT 1608	*B. multivorans*	50,362	62.9	-	PHAGE_Aeromo_vB_AsaM_56_NC_019527(15)
DVT 1608_prophage 2	DVT 1608	*B. multivorans*	37,188	63.4	1	PHAGE_Burkho_KS5_NC_015265(35)
DVT 1627_prophage 1	DVT 1627	*B. cenocepacia*	34,069	62.4	7	PHAGE_Burkho_KS9_NC_013055(32)
DVT 599_prophage 1	DVT 599	*B. cenocepacia*	26,389	65.4	10	PHAGE_Salmon_SEN34_NC_028699(19)
DVT 599_prophage 2	DVT 599	*B. cenocepacia*	29,200	68.2	5	PHAGE_Burkho_KL3_NC_015266(24)
DVT 614_prophage 1	DVT 614	*B. cenocepacia*	18,756	62.7	6	PHAGE_Burkho_KS9_NC_013055(14)
DVT 614_prophage 2	DVT 614	*B. cenocepacia*	14,871	61.7	7	PHAGE_Burkho_KS9_NC_013055(20)
DVT 790_prophage 1	DVT 790	*B. cenocepacia*	8,265	63.1	11	PHAGE_Stx2_c_Stx2a_F451_NC_049924(3)
DVT 790_prophage 2	DVT 790	*B. cenocepacia*	18,495	62.4	6	PHAGE_Burkho_KS9_NC_013055(14)

Appendix B

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