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

Hayley R. Nordstrom[1], Marissa P. Griffith[1], Vatsala Rangachar Srinivasa[1], Nathan R. Wallace[1], Anna Li[2], Vaughn S. Cooper[2,3], Ryan K. Shields[1], Daria Van Tyne[1,3*]

[1]Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

[2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

[3]Center for Evolutionary Biology and Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

*Address correspondence to: Daria Van Tyne, vantyne@pitt.edu

Keywords: *Burkholderia*, prophage, antibiotic resistance, phage therapy

Running title: *Burkholderia* prophage diversity

Abstract (200-word limit)

Burkholderia spp. are often resistant to antibiotics, and infections with these organisms are difficult to treat. A potential alternative treatment for *Burkholderia* spp. infections is bacteriophage (phage) therapy; however, it can be difficult to locate phages that target these bacteria. Prophages incorporated into the bacterial genome have been identified within *Burkholderia* spp. and may represent a source of useful phages for therapy. Here we investigate whether prophages within *Burkholderia* spp. clinical isolates can kill conspecific and heterospecific isolates. Thirty-two *Burkholderia* spp. isolates were induced for prophage release, and harvested prophages were tested for lytic activity against the same 32 isolates. Lytic phages were passaged and their host ranges were determined, resulting in four unique phages of prophage origin that showed different ranges of lytic activity. We also analyzed the prophage content of 35 *Burkholderia* spp. clinical isolate genomes, and identified several prophages present in the genomes of multiple isolates of the same species. Finally, we observed that *B. cenocepacia* isolates were more phage-susceptible than *Burkholderia multivorans* isolates. Overall, our findings suggest that prophages present within *Burkholderia* spp. genomes are a potentially useful starting point for the isolation and development of novel phages for use in phage therapy.

Introduction

Burkholderia is a genus of gram-negative bacteria that encompasses nearly 50 different species [1]. These organisms are abundant in the environment, found readily in soil and water, and associated with the rhizospheres of several species of plants [2, 3]. *Burkholderia* spp. are also opportunistic human pathogens, particularly the members of the *Burkholderia cepacia* complex (Bcc), a group of at least 20 species which possess high levels of intrinsic resistance to multiple classes of antibiotics [4]. Two Bcc species make up the majority of *Burkholderia* spp. clinical isolates in the United States: *Burkholderia multivorans* and *Burkholderia cenocepacia* [5, 6]. While infections caused by *Burkholderia* spp. are relatively rare in healthy people, these bacteria cause difficult-to-treat infections in patients with compromised immune systems or chronic conditions such as cystic fibrosis (CF) and chronic granulomatous disease [7, 8]. Chronic infection with Bcc, and in particular *B. cenocepacia*, in CF patients is associated with increased morbidity and mortality, decreased lung function, and shorter life expectancy [4, 8-10]. Treatment of *Burkholderia* spp. infections is further complicated by intrinsic resistance to many classes of antibiotics [11, 12]. This makes *Burkholderia* spp. some of the most challenging bacteria to eradicate, and increases the risk of transmission to vulnerable patients. Current treatment protocols for managing *Burkholderia* spp. infections typically include long courses of multiple antibiotics resulting in high rates of treatment failure [13]. Concern over these clinically challenging pathogens has led to a need for alternative treatment strategies.

Bacteriophage (phage) therapy is the use of naturally occurring viruses that infect bacteria to treat infections. Compassionate use of phage therapy has risen dramatically in the last decade, with numerous successful cases reported [14-16]. While phage therapy for *Burkholderia* spp. infections would be advantageous, to date only three compassionate use cases of *Burkholderia*-targeting phages have been reported [17-19]. However, studies investigating the use of phages to treat *Burkholderia* spp. infection *in vivo* have shown efficacy both in *Galleria mellonella* [20, 21]

and murine lung infection models [22, 23]. Phage therapy for *Burkholderia* spp. infections nonetheless is currently limited by the large genomic diversity of the *Burkholderia* genus as well as the relatively small number of *Burkholderia*-targeting phages that have been isolated to date [5]. One alternative source of *Burkholderia*-targeting phages are prophages found within the genomes of *Burkholderia* spp. isolates [24]. This is because prophages that have integrated into bacterial genomes are the result of successful prior phage infection and therefore could be useful for phage therapy [25, 26]. Engineering of prophages to render them obligately lytic and deploy them for phage therapy is a strategy that has been used previously, though not in *Burkholderia* [14]. Prophages found within the genomes of *Burkholderia* spp. may therefore represent a source of phages for potential therapeutic use.

In this study, we investigated whether prophages from *Burkholderia* spp. clinical isolates could lyse other clinical isolates belonging to the same (conspecific) or different (heterospecific) species. We induced prophage release from *Burkholderia* spp. clinical isolates using mitomycin C and subsequently propagated prophages that lysed alternative *Burkholderia* spp. isolates. We isolated four different prophages with varying activities in this manner. Additionally, we characterized the prophage content of 35 *Burkholderia* spp. clinical isolates, and explored associations between bacterial species, prophage content, and phage susceptibility. Taken together, this study represents a first step toward addressing the limited availability of *Burkholderia*-targeting phages, presents an alternative strategy for phage discovery, and uncovers valuable insights regarding prophage carriage among *Burkholderia* spp. clinical isolates.

Methods

Bacterial isolates and induction of prophage release

All *Burkholderia* spp. isolates used in this study were collected from patients at the University of Pittsburgh Medical Center (UPMC) as part of routine clinical care. Most isolates were

collected as part of the Enhanced Detection System for Healthcare Associated Transmission [27], and others were collected from patients being evaluated for compassionate use phage therapy. Isolate collection was approved by the University of Pittsburgh Institutional Review Board under protocols PRO07060222 and STUDY19110005.

To induce prophage release, 10 µL of stationary phase liquid culture of each bacterial isolate was inoculated into 5 mL of Luria Broth (LB) containing 2.5 µg/mL mitomycin C. Cultures were grown overnight shaking at 37ºC. The next day bacterial cells were pelleted, and liquid lysates were filtered through a 0.22 µm syringe filter to remove bacteria. The remaining lysates were presumed to contain prophages that were released during growth.

Isolation of phages and host range testing

Lytic bacteriophage activity was identified with a soft agar overlay assay [28]. Briefly, square petri plates were prepared containing LB bottom agar (LB media with 1.5% agar, 1 mM $CaCl_2$ and 1 mM $MgCl_2$). Bacterial isolates were inoculated into LB media and incubated overnight at 37°C. The following day, bacterial soft agar lawns were created by mixing 50 µL of overnight bacterial culture with 5 mL of LB top agarose (LB media with 0.5% agarose, 1 mM $CaCl_2$, and 1 mM $MgCl_2$) cooled to 55°C, plated on top of bottom agar plates and allowed to solidify. After top agar bacterial lawns had solidified, 5 µL of potential phage-containing lysates were spotted on top of the lawn. Plates were incubated upright at 37ºC overnight. The next day, plates were examined for evidence of lytic phage activity in the form of plaques or clearing of the bacterial lawn. Individual clear plaques were “picked” with a pipette tip, transferred into 100 µL of suspension media (SM) buffer (50 mM TrisCl pH 7.5, 100 mM NaCl, 8 mM $MgSO_4$) and were incubated overnight at 37ºC. Areas of full clearance where individual plaques were not visible were noted, and phage-mediated killing was confirmed by plating 10-fold serial dilutions of prophage lysate to observe and pick individual plaques. To propagate and isolate individual phages, individual plaques were picked and prepared in 100 µL SM buffer as described above. Serial 10-fold

dilutions of each plaque pick were made in SM buffer, and 5 µL of each dilution was spotted onto a plate containing 5 mL of LB top agarose mixed with 50 µL of bacterial culture of the propagating isolate and layered on top of a LB 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.

To prepare high-titer liquid lysates of each phage, individual plaques picked after four rounds of plaque purification were transferred to 100 µL of SM buffer and were then mixed with 100 µL of overnight 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 10 mL of SM buffer and incubated at 37ºC for 2 hours. The SM lysate was then collected and centrifuged at 4,000 g for 10 minutes to pellet bacteria. Supernatants were then filter sterilized through a 0.22 µm membrane syringe filter and were stored for future use.

Host range testing was performed using the soft agar overlay spot screening method [28]. Briefly, 5 uL of 10-fold serial dilutions of each phage lysate was spotted onto top agar lawns of each bacterial isolate and incubated at 37 ºC overnight. The following day, each phage-bacteria pairing was assessed for visibility of plaques. For pairings where lysis was noted, phage titer in plaque-forming units (PFU)/mL was calculated.

Whole genome sequencing and analysis

Bacterial genomic DNA was extracted from 1 mL 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 from aliquots of high-titer liquid lysate using the same kit, or via phenol chloroform extraction followed by ethanol precipitation. Briefly, 500 µL phenol:chloroform:isoamyl alcohol (25:24:1) was added to 500 µL of each lysate,

samples were vortexed and then centrifuged at 16,000 g for 1 minute. The upper aqueous phase was transferred to a new tube and 500 µL of chloroform was added. Samples were vortexed and centrifuged again at 16,000 g for 1 minute, and the upper aqueous phase was again transferred to a new tube. Then 1 µL glycogen, 0.1 x volume 3 M sodium acetate, and 2.5 x volume 100% ethanol were added, and samples were incubated overnight at -20ºC. The next day, samples were centrifuged at 16,000 g for 30 minutes at 4ºC, then the supernatant was removed and the DNA pellet was washed with 150 µL 70% ethanol. DNA pellets were resuspended in 100 µL 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).

Genomes were assembled with SPAdes v3.11 to generate contigs with a 200 bp minimum length cut-off [29]. 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 [30]. A core genome SNP alignment was generated with snippy v4.6 (https://github.com/tseemann/snippy), then the core alignment was used to construct a phylogenetic tree with RAxML with the GTRCAT substitution model and 1000 iterations [31]. Bacterial species were assigned by average nucleotide identity (ANI) comparisons with previously sequenced *Burkholderia* species using fastANI [32]. Prophages were identified in each bacterial genome using PHASTER [33]. Prophages of any length that were predicted to be intact, questionable, or incomplete by PHASTER were included. Prophage sequences were compared to one another with BLASTN [34], 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

159 performed and visualized using Cytoscape [35]. Predicted family and genus of each
160 bacteriophage was determined by closest BLAST match in the NCBI nr database.
161
162 *EM imaging*
163 1-5 µL of bacteriophage BCC02 suspension was added to a copper grid and negatively
164 stained with 1% uranyl acetate. Phage suspension was imaged by transmission electron
165 microscopy on a JEW 1400 Flash Transmission Electron Microscope. Imaging was performed by
166 the University of Pittsburgh Center for Biologic Imaging.
167
168 *Statistical analysis*
169 Statistical analyses were performed in GraphPad Prism version 8.0.0 (GraphPad
170 Software, San Diego, California USA). Linear regression and two-tailed t-tests were performed to
171 assess significance of associations between prophage abundance and phage susceptibility. A
172 Fisher's Exact Test was used to assess the association between Bmulti_pp1 and phage
173 resistance in *B. multivorans* isolates.
174
175
176 **Results**
177 **Prophage Induction and Isolation**
178 To test whether prophages found within the genomes of *Burkholderia* spp. clinical isolates
179 could target other *Burkholderia* spp. isolates, we first created a library of potential prophage-
180 containing lysates. To do this, we inoculated 32 *Burkholderia* spp. clinical isolates collected from
181 28 unique patients into liquid culture in the presence of the mutagen, mitomycin C, which prompts
182 prophage excision (Figure 1) [36, 37]. We then performed an all-by-all screen of the 32 prophage-
183 containing "source" lysates against the same 32 *Burkholderia* spp. "target" isolates, using a spot-
184 plaque screening method to perform 1,024 pairwise tests (Figure 1). For 11 of these tests, a

prophage-containing source lysate inhibited the growth of the target bacterial lawn (1.1% hit rate). Each positive pairing was retested to confirm that the observed inhibitory activity was due to phage activity by serially diluting the source lysate and looking for individual plaques. The 10 source-target pairings with confirmed phage activity were then subjected to four rounds of picking and passaging of single plaques to isolate individual phages. Three of these pairings did not maintain lytic activity through picking and passaging, meaning that during one of the passages no lytic plaques were visible perhaps due to instability between lysis and lysogeny. The seven remaining source-target pairs yielded viable phages, which were amplified into high-titer lysates and designated as BCC02 through BCC08 (Table S1).

Whole Genome Sequencing of *Burkholderia* spp. Clinical Isolates

To explore the genetic diversity of the *Burkholderia* spp. clinical isolates studied here, we sequenced 35 bacterial genomes, including the 32 isolates used for prophage induction and testing, plus three additional clinical isolates. Isolates were sequenced on the Illumina platform and a core genome phylogeny was constructed (Figure 2). The species of each isolate was determined using fastANI [32] to compare average nucleotide identity (ANI) between each genome and available genomes of type strains of *Burkholderia* spp. The most frequently sampled species were *B. multivorans* (16 isolates collected from 13 patients) and *B. cenocepacia* (14 isolates collected from 13 patients). One isolate each belonged to *Burkholderia gladioli*, *Burkholderia pseudomultivorans*, *Burkholderia vietnamiensis*, *Burkholderia seminalis*, and *Burkholderia cepacia* (Figure 2). All isolates except DVT1600 (*Burkholderia gladioli*) fell into the *Burkholderia cepacia* complex (Figure 2).

Phage Host-Range Screening

To determine the infectivity profile of each isolated prophage-derived phage as well as the phage susceptibility of each *Burkholderia* spp. clinical isolate, we performed an all-by-all phage

activity screen. We tested all seven prophage-derived phages as well as two additional *Burkholderia*-targeting bacteriophages of environmental origin, DSMZ107315 and Bch7 [19], against all 35 *Burkholderia* spp. clinical isolates (Figure 2). Eight isolates, all of which were *B. multivorans*, were resistant to all phages tested. However, the other 27 isolates (77.1% of all isolates tested) were susceptible to at least one phage. We observed variability in the host range of each phage, with phages able to lyse between 2 (BCC06) and 12 (Bch7) of the 35 isolates. All phages were able to infect and lyse multiple isolates, and in most cases, phages were able to lyse bacteria belonging to different species (Figure 2). We observed similar host ranges for phages BCC02, BCC03, and BCC04, as well as for phages BCC05 and BCC06. This finding, along with the fact that these groups contained phages derived from the same donor bacterial isolates (Table S1), suggested that these might represent duplicate isolations of the same phage.

Whole Genome Sequencing of Phages

To explore the genetic diversity of the phages tested in this study, phage lysates were subjected to genomic DNA extraction and sequencing on the Illumina platform. High-coverage phage-derived contigs were analyzed and were compared to each other, to bacterial prophage genomes, and to publicly available genomes (Table 1). Phage genomes ranged in size from 21-68 kb and varied in GC content from 54.7-67.1% (Table 1). Phages BCC02, BCC03 and BCC04, which were all isolated from the DVT1180 source isolate, were found to be genetically identical to one another. Phage BCC02 therefore was chosen as the representative phage for follow-on work. Likewise, phages BCC05 and BCC06, which were both isolated from the DVT1166 source isolate, were found to be identical except for one single nucleotide polymorphism (SNP) within a gene predicted to encode a phage tail fiber protein, suggesting divergent evolution during the course of phage isolation. However, as we observed similar host ranges between BCC05 and BCC06, for the purposes of our analysis we also considered these two phages to be identical and chose BCC05 as the representative 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 1). Transmission electron microscopy (TEM) imaging of phage BCC02 confirmed that it has a contractile tail and icosahedral head (Figure 3). Overall, genomic analyses revealed that our approach yielded four novel *Burkholderia*-targeting bacteriophages (BCC02, BCC05, BCC07, and BCC08) of prophage origin that were distinct from each other and from other known phages.

Analysis of Prophage Carriage in *Burkholderia* spp. Clinical Isolates

To understand the diversity of prophages present in the *Burkholderia* spp. clinical isolates we collected, we extracted prophage sequences from the genomes of the 35 isolates used for screening. We searched each isolate genome for sequences of likely prophage origin using PHASTER [33]. A total of 114 prophage sequences were identified (range = 1-7, median = 3 prophages per genome) (Table S2) (Figure 4a). We tested whether prophage abundance was associated with phage susceptibility and found that these two features were only loosely correlated with one another (linear regression $P = 0.0675$) (Figure S1, S2). However, we did find that *B. multivorans* isolates contained more prophages compared with *B. cenocepacia* isolates (3.8 average prophages in *B. multivorans* vs. 2.4 average prophages in *B. cenocepacia*, $P = 0.0266$) (Figure 4b). Additionally, *B. multivorans* isolates were susceptible to fewer phages compared with *B. cenocepacia* isolates ($P = 0.0005$) (Figure 4c), in agreement with other studies demonstrating an inverse correlation between prophage abundance and phage susceptibility in different bacterial species [38-40].

To determine the extent of relatedness among prophages encoded in *Burkholderia* spp. clinical isolate genomes, we compared all extracted prophage sequences to one another using nucleotide BLAST [34]. Prophages that shared >90% sequence coverage and >90% sequence identity were grouped into clusters, and clusters were visualized using Cytoscape [35] (Figure 5) (Table S2). Twenty clusters of similar prophages were identified, which ranged in size from 2-4

with the exception of one large cluster containing 11 prophages. Overall, prophage clusters tended to group with related host strains. For example, 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*) specifically were collected from one patient while DVT599 and DVT1154 (*B. cenocepacia*) were collected from another. The prophages present in these isolates clustered together, as expected (Figure 5) (Table S2). Isolated phages BCC02/BCC03/BCC04, BCC05/BCC06, BCC07 and BCC08 did not fall into any of these clusters, however. Finally, one cluster, which we named Bmulti_pp1, contained 11 prophage sequences from 10 *B. multivorans* isolates. Presence of this prophage was significantly associated with phage resistance among the *B. multivorans* isolates we tested (P = 0.035) (Figure S3). This finding suggests that Bmulti_pp1 may provide protection against phage-mediated lysis in *B. multivorans.*

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 cellular and ecological levels. These interactions are influenced by many factors, including genetics, cellular development, community dynamics, and environmental conditions [41]. In this study, we show that in some cases, the relationship between bacteria and their prophages can be exploited to isolate bacteriophages with potential therapeutic use, and that prophage induction with mitomycin C is an effective method to accomplish this in *Burkholderia* isolates.

Polylysogeny in *Burkholderia* species appears to be common [24], and prophages present in *Burkholderia* genomes likely represent a rich hunting ground for clinically useful bacteriophages [42]. Triggering the bacterial DNA damage response using a mutagen like mitomycin C is a simple way to activate the lysogenic-lytic switch for some prophages [43], and the prophages we induced with this method showed lytic activity against both conspecific and heterospecific bacterial

isolates. However, further characterization and rational engineering of temperate phages usually is considered to be necessary before they can be used clinically. Engineering of *Burkholderia* prophages to render them obligate lytic, either through site-directed mutagenesis or experimental evolution, has been previously described [44, 45]. Similar approaches could be taken with the prophage-derived phages described here to attempt to convert them from temperate to lytic phages. Such exploration of the use of temperate bacteriophages in phage therapy has the potential to expand the pool of useful tools against the escalating threat of multidrug-resistant bacteria when naturally occurring obligately lytic phages are rare. Several strategies for their use have already been identified, including in *Burkholderia*.

In contrast to prior studies [46, 47], here we performed a systematic screen of lytic phage activity among induced prophages using more than 30 genetically diverse *Burkholderia* spp. clinical isolates. The four prophage-derived phages we studied showed combined activity against 60% (21/35) of the clinical isolates that were tested. The isolated prophage BCC02, while a temperate phage, was able to lyse 31% (11/35) 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 [48]. The four novel prophage-derived phages isolated and described in this study have potential for further development toward therapeutic application. With the exception of BCC05 and BCC06, the host ranges of bacteriophages of prophage origin were comparable to those of environmental origin, in agreement with previous literature [48, 49]. Additionally, identification of phages with varying host ranges and infection dynamics allows for the development of multi-phage cocktails in order to reduce the probability of phage resistance [46].

Our findings demonstrate that clinically significant *Burkholderia* isolates host a variety of prophage elements, in agreement with previous studies showing that lysogeny is relatively common in this genus [3, 49, 50]. While our study was likely underpowered to identify a strong correlation between prophage abundance and phage susceptibility, we found more prophages

and greater overall phage resistance in *B. multivorans* isolates compared to *B. cenocepacia* isolates. A prior study similarly noted the relative phage resistance of *B. multivorans* isolates [49]. We also identified one prophage that was associated with resistance to phage lysis, which may indicate some evolutionary or ecological relevance, possibly enabling superinfection immunity. The occurrence of this prophage could indicate ancestral integration maintained through a fitness advantage, or direct phage transmission between clinical strains in close contact with one another. Exploring the effects of this prophage on infection dynamics of other phages requires further study.

This study had several limitations. The small volume spot-screening method used to isolate induced phages may have missed phages that were present in lysates at low concentration. Additionally, slow-growing clinical isolates may have hindered the detection of lytic activity, since some temperate phages are known to induce lysis only when host density is high [41]. All genomes used for our analysis were draft genomes, and many were not able to be completely assembled 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 unable to conclude that these newly characterized phages would be useful for clinical therapy without further testing in a relevant *in vivo* model of infection.

Overall, the data generated in this study adds to the literature characterizing *Burkholderia*-targeting bacteriophages, as well as prophage abundance and diversity in clinically relevant *Burkholderia* species. We isolated four novel bacteriophages with lytic activity against a variety of Bcc isolates, identified prophage-derived phages with broad host range, and detected an association between one prophage and phage resistance in *B. multivorans*. The isolated phages of prophage origin may also have clinical utility, and are a potentially useful starting point for the development of novel phages for use in phage therapy.

341

342

Acknowledgements

We gratefully acknowledge Donna Stolz for assistance with EM imaging of BCC02. Many clinical *Burkholderia* spp. isolates were kindly provided by Lee Harrison from the EDS-HAT project (R01AI127472). This work was supported by grant BOMBER19R0 from the Cystic Fibrosis Foundation to DVT, and by the Department of Medicine at the University of Pittsburgh School of Medicine. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

HRN and DVT designed the study. RKS provided clinical isolates. HRN, MPG, VRS, NRW, and AL performed experiments and generated data. HRN and DVT wrote the manuscript. All authors reviewed and edited the manuscript.

Tables

Table 1. Genome summary of *Burkholderia*-targeting phages

Phage ID	Source Isolate	Source species	Length (bp)	GC %	Predicted Genus[1]	NCBI Similar Phage[2]
BCC02/03/04	DVT1180	*B. multivorans*	34,126	64.2	*Peduovirinae; Kisquinquevirus*	*Burkholderia* Phage KS5 (GU911303.1)
BCC05/06	DVT1166	*B. multivorans*	30,957	62.8	*Peduoviridae; Duodecimduovirus*	*Burkholderia* Phage phiE12-2 (NC_009236.1)
BCC07	DVT1155	*B. seminalis*	38,216	66.7	*Peduoviridae; Aptresvirus*	*Burkholderia* Phage Mana (NC_055863.1)
BCC08	DVT1155	*B. seminalis*	28,709	63.7	*Peduoviridae; Kayeltresvirus*	*Burkholderia* Phage KL3 (GU911304.1)
Bch7	Env	-	68,166	54.7	*Bcepfunavirus*	*Burkholderia* Phage Maja (MT708549.1)
DSMZ 107315	Env	-	22,967	61.5	*Peduovirinae; Kisquattuordecimvirus*	*Burkholderia* Phage FLC5 (NC_055722.1)

[1]Predictions were made based on nucleotide BLAST comparison with available phage genomes in NCBI.

[2]Most similar phage identified in NCBI through nucleotide BLAST comparison of sequence coverage and identity.

Env = Environmental.

References

1. Sawana, A., M. Adeolu, and R. S. Gupta. "Molecular Signatures and Phylogenomic Analysis of the Genus Burkholderia: Proposal for Division of This Genus into the Emended Genus Burkholderia Containing Pathogenic Organisms and a New Genus Paraburkholderia Gen. Nov. Harboring Environmental Species." *Front Genet* 5 (2014): 429.
2. Coenye, T., P. Vandamme, J. R. Govan, and J. J. LiPuma. "Taxonomy and Identification of the Burkholderia Cepacia Complex." *J Clin Microbiol* 39, no. 10 (2001): 3427-36.
3. Mahenthiralingam, E., T. A. Urban, and J. B. Goldberg. "The Multifarious, Multireplicon Burkholderia Cepacia Complex." *Nat Rev Microbiol* 3, no. 2 (2005): 144-56.
4. Somayaji, R., Y. C. W. Yau, E. Tullis, J. J. LiPuma, F. Ratjen, and V. Waters. "Clinical Outcomes Associated with Burkholderia Cepacia Complex Infection in Patients with Cystic Fibrosis." *Ann Am Thorac Soc* 17, no. 12 (2020): 1542-48.
5. Lauman, P., and J. J. Dennis. "Advances in Phage Therapy: Targeting the Burkholderia Cepacia Complex." *Viruses* 13, no. 7 (2021).
6. LiPuma, J. J., T. Spilker, L. H. Gill, P. W. Campbell, 3rd, L. Liu, and E. Mahenthiralingam. "Disproportionate Distribution of Burkholderia Cepacia Complex Species and Transmissibility Markers in Cystic Fibrosis." *Am J Respir Crit Care Med* 164, no. 1 (2001): 92-6.
7. Govan, J. R., and V. Deretic. "Microbial Pathogenesis in Cystic Fibrosis: Mucoid Pseudomonas Aeruginosa and Burkholderia Cepacia." *Microbiol Rev* 60, no. 3 (1996): 539-74.
8. Jones, A. M., M. E. Dodd, and A. K. Webb. "Burkholderia Cepacia: Current Clinical Issues, Environmental Controversies and Ethical Dilemmas." *Eur Respir J* 17, no. 2 (2001): 295-301.

9. Branstetter, J. W., A. Yarbrough, and C. Poole. "Management of Cepacia Syndrome with a Combination of Intravenous and Inhaled Antimicrobials in a Non-Cystic Fibrosis Pediatric Patient." *J Pediatr Pharmacol Ther* 25, no. 8 (2020): 730-34.

10. Zahariadis, G., M. H. Levy, and J. L. Burns. "Cepacia-Like Syndrome Caused by Burkholderia Multivorans." *Can J Infect Dis* 14, no. 2 (2003): 123-5.

11. Burns, J. L., C. D. Wadsworth, J. J. Barry, and C. P. Goodall. "Nucleotide Sequence Analysis of a Gene from Burkholderia (Pseudomonas) Cepacia Encoding an Outer Membrane Lipoprotein Involved in Multiple Antibiotic Resistance." *Antimicrob Agents Chemother* 40, no. 2 (1996): 307-13.

12. Hancock, R. E. "Peptide Antibiotics." *Lancet* 349, no. 9049 (1997): 418-22.

13. Middleton, P. G., T. J. Kidd, and B. Williams. "Combination Aerosol Therapy to Treat Burkholderia Cepacia Complex." *Eur Respir J* 26, no. 2 (2005): 305-8.

14. Dedrick, R. M., C. A. Guerrero-Bustamante, R. A. Garlena, D. A. Russell, K. Ford, K. Harris, K. C. Gilmour, J. Soothill, D. Jacobs-Sera, R. T. Schooley, G. F. Hatfull, and H. Spencer. "Engineered Bacteriophages for Treatment of a Patient with a Disseminated Drug-Resistant *Mycobacterium Abscessus*." *Nat Med* 25, no. 5 (2019): 730-33.

15. Suh, G. A., T. P. Lodise, P. D. Tamma, J. M. Knisely, J. Alexander, S. Aslam, K. D. Barton, E. Bizzell, K. M. C. Totten, J. L. Campbell, B. K. Chan, S. A. Cunningham, K. E. Goodman, K. E. Greenwood-Quaintance, A. D. Harris, S. Hesse, A. Maresso, V. Nussenblatt, D. Pride, M. J. Rybak, Z. Sund, D. van Duin, D. Van Tyne, R. Patel, and Group Antibacterial Resistance Leadership. "Considerations for the Use of Phage Therapy in Clinical Practice." *Antimicrob Agents Chemother* 66, no. 3 (2022): e0207121.

16. Schooley, R. T., B. Biswas, J. J. Gill, A. Hernandez-Morales, J. Lancaster, L. Lessor, J. J. Barr, S. L. Reed, F. Rohwer, S. Benler, A. M. Segall, R. Taplitz, D. M. Smith, K. Kerr, M. Kumaraswamy, V. Nizet, L. Lin, M. D. McCauley, S. A. Strathdee, C. A. Benson, R. K. Pope, B. M. Leroux, A. C. Picel, A. J. Mateczun, K. E. Cilwa, J. M. Regeimbal, L. A.

Estrella, D. M. Wolfe, M. S. Henry, J. Quinones, S. Salka, K. A. Bishop-Lilly, R. Young, and T. Hamilton. "Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails to Treat a Patient with a Disseminated Resistant Acinetobacter Baumannii Infection." *Antimicrob Agents Chemother* 61, no. 10 (2017).

17. Aslam, S., A. M. Courtwright, C. Koval, S. M. Lehman, S. Morales, C. L. Furr, F. Rosas, M. J. Brownstein, J. R. Fackler, B. M. Sisson, B. Biswas, M. Henry, T. Luu, B. N. Bivens, T. Hamilton, C. Duplessis, C. Logan, N. Law, G. Yung, J. Turowski, J. Anesi, S. A. Strathdee, and R. T. Schooley. "Early Clinical Experience of Bacteriophage Therapy in 3 Lung Transplant Recipients." *Am J Transplant* 19, no. 9 (2019): 2631-39.

18. Smith, Mallory. *Salt in My Soul: An Unfinished Life*: Random House, 2019.

19. Haidar, G., B. K. Chan, S. T. Cho, K. Hughes Kramer, H. R. Nordstrom, N. R. Wallace, M. E. Stellfox, M. Holland, E. G. Kline, J. M. Kozar, S. D. Kilaru, J. M. Pilewski, J. J. LiPuma, V. S. Cooper, R. K. Shields, and D. Van Tyne. "Phage Therapy in a Lung Transplant Recipient with Cystic Fibrosis Infected with Multidrug-Resistant Burkholderia Multivorans." *Transpl Infect Dis* 25, no. 2 (2023): e14041.

20. Roszniowski, B., A. Latka, B. Maciejewska, D. Vandenheuvel, T. Olszak, Y. Briers, G. S. Holt, M. A. Valvano, R. Lavigne, D. L. Smith, and Z. Drulis-Kawa. "The Temperate Burkholderia Phage Ap3 of the Peduovirinae Shows Efficient Antimicrobial Activity against B. Cenocepacia of the Iiia Lineage." *Appl Microbiol Biotechnol* 101, no. 3 (2017): 1203-16.

21. Seed, K. D., and J. J. Dennis. "Experimental Bacteriophage Therapy Increases Survival of Galleria Mellonella Larvae Infected with Clinically Relevant Strains of the Burkholderia Cepacia Complex." *Antimicrob Agents Chemother* 53, no. 5 (2009): 2205-8.

22. Carmody, L. A., J. J. Gill, E. J. Summer, U. S. Sajjan, C. F. Gonzalez, R. F. Young, and J. J. LiPuma. "Efficacy of Bacteriophage Therapy in a Model of Burkholderia Cenocepacia Pulmonary Infection." *J Infect Dis* 201, no. 2 (2010): 264-71.

438 23. Semler, D. D., A. D. Goudie, W. H. Finlay, and J. J. Dennis. "Aerosol Phage Therapy
439 Efficacy in Burkholderia Cepacia Complex Respiratory Infections." *Antimicrob Agents*
440 *Chemother* 58, no. 7 (2014): 4005-13.
441 24. Roszniowski, B., S. McClean, and Z. Drulis-Kawa. "Burkholderia Cenocepacia
442 Prophages-Prevalence, Chromosome Location and Major Genes Involved." *Viruses* 10,
443 no. 6 (2018).
444 25. Dedrick, R. M., B. E. Smith, R. A. Garlena, D. A. Russell, H. G. Aull, V. Mahalingam, A.
445 M. Divens, C. A. Guerrero-Bustamante, K. M. Zack, L. Abad, C. H. Gauthier, D. Jacobs-
446 Sera, and G. F. Hatfull. "Mycobacterium Abscessus Strain Morphotype Determines
447 Phage Susceptibility, the Repertoire of Therapeutically Useful Phages, and Phage
448 Resistance." *MBio* 12, no. 2 (2021).
449 26. Raya, R. R., and M. H'Bert E. "Isolation of Phage Via Induction of Lysogens." *Methods*
450 *Mol Biol* 501 (2009): 23-32.
451 27. Sundermann, A. J., J. Chen, P. Kumar, A. M. Ayres, S. T. Cho, C. Ezeonwuka, M. P.
452 Griffith, J. K. Miller, M. M. Mustapha, A. W. Pasculle, M. I. Saul, K. A. Shutt, V. Srinivasa,
453 K. Waggle, D. J. Snyder, V. S. Cooper, D. Van Tyne, G. M. Snyder, J. W. Marsh, A.
454 Dubrawski, M. S. Roberts, and L. H. Harrison. "Whole-Genome Sequencing Surveillance
455 and Machine Learning of the Electronic Health Record for Enhanced Healthcare
456 Outbreak Detection." *Clin Infect Dis* 75, no. 3 (2022): 476-82.
457 28. Russell, DA, MRJ Clokie, AM Kropinski, and R Lavigne. "Bacteriophages: Methods and
458 Protocols." (2018).
459 29. Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M.
460 Lesin, S. I. Nikolenko, S. Pham, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi,
461 G. Tesler, M. A. Alekseyev, and P. A. Pevzner. "Spades: A New Genome Assembly
462 Algorithm and Its Applications to Single-Cell Sequencing." *J Comput Biol* 19, no. 5
463 (2012): 455-77.

30. Aziz, R. K., D. Bartels, A. A. Best, M. DeJongh, T. Disz, R. A. Edwards, K. Formsma, S. Gerdes, E. M. Glass, M. Kubal, F. Meyer, G. J. Olsen, R. Olson, A. L. Osterman, R. A. Overbeek, L. K. McNeil, D. Paarmann, T. Paczian, B. Parrello, G. D. Pusch, C. Reich, R. Stevens, O. Vassieva, V. Vonstein, A. Wilke, and O. Zagnitko. "The Rast Server: Rapid Annotations Using Subsystems Technology." *BMC Genomics* 9 (2008): 75.

31. Stamatakis, A. "Raxml Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies." *Bioinformatics* 30, no. 9 (2014): 1312-3.

32. Jain, C., R. Lm Rodriguez, A. M. Phillippy, K. T. Konstantinidis, and S. Aluru. "High Throughput Ani Analysis of 90k Prokaryotic Genomes Reveals Clear Species Boundaries." *Nat Commun* 9, no. 1 (2018): 5114.

33. Arndt, D., A. Marcu, Y. Liang, and D. S. Wishart. "Phast, Phaster and Phastest: Tools for Finding Prophage in Bacterial Genomes." *Brief Bioinform* (2017).

34. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. "Basic Local Alignment Search Tool." *J Mol Biol* 215, no. 3 (1990): 403-10.

35. Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker. "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks." *Genome Res* 13, no. 11 (2003): 2498-504.

36. Oliveira, J., J. Mahony, L. Hanemaaijer, Trhm Kouwen, H. Neve, J. MacSharry, and D. van Sinderen. "Detecting Lactococcus Lactis Prophages by Mitomycin C-Mediated Induction Coupled to Flow Cytometry Analysis." *Front Microbiol* 8 (2017): 1343.

37. Otsuji, N., M. Sekiguchi, T. Iijima, and Y. Takagi. "Induction of Phage Formation in the Lysogenic Escherichia Coli K-12 by Mitomycin C." *Nature* 184(Suppl 14) (1959): 1079-80.

38. Bondy-Denomy, J., J. Qian, E. R. Westra, A. Buckling, D. S. Guttman, A. R. Davidson, and K. L. Maxwell. "Prophages Mediate Defense against Phage Infection through Diverse Mechanisms." *ISME J* 10, no. 12 (2016): 2854-66.

490 39. Dedrick, R. M., D. Jacobs-Sera, C. A. Bustamante, R. A. Garlena, T. N. Mavrich, W. H.
491 Pope, J. C. Reyes, D. A. Russell, T. Adair, R. Alvey, J. A. Bonilla, J. S. Bricker, B. R.
492 Brown, D. Byrnes, S. G. Cresawn, W. B. Davis, L. A. Dickson, N. P. Edgington, A. M.
493 Findley, U. Golebiewska, J. H. Grose, C. F. Hayes, L. E. Hughes, K. W. Hutchison, S.
494 Isern, A. A. Johnson, M. A. Kenna, K. K. Klyczek, C. M. Mageeney, S. F. Michael, S. D.
495 Molloy, M. T. Montgomery, J. Neitzel, S. T. Page, M. C. Pizzorno, M. K. Poxleitner, C. A.
496 Rinehart, C. J. Robinson, M. R. Rubin, J. N. Teyim, E. Vazquez, V. C. Ware, J.
497 Washington, and G. F. Hatfull. "Prophage-Mediated Defence against Viral Attack and
498 Viral Counter-Defence." *Nat Microbiol* 2 (2017): 16251.
499 40. Harrison, E., and M. A. Brockhurst. "Ecological and Evolutionary Benefits of Temperate
500 Phage: What Does or Doesn't Kill You Makes You Stronger." *Bioessays* 39, no. 12
501 (2017).
502 41. Howard-Varona, C., K. R. Hargreaves, S. T. Abedon, and M. B. Sullivan. "Lysogeny in
503 Nature: Mechanisms, Impact and Ecology of Temperate Phages." *ISME J* 11, no. 7
504 (2017): 1511-20.
505 42. Monteiro, R., D. P. Pires, A. R. Costa, and J. Azeredo. "Phage Therapy: Going
506 Temperate?" *Trends Microbiol* 27, no. 4 (2019): 368-78.
507 43. Tomasz, M. "Mitomycin C: Small, Fast and Deadly (but Very Selective)." *Chem Biol* 2,
508 no. 9 (1995): 575-9.
509 44. Lynch, K. H., K. D. Seed, P. Stothard, and J. J. Dennis. "Inactivation of Burkholderia
510 Cepacia Complex Phage Ks9 Gp41 Identifies the Phage Repressor and Generates Lytic
511 Virions." *J Virol* 84, no. 3 (2010): 1276-88.
512 45. Yao, G., T. Le, A. M. Korn, H. N. Peterson, M. Liu, C. F. Gonzalez, and J. J. Gill. "Phage
513 Milagro: A Platform for Engineering a Broad Host Range Virulent Phage for
514 Burkholderia." *J Virol* 97, no. 11 (2023): e0085023.

515 46. Lauman, P., and J. J. Dennis. "Synergistic Interactions among Burkholderia Cepacia
516 Complex-Targeting Phages Reveal a Novel Therapeutic Role for Lysogenization-
517 Capable Phages." *Microbiol Spectr* 11, no. 3 (2023): e0443022.
518 47. Al-Anany, A. M., R. Fatima, and A. P. Hynes. "Temperate Phage-Antibiotic Synergy
519 Eradicates Bacteria through Depletion of Lysogens." *Cell Rep* 35, no. 8 (2021): 109172.
520 48. Seed, K. D., and J. J. Dennis. "Isolation and Characterization of Bacteriophages of the
521 Burkholderia Cepacia Complex." *FEMS Microbiol Lett* 251, no. 2 (2005): 273-80.
522 49. Langley, R., D. T. Kenna, P. Vandamme, R. Ure, and J. R. W. Govan. "Lysogeny and
523 Bacteriophage Host Range within the Burkholderia Cepacia Complex." *J Med Microbiol*
524 52, no. Pt 6 (2003): 483-90.
525 50. Weiser, R., Z. L. Yap, A. Otter, B. V. Jones, J. Salvage, J. Parkhill, and E.
526 Mahenthiralingam. "A Novel Inducible Prophage from Burkholderia Vietnamiensis G4 Is
527 Widely Distributed across the Species and Has Lytic Activity against Pathogenic
528 Burkholderia." *Viruses* 12, no. 6 (2020).
529

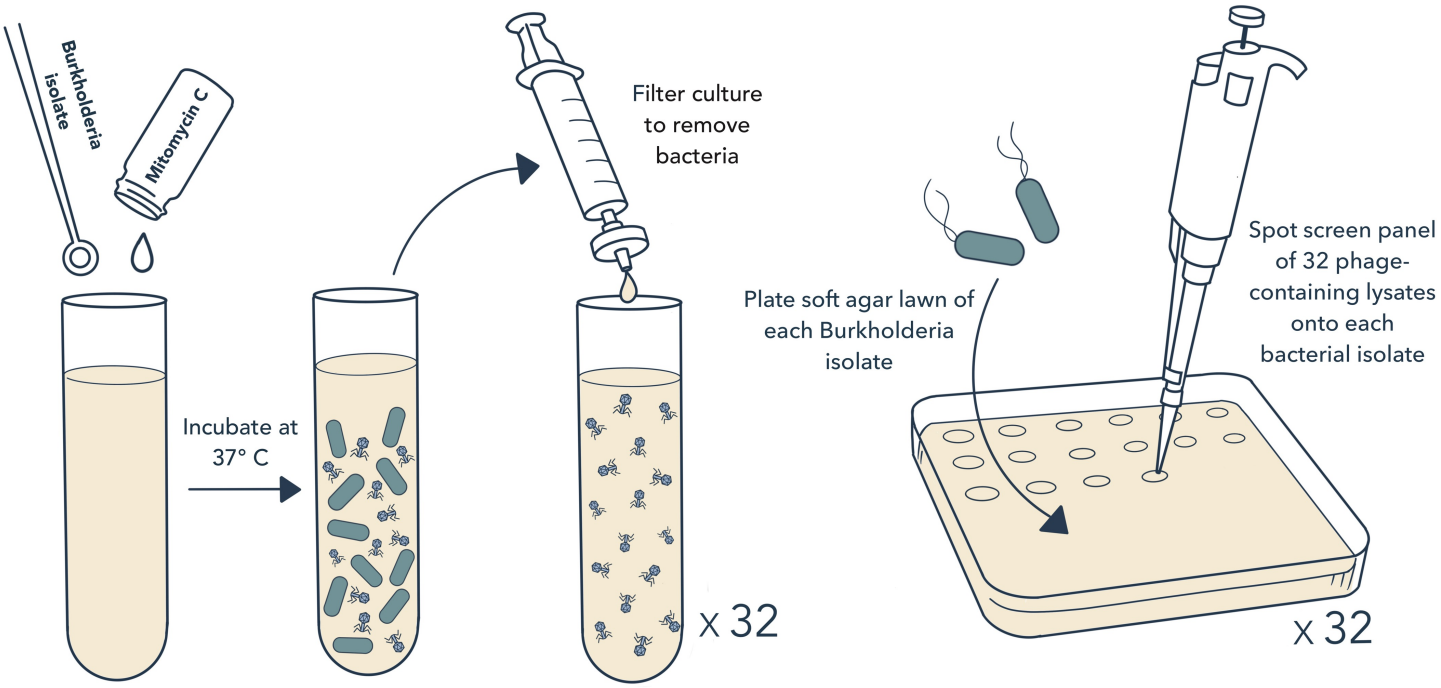
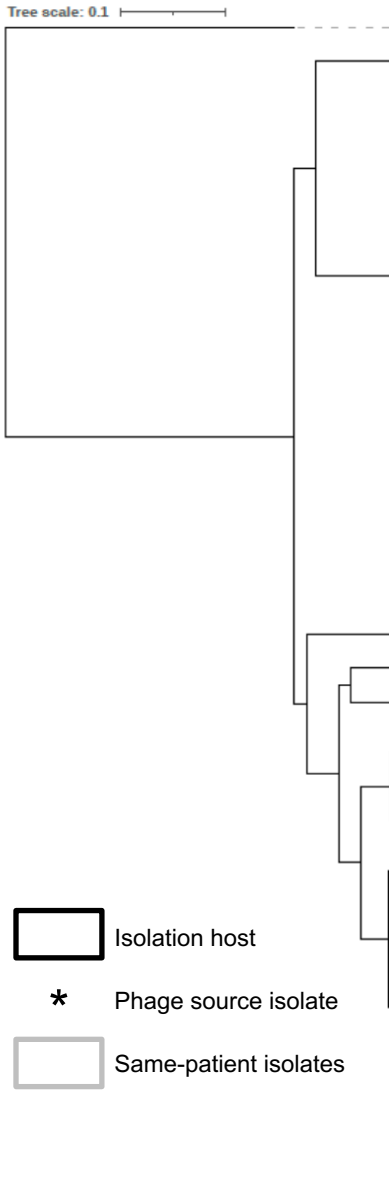


Figure 1. Approach for prophage induction and testing. To induce prophage release, stationary phase liquid culture of 32 *Burkholderia* spp. clinical isolates were individually inoculated into LB media containing mitomycin C. Cultures were grown overnight at 37ºC. The next day, bacterial cells were pelleted and liquid lysates were filtered through a 0.22µm filter. Filtered lysates were then spotted onto soft agar lawns containing each of the same 32 *Burkholderia* spp. clinical isolates. Plates were examined for growth inhibition, then inhibitory lysates were retested to confirm phage activity.



Species		BCC02	BCC03	BCC04	BCC05	BCC06	BCC07	BCC08	DSMZ 107315	Bch7
DVT1600	*B. gladioli*						3.6		6.1	7.1
DVT613	*B. pseudomultivorans*	2.8								6.3
DVT1176	*B. multivorans*									
DVT1180		*	*	*						
DVT1161								3.9		
DVT1181										6.5
DVT1140										6.8
DVT1171										5.8
DVT1172										6.6
DVT1166					*	*				
DVT1177										
DVT1153		4.3	4.3	4.6						
DVT1159										
DVT1608										
DVT1167		2.3		2.2						
DVT1173										
DVT1178										
DVT1170		3.6	3.8	4.3						
DVT1157	*B. vietnamiensis*							3.3		
DVT1155	*B. seminalis*						*	*		4.3
DVT1152	*B. cepacia*							6.6		
DVT1154	*B. cenocepacia*				4.3	**4.3**				
DVT599					**4.0**	4.6				
DVT1139		**5.3**	4.3	4.3						
DVT1165		5.3	5.3	**6.6**						
DVT1627										6.6
DVT1174							2.8			4.9
DVT790		3.4	2.6	3.3			3.8	**6.0**	**6.0**	5.3
DVT614		4.6	**4.9**	4.9			**5.4**	6.3		5.9
DVT1175		4.9	4.3	4.3			5.8	8.3		6.2
DVT1156									4.3	
DVT1160		3.4		3.2	3.1				6.8	
DVT753		3.6		3.1					6.3	
DVT1163									2.1	
DVT1179									2.6	

Figure 2. Phage activity against genetically diverse *Burkholderia* spp. clinical isolates. Bacterial isolates are ordered according to their core genome phylogeny and are grouped by species. Grey boxes indicate isolates collected from the same patient. Infectivity is shown as the $\log_{10}$ titer (PFU/mL) of each phage against each isolate. Bolded values indicate the *Burkholderia* spp. target isolate that each phage was isolated and propagated on. Asterisks mark the source *Burkholderia* spp. isolate for each phage. Green shading corresponds to phage activity titer, with darker shading indicating a higher titer. Empty cells indicate no phage activity.

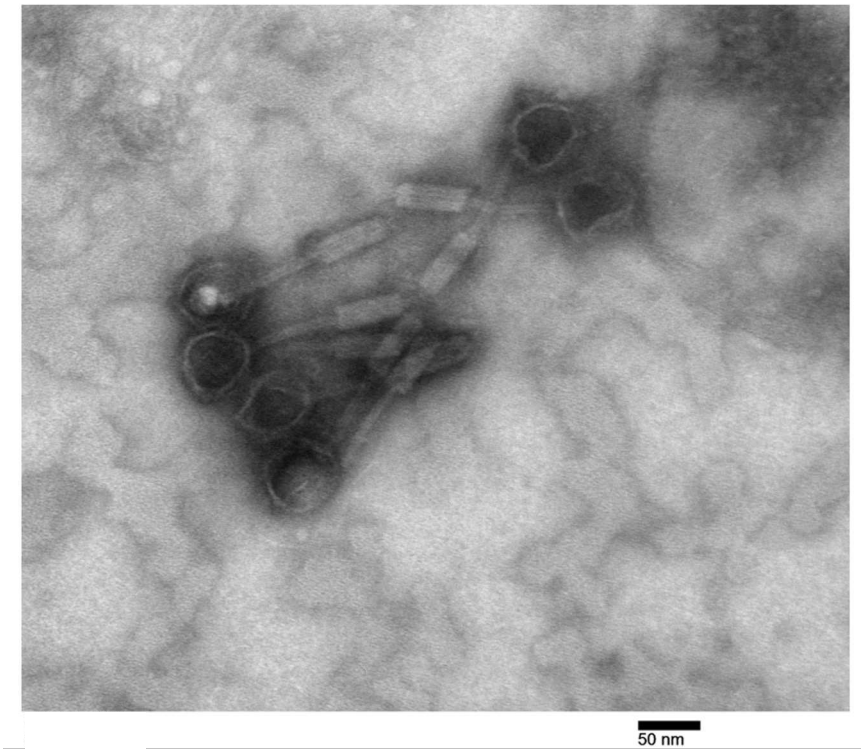


Figure 3. Electron micrograph of prophage-derived phage BCC02. Transmission electron micrograph showing *Burkholderia* phage BCC02. Image was taken at 200,000X magnification. Icosahedral head, tail, and contractile tail sheath are visible for 6 virions.

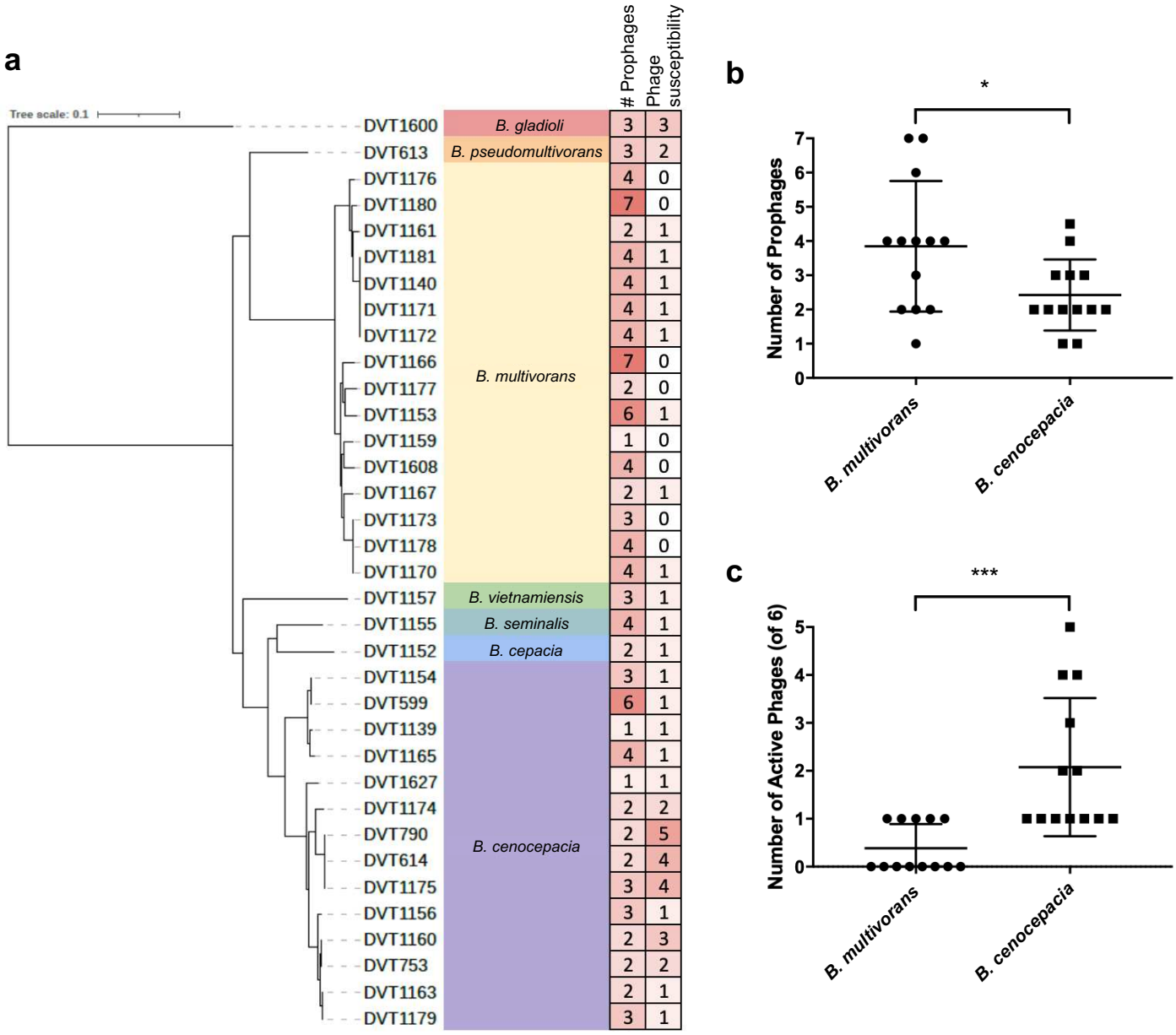


Figure 4. Prophage abundance and phage susceptibility of diverse *Burkholderia* spp. clinical isolates. (a) Core genome phylogeny of 35 *Burkholderia* spp. clinical isolates showing the number of prophages and phage susceptibility of each isolate. For phage susceptibility, BCC02/BCC03/BCC04 and BCC05/BCC06 were each combined into a single count. Darker red shading corresponds with higher values. (b) Prophage abundance among *B. multivorans* and *B. cenocepacia* genomes. (c) Phage susceptibility among *B. multivorans* and *B. cenocepacia* isolates. *P*-values are from unpaired two-tailed t-tests. * $P < 0.05$; *** $P < 0.001$.

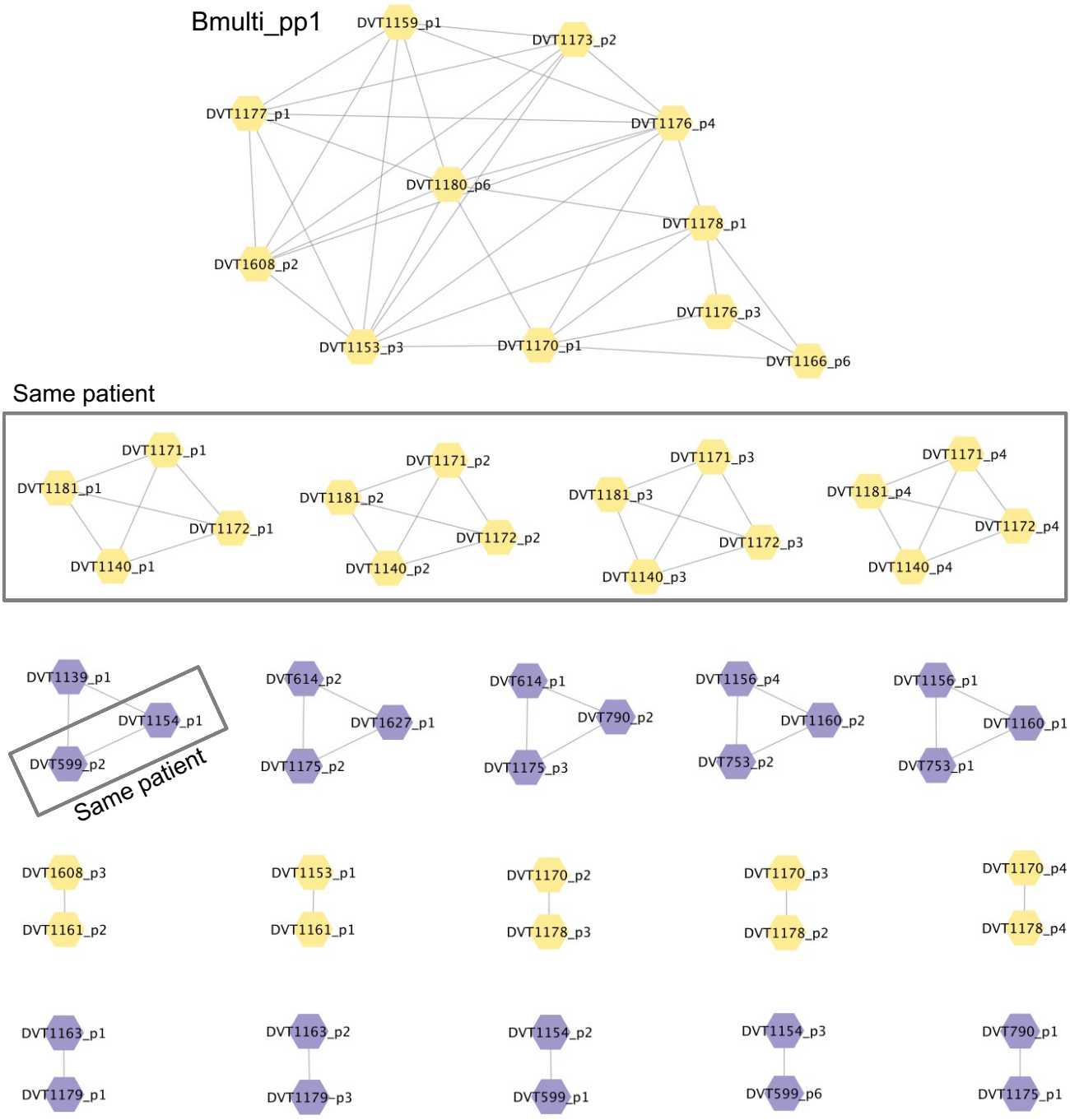


Figure 5. Clusters of prophages encoded by *Burkholderia* spp. clinical isolate genomes. Bacterial isolate names and prophage number are listed inside the nodes of each cluster, and lines connect prophages that share >90% sequence coverage and >90% sequence identity. Yellow nodes indicate prophages in *B. multivorans* isolate genomes and purple nodes indicate prophages in *B. cenocepacia* isolate genomes. Isolates from the same patient (two separate patients) are boxed.

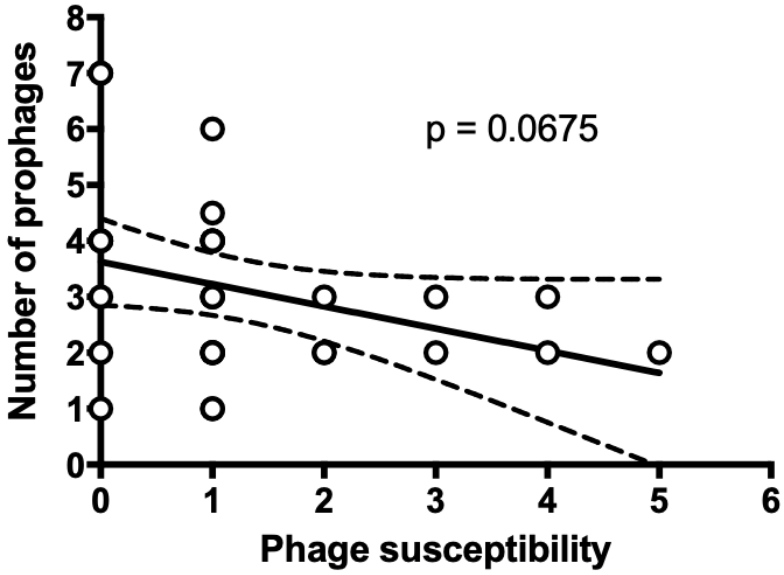


Figure S1: Prophage abundance versus phage susceptibility among 35 *Burkholderia* spp. isolates. Isolates are plotted according to the number of phages they are susceptible to (x-axis) and the number of prophages encoded in the genome (y-axis). Multiple isolates with the same coordinates are plotted on top of one another. Linear regression and 95% confidence interval are shown.

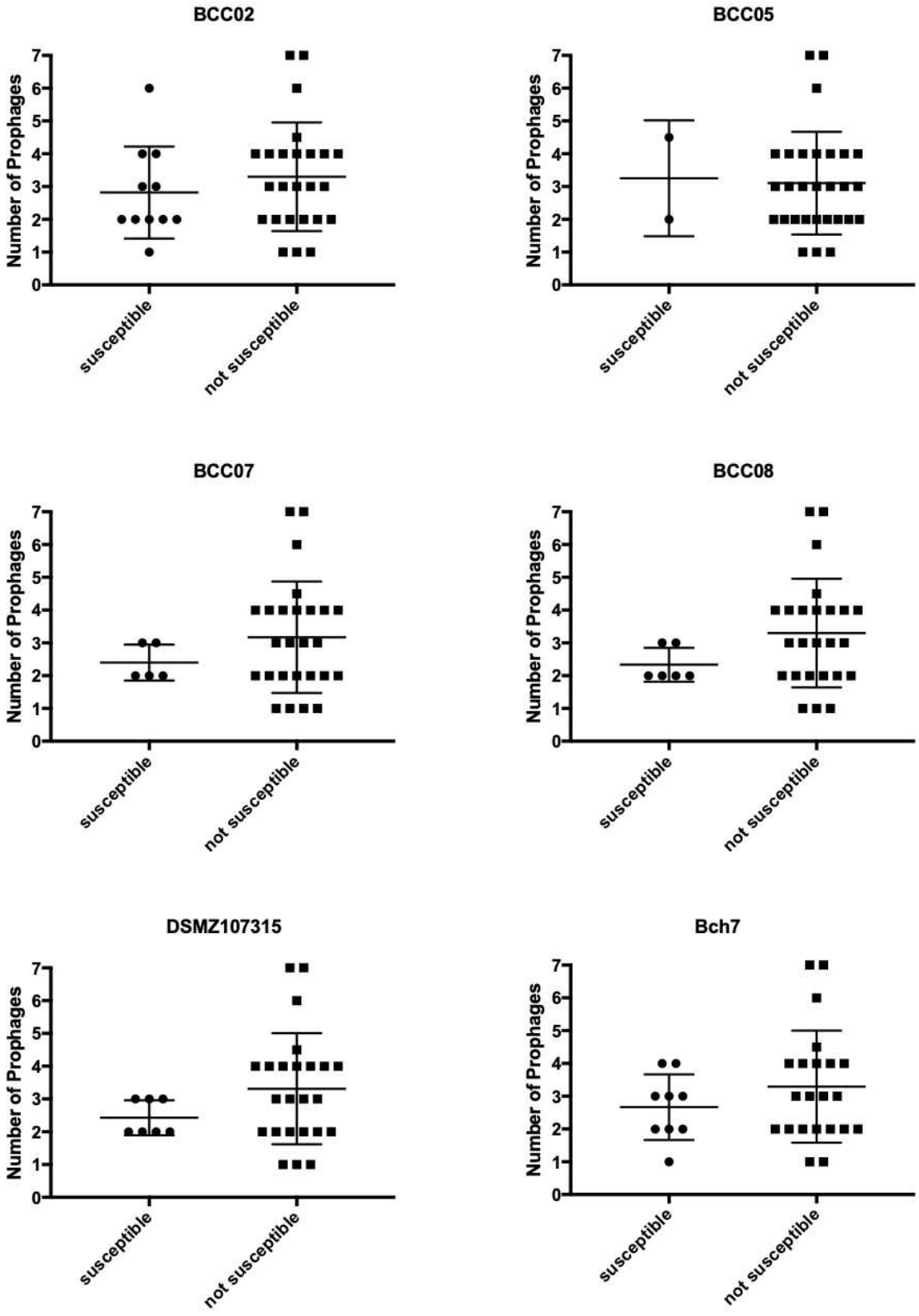


Figure S2: Prophage abundance among isolates that are or are not susceptible to each phage used in the study. Prophage abundance in isolates that were and were not susceptible to each phage. *P*-values from unpaired two-tailed t-tests were not significant in all cases.

a

	species	BCC02	BCC03	BCC04	BCC05	BCC06	BCC07	BCC08	DSMZ 107315	Bch7	Bmulti_pp1
DVT1176	B. multivorans										X
DVT1180		*	*	*							X
DVT1161								3.9			
DVT1181										6.5	
DVT1140										6.8	
DVT1171										5.8	
DVT1172										6.6	
DVT1166					*	*					X
DVT1177											X
DVT1153		4.3	4.3	4.6							X
DVT1159											X
DVT1608											X
DVT1167		2.3		2.2							
DVT1173											X
DVT1178											X
DVT1170		3.6	3.8	4.3							X

b

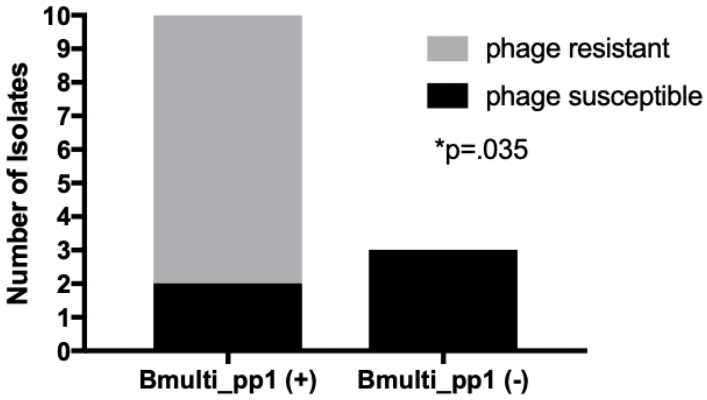


Figure S3: Prophage Bmulti_pp1 is associated with phage resistance in *B. multivorans*. (a) Phage susceptibility of 16 *B. multivorans* isolates, with isolates encoding prophage Bmulti_pp1 indicated with an X to the right of the figure. (b) Number of isolates that were resistant (grey) or susceptible (black) to any phage among isolates with (+) or without (-) prophage Bmulti_pp1. Four isolates from the same patient (grey box in panel (a)) were only counted once in this comparison. *P*-value is from Fisher's Exact Test comparing Bmulti_pp1 (+) and (-) groups.

Table S1. *Burkholderia* spp. prophage-derived phages

Phage ID	Source isolate	Source species	Target isolate	Target species
BCC02	DVT1180	*B. multivorans*	DVT1139	*B. cenocepacia*
BCC03	DVT1180	*B. multivorans*	DVT614	*B. cenocepacia*
BCC04	DVT1180	*B. multivorans*	DVT1165	*B. cenocepacia*
BCC05	DVT1166	*B. multivorans*	DVT599	*B. cenocepacia*
BCC06	DVT1166	*B. multivorans*	DVT1154	*B. cenocepacia*
BCC07	DVT1155	*B. seminalis*	DVT614	*B. cenocepacia*
BCC08	DVT1155	*B. seminalis*	DVT790	*B. cenocepacia*

Table S2. Prophages identified in *Burkholderia* spp. bacterial isolate genomes

Prophage ID	Host Isolate	Host Species	Length (bp)	% GC	Cluster	Isolated phage	Most Common Phage (# genes that match)	status
DVT1139_prophage1_contig44	DVT1139	*B. cenocepacia*	22693	69.1	Bceno_pp6	-	PHAGE_Burkho_KL3_NC_015266(24)	intact
DVT1140_prophage1_contig6	DVT1140	*B. multivorans*	39836	63.8	Bmulti_pp2 (same patient)	-	PHAGE_Burkho_KS10_NC_011216(43)	intact
DVT1140_prophage2_contig10	DVT1140	*B. multivorans*	32331	62.2	Bmulti_pp3 (same patient)	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(8)	intact
DVT1140_prophage3_contig29	DVT1140	*B. multivorans*	32989	65.2	Bmulti_pp4 (same patient)	-	PHAGE_Burkho_KS5_NC_015265(37)	intact
DVT1140_prophage4_contig3	DVT1140	*B. multivorans*	10969	61.9	Bmulti_pp5 (same patient)	-	PHAGE_Ralsto_RS_PII_1_NC_047804(3)	incomplete
DVT1152_prophage1_contig10	DVT1152	*B. cepacia*	36096	63.3	-	-	PHAGE_Mannhe_vB_MhM_3927AP2_NC_028766(14)	intact
DVT1152_prophage2_contig5	DVT1152	*B. cepacia*	28889	63.2	-	-	PHAGE_Escher_vB_EcoM_ECO1230_10_NC_027995(8)	incomplete
DVT1153_prophage1_contig21	DVT1153	*B. multivorans*	47703	62.2	Bmulti_pp12	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(8)	intact
DVT1153_prophage2_contig39	DVT1153	*B. multivorans*	34871	62.5	-	-	PHAGE_Salmon_SEN34_NC_028699(21)	questionable
DVT1153_prophage3_contig15	DVT1153	*B. multivorans*	14848	65.2	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(20)	incomplete
DVT1153_prophage4_contig19	DVT1153	*B. multivorans*	4846	59.3	-	-	PHAGE_Burkho_phi1026b_NC_005284(2)	incomplete
DVT1153_prophage5_contig36	DVT1153	*B. multivorans*	21955	64.7	-	-	PHAGE_Burkho_KS5_NC_015265(18)	incomplete
DVT1153_prophage6_contig40	DVT1153	*B. multivorans*	49434	65	-	-	PHAGE_Stx2_c_1717_NC_011357(3)	incomplete
DVT1154_prophage1_contig22	DVT1154	*B. cenocepacia*	29199	68.1	Bceno_pp6 (same patient)	-	PHAGE_Burkho_phiE202_NC_009234(24)	intact
DVT1154_prophage2_contig28	DVT1154	*B. cenocepacia*	49434	65	Bceno_pp18	-	PHAGE_Salmon_SEN34_NC_028699(20)	intact
DVT1154_prophage3_contig6	DVT1154	*B. cenocepacia*	19213	65	Bceno_pp19	-	PHAGE_Burkho_BcepC6B_NC_005887(5)	incomplete
DVT1155_prophage1_contig4	DVT1155	*B. seminalis*	44646	62.9	-	-	PHAGE_Rhodof_P26218_NC_029061(7)	intact
DVT1155_prophage2_contig10	DVT1155	*B. seminalis*	38090	64.9	-	BCC07	PHAGE_Burkho_AP3_NC_047752(38)	intact
DVT1155_prophage3_contig20	DVT1155	*B. seminalis*	37802	63.6	-	BCC08	PHAGE_Burkho_KL3_NC_015266(39)	intact
DVT1155_prophage4_contig21	DVT1155	*B. seminalis*	37166	61.8	-	-	PHAGE_Burkho_ST79_NC_021343(41)	intact
DVT1156_prophage1_contig3	DVT1156	*B. cenocepacia*	7515	68	Bceno_pp10	-	PHAGE_Escher_phAPEC8_NC_020079(4)	incomplete
DVT1156_prophage2_contig35	DVT1156	*B. cenocepacia*	10085	64.2	-	-	PHAGE_Burkho_phi6442_NC_009235(3)	incomplete
DVT1156_prophage3_contig70	DVT1156	*B. cenocepacia*	11199	59.3	-	-	PHAGE_Rhizob_RR1_A_NC_021560(1)	incomplete
DVT1156_prophage4_contig71	DVT1156	*B. cenocepacia*	8814	66.7	Bceno_pp9	-	PHAGE_Cellul_phi38:1_NC_021796(1)	incomplete
DVT1157_prophage1_contig11	DVT1157	*B. vietnamensis*	47046	65.5	-	-	PHAGE_Burkho_BcepC6B_NC_005887(37)	intact
DVT1157_prophage2_contig18	DVT1157	*B. vietnamensis*	41263	61.5	-	-	PHAGE_Burkho_Bcep176_NC_007497(24)	intact
DVT1157_prophage3_contig21	DVT1157	*B. vietnamensis*	25312	63.2	-	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(9)	incomplete
DVT1159_prophage1_contig37	DVT1159	*B. multivorans*	37188	63.4	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(35)	intact
DVT1160_prophage1_contig26	DVT1160	*B. cenocepacia*	7515	68	Bceno_pp10	-	PHAGE_Escher_ESCO5_NC_047776(4)	incomplete
DVT1160_prophage2_contig71	DVT1160	*B. cenocepacia*	8810	66.7	Bceno_pp9	-	PHAGE_Klebsi_ST437_OXA245phi4.1_NC_049448(1)	incomplete
DVT1161_prophage1_contig22	DVT1161	*B. multivorans*	38405	62.3	Bmulti_pp12	-	PHAGE_Escher_vB_EcoM_ECO1230_10_NC_027995(8)	questionable
DVT1161_prophage2_contig8	DVT1161	*B. multivorans*	17709	64.3	Bmulti_pp11	-	PHAGE_Pectob_CBB_NC_041878(2)	incomplete
DVT1163_prophage1_contig12	DVT1163	*B. cenocepacia*	44021	65	Bceno_pp16	-	PHAGE_Salmon_SEN34_NC_028699(14)	intact
DVT1163_prophage2_contig25	DVT1163	*B. cenocepacia*	12189	61.2	Bceno_pp17	-	PHAGE_Salmon_SEN34_NC_028699(3)	incomplete
DVT1165_prophage1_contig8	DVT1165	*B. cenocepacia*	34706	63.3	-	-	PHAGE_Burkho_BcepMu_NC_005882(46)	intact
DVT1165_prophage2_contig39	DVT1165	*B. cenocepacia*	30623	65.5	-	-	PHAGE_Myxoco_Mx8_NC_003085(7)	intact
DVT1165_prophage3_contig41	DVT1165	*B. cenocepacia*	40032	63.5	-	-	PHAGE_Burkho_KS10_NC_011216(43)	intact
DVT1165_prophage4_contig19	DVT1165	*B. cenocepacia*	33431	62.5	-	-	PHAGE_Pseudo_PAJU2_NC_011373(4)	incomplete
DVT1166_prophage1_contig10	DVT1166	*B. multivorans*	29994	63	-	BCC05/BCC06	PHAGE_Burkho_phiE12_2_NC_009236(25)	intact
DVT1166_prophage2_contig19	DVT1166	*B. multivorans*	23631	65.3	-	-	PHAGE_Salmon_SEN34_NC_028699(23)	intact
DVT1166_prophage3_contig12	DVT1166	*B. multivorans*	8333	64.8	-	-	PHAGE_Bacill_vB_BtS_BMBtp14_NC_048640(2)	incomplete
DVT1166_prophage4_contig19	DVT1166	*B. multivorans*	19712	61.2	-	-	PHAGE_Burkho_phi1026b_NC_005284(3)	incomplete
DVT1166_prophage5_contig21	DVT1166	*B. multivorans*	18687	64.8	-	-	PHAGE_Burkho_KS5_NC_015265(9)	incomplete
DVT1166_prophage6_contig38	DVT1166	*B. multivorans*	22687	64.3	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(11)	incomplete
DVT1166_prophage7_contig45	DVT1166	*B. multivorans*	11827	58.2	-	-	PHAGE_Burkho_phiE12_2_NC_009236(3)	incomplete
DVT1167_prophage1_contig8	DVT1167	*B. multivorans*	62320	62.7	-	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(9)	questionable
DVT1167_prophage2_contig16	DVT1167	*B. multivorans*	12701	62	-	-	PHAGE_Salmon_SEN34_NC_028699(5)	incomplete
DVT1170_prophage1_contig24	DVT1170	*B. multivorans*	41030	64.4	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(35)	intact
DVT1170_prophage2_contig27	DVT1170	*B. multivorans*	13392	64.4	Bmulti_pp13	-	PHAGE_Erwini_PEp14_NC_016767(3)	incomplete
DVT1170_prophage3_contig32	DVT1170	*B. multivorans*	20473	63.7	Bmulti_pp14	-	PHAGE_Burkho_phiE125_NC_003309(3)	incomplete
DVT1170_prophage4_contig53	DVT1170	*B. multivorans*	31843	63.8	Bmulti_pp15	-	PHAGE_Burkho_vB_BmuP_KL4_NC_047958(11)	incomplete
DVT1171_prophage1_contig9	DVT1171	*B. multivorans*	39836	63.8	Bmulti_pp2 (same patient)	-	PHAGE_Burkho_KS10_NC_011216(43)	intact
DVT1171_prophage2_contig20	DVT1171	*B. multivorans*	32331	62.2	Bmulti_pp3 (same patient)	-	PHAGE_Escher_vB_EcoM_ECO1230_10_NC_027995(7)	intact
DVT1171_prophage3_contig43	DVT1171	*B. multivorans*	33430	65.2	Bmulti_pp4 (same patient)	-	PHAGE_Burkho_KS5_NC_015265(37)	intact
DVT1171_prophage4_contig6	DVT1171	*B. multivorans*	12231	60.8	Bmulti_pp5 (same patient)	-	PHAGE_Ralsto_RS_PII_1_NC_047804(3)	incomplete
DVT1172_prophage1_contig10	DVT1172	*B. multivorans*	39836	63.8	Bmulti_pp2 (same patient)	-	PHAGE_Burkho_KS10_NC_011216(43)	intact
DVT1172_prophage2_contig11	DVT1172	*B. multivorans*	32331	62.2	Bmulti_pp3 (same patient)	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(7)	questionable
DVT1172_prophage3_contig41	DVT1172	*B. multivorans*	32970	65.2	Bmulti_pp4 (same patient)	-	PHAGE_Burkho_KS5_NC_015265(38)	intact
DVT1172_prophage4_contig15	DVT1172	*B. multivorans*	10969	61.9	Bmulti_pp5 (same patient)	-	PHAGE_Ralsto_RS_PII_1_NC_047804(3)	incomplete
DVT1173_prophage1_contig22	DVT1173	*B. multivorans*	17089	62.4	-	-	PHAGE_Entero_SfV_NC_003444(4)	intact
DVT1173_prophage2_contig23	DVT1173	*B. multivorans*	37187	63.3	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(35)	intact
DVT1173_prophage3_contig22	DVT1173	*B. multivorans*	16888	62.3	-	-	PHAGE_Burkho_phi1026b_NC_005284(2)	incomplete
DVT1174_prophage1_contig4	DVT1174	*B. cenocepacia*	40429	62.4	-	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(9)	questionable
DVT1174_prophage2_contig43	DVT1174	*B. cenocepacia*	16336	62.1	-	-	PHAGE_Entero_fiAA91_ss_NC_022750(2)	intact
DVT1175_prophage1_contig50	DVT1175	*B. cenocepacia*	8307	63.1	Bceno_pp20	-	PHAGE_Stx2_c_1717_NC_011357(3)	questionable
DVT1175_prophage2_contig72	DVT1175	*B. cenocepacia*	16371	62.3	Bceno_pp7	-	PHAGE_Burkho_KS9_NC_013055(21)	intact
DVT1175_prophage3_contig78	DVT1175	*B. cenocepacia*	18522	62.4	Bceno_pp8	-	PHAGE_Burkho_KS9_NC_013055(14)	questionable
DVT1176_prophage1_contig17	DVT1176	*B. multivorans*	44249	62.3	-	-	PHAGE_Burkho_Bcep176_NC_007497(35)	incomplete
DVT1176_prophage2_contig30	DVT1176	*B. multivorans*	22884	62.5	-	-	PHAGE_Salmon_SEN34_NC_028699(3)	incomplete
DVT1176_prophage3_contig35	DVT1176	*B. multivorans*	26076	64.5	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(16)	incomplete
DVT1176_prophage4_contig60	DVT1176	*B. multivorans*	14004	65.5	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(17)	incomplete
DVT1177_prophage1_contig16	DVT1177	*B. multivorans*	37188	63.4	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(35)	intact
DVT1177_prophage2_contig30	DVT1177	*B. multivorans*	9446	61.6	-	-	PHAGE_Escher_SH2026Stx1_NC_049919(3)	incomplete
DVT1178_prophage1_contig21	DVT1178	*B. multivorans*	39737	64	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(35)	intact
DVT1178_prophage2_contig27	DVT1178	*B. multivorans*	20473	63.7	Bmulti_pp14	-	PHAGE_Burkho_phiE125_NC_003309(3)	incomplete
DVT1178_prophage3_contig29	DVT1178	*B. multivorans*	13392	64.4	Bmulti_pp13	-	PHAGE_Serrat_Parlo_NC_048758(3)	incomplete
DVT1178_prophage4_contig96	DVT1178	*B. multivorans*	20455	61.2	Bmulti_pp15	-	PHAGE_Burkho_vB_BmuP_KL4_NC_047958(12)	incomplete
DVT1179_prophage1_contig22	DVT1179	*B. cenocepacia*	44021	65	Bceno_pp16	-	PHAGE_Salmon_SEN34_NC_028699(14)	intact
DVT1179_prophage2_contig129	DVT1179	*B. cenocepacia*	7338	59.8	-	-	PHAGE_Stx2_c_1717_NC_011357(3)	questionable
DVT1179_prophage3_contig91	DVT1179	*B. cenocepacia*	12189	61.2	Bceno_pp17	-	PHAGE_Salmon_SEN34_NC_028699(3)	incomplete
DVT1180_prophage1_contig3	DVT1180	*B. multivorans*	34935	64.2	-	-	PHAGE_Pseudo_NP1_NC_031058(5)	intact
DVT1180_prophage2_contig53	DVT1180	*B. multivorans*	17357	60.8	-	-	PHAGE_Salmon_118970_sal3_NC_031940(7)	intact
DVT1180_prophage3_contig50	DVT1180	*B. multivorans*	12911	66.6	-	BCC02/BCC03/BCC04	PHAGE_Burkho_KS5_NC_015265(19)	incomplete
DVT1180_prophage4_contig53	DVT1180	*B. multivorans*	18839	60.6	-	-	PHAGE_Burkho_Bcep176_NC_007497(9)	incomplete
DVT1180_prophage5_contig85	DVT1180	*B. multivorans*	19107	65.1	-	BCC02/BCC03/BCC04	PHAGE_Burkho_KS5_NC_015265(15)	incomplete
DVT1180_prophage6_contig113	DVT1180	*B. multivorans*	13341	65.3	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(19)	incomplete
DVT1180_prophage7_contig120	DVT1180	*B. multivorans*	13133	63.8	-	-	PHAGE_Burkho_KS5_NC_015265(12)	incomplete
DVT1181_prophage1_contig6	DVT1181	*B. multivorans*	39836	63.8	Bmulti_pp2 (same patient)	-	PHAGE_Burkho_KS10_NC_011216(43)	intact
DVT1181_prophage2_contig16	DVT1181	*B. multivorans*	32331	62.2	Bmulti_pp3 (same patient)	-	PHAGE_Escher_vB_EcoM_ECOO78_NC_041926(7)	intact
DVT1181_prophage3_contig35	DVT1181	*B. multivorans*	32989	65.2	Bmulti_pp4 (same patient)	-	PHAGE_Burkho_KS5_NC_015265(37)	intact
DVT1181_prophage4_contig8	DVT1181	*B. multivorans*	12231	60.8	Bmulti_pp5 (same patient)	-	PHAGE_Ralsto_RS_PI_1_NC_047816(3)	incomplete
DVT1600_prophage1_contig13	DVT1600	*B. gladioliii*	38172	61.2	-	-	PHAGE_Burkho_KS9_NC_013055(22)	intact
DVT1600_prophage2_contig18	DVT1600	*B. gladioliii*	34696	61.3	-	-	PHAGE_Sphing_Lacusarx_NC_041927(4)	intact
DVT1600_prophage3_contig22	DVT1600	*B. gladioliii*	39615	64.3	-	-	PHAGE_Burkho_AP3_NC_047752(35)	intact
DVT1608_prophage1_contig6	DVT1608	*B. multivorans*	50362	62.9	-	-	PHAGE_Aeromo_vB_AsaM_56_NC_019527(15)	intact
DVT1608_prophage2_contig19	DVT1608	*B. multivorans*	37188	63.4	Bmulti_pp1	-	PHAGE_Burkho_KS5_NC_015265(35)	intact
DVT1608_prophage3_contig1	DVT1608	*B. multivorans*	18352	64.6	Bmulti_pp11	-	PHAGE_Pectob_CBB_NC_041878(2)	incomplete
DVT1608_prophage4_contig31	DVT1608	*B. multivorans*	23671	60.5	-	-	PHAGE_Burkho_vB_BmuP_KL4_NC_047958(9)	incomplete
DVT1627_prophage1_contig27	DVT1627	*B. cenocepacia*	34069	62.4	Bceno_pp7	-	PHAGE_Burkho_KS9_NC_013055(32)	intact
DVT599_prophage1_contig3	DVT599	*B. cenocepacia*	26389	65.4	Bceno_pp18	-	PHAGE_Salmon_SEN34_NC_028699(19)	questionable
DVT599_prophage2_contig15	DVT599	*B. cenocepacia*	29200	68.2	Bceno_pp6 (same patient)	-	PHAGE_Burkho_KL3_NC_015266(24)	intact
DVT599_prophage3_contig3	DVT599	*B. cenocepacia*	23915	66.1	-	-	PHAGE_Salmon_118970_sal3_NC_031940(2)	incomplete
DVT599_prophage4_contig4	DVT599	*B. cenocepacia*	13641	61.6	-	-	PHAGE_Ralsto_RsoM1USA_NC_049432(2)	incomplete
DVT599_prophage5_contig25	DVT599	*B. cenocepacia*	10967	60.4	-	-	PHAGE_Burkho_vB_BmuP_KL4_NC_047958(4)	incomplete
DVT599_prophage6_contig25	DVT599	*B. cenocepacia*	22203	65.2	Bceno_pp19	-	PHAGE_Burkho_BcepC6B_NC_005887(5)	incomplete
DVT613_prophage1_contig9	DVT613	*B. pseudomultivorans*	8119	71.2	-	-	PHAGE_Bacill_G_NC_023719(2)	incomplete
DVT613_prophage2_contig45	DVT613	*B. pseudomultivorans*	7720	66.4	-	-	PHAGE_Plankt_PaV_LD_NC_016564(1)	incomplete
DVT613_prophage3_contig100	DVT613	*B. pseudomultivorans*	9479	59.1	-	-	PHAGE_Plankt_PaV_LD_NC_016564(1)	incomplete
DVT614_prophage1_contig13	DVT614	*B. cenocepacia*	18756	62.7	Bceno_pp8	-	PHAGE_Burkho_KS9_NC_013055(14)	questionable
DVT614_prophage2_contig84	DVT614	*B. cenocepacia*	14871	61.7	Bceno_pp7	-	PHAGE_Burkho_KS9_NC_013055(20)	questionable
DVT753_prophage1_contig2	DVT753	*B. cenocepacia*	7515	68.1	Bceno_pp10	-	PHAGE_Escher_phAPEC8_NC_020079(4)	incomplete
DVT753_prophage2_contig40	DVT753	*B. cenocepacia*	11919	67.5	Bceno_pp9	-	PHAGE_Bacill_SP_15_NC_031245(1)	incomplete
DVT790_prophage1_contig50	DVT790	*B. cenocepacia*	8265	63.1	Bceno_pp20	-	PHAGE_Stx2_c_Stx2a_F451_NC_049924(3)	questionable
DVT790_prophage2_contig94	DVT790	*B. cenocepacia*	18495	62.4	Bceno_pp8	-	PHAGE_Burkho_KS9_NC_013055(14)	questionable