**φX216, a P2-like bacteriophage with broad *Burkholderia pseudomallei* and *B. mallei* strain infectivity**

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**Abstract**

**Background:** *Burkholderia pseudomallei* and *B. mallei* are closely related Category B Select Agents of bioterrorism and the causative agents of the diseases melioidosis and glanders, respectively. Rapid phage-based diagnostic tools would greatly benefit early recognition and treatment of these diseases. There is extensive strain-to-strain variation in *B. pseudomallei* genome content due in part to the presence or absence of integrated prophages. Several phages have previously been isolated from *B. pseudomallei* lysogens, for example ϕK96243, ϕ1026b and ϕ52237.

**Results:** We have isolated a P2-like bacteriophage, ϕX216, which infects 78% of all *B. pseudomallei* strains tested. ϕX216 also infects *B. mallei*, but not other *Burkholderia* species, including the closely related *B. thailandensis* and *B. oklahomensis*. The nature of the ϕX216 host receptor remains unclear but evidence indicates that in *B. mallei* ϕX216 uses lipopolysaccharide O-antigen but a different receptor in *B. pseudomallei*. The 37,637 bp genome of ϕX216 encodes 47 predicted open reading frames and shares 99.8% pairwise identity and an identical strain host range with bacteriophage ϕ52237. Closely related P2-like prophages appear to be widely distributed among *B. pseudomallei* strains but both ϕX216 and ϕ52237 readily infect prophage carrying strains.

**Conclusions:** The broad strain infectivity and high specificity for *B. pseudomallei* and *B. mallei* indicate that ϕX216 will provide a good platform for the development of phage-based diagnostics for these bacteria.

**Keywords:** Bacteriophage, *Burkholderia pseudomallei*, *B. mallei*, P2, Prophage distribution, Phage-based diagnostics

**Introduction**

*Burkholderia pseudomallei* and *B. mallei* are facultative intracellular Gram-negative human and animal pathogens and the causative agents of the endemic diseases melioidosis and glanders, respectively [1-4]. Because of their intrinsic antibiotic resistance and high mortality caused by the respective diseases despite aggressive treatment, *B. pseudomallei* and *B. mallei* are classed as Category B Select Agents of bioterrorism. *B. pseudomallei* is a ubiquitous Gram-negative soil bacterium endemic to southeast Asia and northern Australia and possesses a genome showing extensive strain-to-strain variation. A significant portion of this genome variation is due to the presence or absence of integrated prophages [5-7]. *B. pseudomallei* strains commonly carry at least one integrated prophage and multiple phages have been isolated from lysogenic *B. pseudomallei* strains [8-10]. *B. mallei*, on the other hand, exists in a zoonotic reservoir and appears to have evolved from *B. pseudomallei* by genome reduction [11]. Previously sequenced *B. mallei* strains do not carry intact prophages but can be infected by many phages isolated from *B. pseudomallei* [8-10,12].

In this study we isolated ϕX216 from spontaneous plaques formed by the Thai *B. pseudomallei* environmental isolate E0237 and determined its DNA sequence. ϕX216 is a member of the widely distributed *Burkholderia* P2-like phage family [8]. It has broad *B. pseudomallei* strain infectivity for members of the *B. pseudomallei* clade. Our data indicate that ϕX216 may serve as a good candidate...
for developing rapid phage-based diagnostic tools for _B. pseudomallei_ and _B. mallei._

**Results and discussion**

**ϕX216 isolation and host range**

_B. pseudomallei_ environmental isolate E0237 was observed to spontaneously form clear phage plaques after plating of overnight liquid cultures on agar plates. The spontaneously released phage, ϕX216 (named for the E0237 laboratory stock number), was plaque purified on _B. pseudomallei_ strain 2698a and used to create medium-titer [10^6 plaque forming units (pfu)/mL] plate lysates with a variety of _B. pseudomallei_ host strains and high-titer (10^8 pfu/mL) liquid lysates using _B. mallei_ ATCC23344. This strain was also chosen for production of larger volume liquid lysates to prevent contamination with other phages as it is not predicted to contain a prophage [8]. One-step growth curves demonstrated that ϕX216 has an approximate 60-minute latent phase, an 80-minute life cycle, and a burst size of 120 pfu per infected cell (Figure 1). ϕX216 formed plaques on 56 of a panel of 72 _B. pseudomallei_ strains composed of 30 environmental and 30 clinical isolates from Thailand, as well as 12 well-characterized strains from various sources, some of which are commonly used laboratory strains (see Additional file 1). At 77.8%, ϕX216 has one of the broadest strain infectivity ranges reported for a _B. pseudomallei_ phage, comparing favorably with the Thai soil phages ST2 (78%, 49/63) and ST96 (67%, 42/63) [13,14]. ϕX216 plaques were 1–2 mm in diameter and mostly-clear on the majority of _B. pseudomallei_ strains although there was some strain-dependent variation in plaque appearance with some forming pinpoint and/or turbid plaques. In addition, ϕX216 was also able to form plaques on all (9/9) _B. mallei_ strains tested. In contrast, ϕX216 did not form plaques on closely related (_B. thailandensis_ and _B. oklahomensis_) or other (_B. ubonensis_, _B. vietnamensis_ and _B. gladioli_ pathovar cocovenenans) _Burkholderia_ species (see Additional file 1). Although fewer isolates of these species were tested, ϕX216 does appear to have specificity for _B. pseudomallei_ and _B. mallei_ as compared with ST2 and ST96, which formed plaques on five of seven tested _B. thailandensis_ strains. Because of the close relatedness of _B. pseudomallei_ and _B. thailandensis_ it will be prudent to assess more _B. thailandensis_ strains as they become available to further support the claim of _B. pseudomallei_ specificity.

Of the 56 _B. pseudomallei_ strains that could be infected with ϕX216, 24 showed decreased relative plaquing efficiencies with the _B. mallei_ lysate. However, when ϕX216 lysates were propagated two to three times on these initially low plaquing efficiency strains, lysates were obtained that then plaqued with titers of 10^5 to 10^6 pfu/mL on those same strains. The reason(s) for low plaquing efficiencies of _B. mallei_ lysates on some _B. pseudomallei_ strains remain unclear but probably reflect some kind of host restrictive mechanism(s).

**ϕX216 host receptor**

Experiments with _B. mallei_ host strains indicated that _B. pseudomallei_ phages ϕ1026b, ϕK96243 and ϕE202 use the lipopolysaccharide (LPS) O-antigen as a host receptor [8-10]. _B. mallei_ O-antigen mutants cannot support infection by these phages and infection is restored if the O-antigen mutation is complemented. ϕX216 is also unable to infect _B. mallei_ O-antigen mutants but, surprisingly, infection is not restored by complementing the mutation (see Additional file 1). As opposed to _B. mallei_, _B. pseudomallei_ O-antigen mutants still support infection by ϕX216. Both an engineered deletion of the _wbiE_ gene in _B. pseudomallei_ Bp82 as well as 10 mapped transposon insertions in the _wbi_ genes of _B. pseudomallei_ 1026b formed ϕX216 plaques with an efficiency comparable to their respective parent strains. Therefore, ϕX216 may use the wild-type _B. mallei_ O-antigen as a host receptor but not in _B. pseudomallei_ where it uses a different receptor that is absent from _B. mallei_ [11].

**ϕX216 genome characterization and chromosomal attachment site**

To ascertain genomic features of ϕX216, we initially determined the entire ϕX216 genome sequence by low-coverage Sanger sequencing of plasmid clones generated by subcloning of ϕX216 DNA fragments and gap closing using sequence information obtained from PCR amplicons.
This was supported by deep sequencing using the Illumina platform. Differences between Sanger and Illumina sequence runs were resolved by Sanger sequencing of specific phage DNA fragments obtained by PCR amplification using purified phage DNA and chromosomal DNA from ϕX216 lysogens as templates. The ϕX216 genome is 37,637 bases in length with a G+C content of 64.8% (GenBank: JX681814). GeneMark software predicted 47 open reading frames (Figure 2). The genome can be subdivided into predicted regions associated with capsid structure and assembly, host lysis, tail structure and assembly, and DNA replication and lysogeny (Figure 2). To determine the chromosomal attachment site, the ϕX216 lysogen Bp523 was isolated. Sequencing of the ϕX216 genome contig. This contig has 100% pairwise identity with the highly active ϕ52237 isolated from B. pseudomallei Pasteur 52237 (GenBank: DQ087285.2) [8]. There are 55 differences observed between ϕX216 and ϕ52237, which were independently confirmed by both Illumina and Sanger sequencing. The majority of these differences, cluster within a six gene region predicted to be associated with tail structure and assembly although only 14 are missense mutations resulting in amino acid alterations. However, these mutations are of no biological consequence since ϕ52237 and ϕX216 were found to have identical host ranges (see Additional file 1).

Illumina sequencing also produced a second 1,141-bp contig independent of the ϕX216 genome contig. This contig has 100% pairwise identity with the highly active IS407a insertion element found in the B. mallei genome [11]. At present we do not know whether this contig is the result of IS407a insertion in a sub-population of ϕX216 virions during preparation of the B. mallei lysates used for Illumina sequencing or an integral part of ϕX216 DNA. However, since the IS407a insertion was absent from the genome sequence obtained by Sanger sequencing it is unlikely an indigenous part of the ϕX216 genome.

Burkholderia P2-like prophage distribution and correlation with ϕX216 host range
Although ϕX216 has a broad B. pseudomallei host range it fails to form plaques on approximately 22% of the strains tested in this study. We sought to determine if this was perhaps due to infection immunity conferred by the presence of related prophages.

To that end, we designed a series of multiplex and individual PCR probes based on six isolated or predicted Burkholderia P2-like phages from Ronning et al. [8]. These included three subgroup A (ϕE202, ϕK96243 and ϕ52237/ϕX216) and three subgroup B (ϕE12-2, GI15, PI-E264-2) P2-like phages (see Additional file 2) [8]. PCR probes were designed to identify candidate P2-like prophages with increasing levels of relatedness to ϕX216/ϕ52237. The P2-like 1 and P2-like 2 probes amplify regions in the capsid gene (gene #6; for gene numbers see GenBank: JX681814) and Fels-2 gene (gene #29) and are conserved in both P2-like A and B subgroups. The P2-like subgroup A-specific probe amplifies in the integrase gene (gene #45). The ϕX216 scrnA and scrnB probes are specific to ϕX216/ϕ52237 and amplify DNA fragments from ϕX216 gene #46 and from the intergenic region between ϕX216 genes #30 and #31, respectively. The GI2 (Genomic island 2) probe amplifies the junction between the bacterial and prophage genomes at rRNA-Phe, predicted to serve as the attB site for Burkholderia subgroup A phages [8,9]. We found that P2-like prophages are very common in B. pseudomallei strains (Table 1). Indeed, PCR analysis revealed that 30 out of 72 B. pseudomallei strains tested allowed amplification of DNA fragments indicative of the presence of a P2-like prophage (see Figure 3 for representative examples). Of those 30, 25 tested positive for subgroup A prophages. Six of those,
### Table 1. *B. pseudomallei* P2-like prophage distribution screen

| Strains with high ϕX216 plaquing efficiency | P2-like prophage PCR probe results | Multiplex |
|--------------------------------------------|-----------------------------------|-----------|
| Strains with high ϕX216 plaquing efficiency | P2-like 1 | P2-like 2 | P2-like group A | ϕX216 scrnA | ϕX216 scrnB | GI2 |
| 2668a | ϕ52237-like | + | + | + | + | + |
| E0237 | ϕ52237-like | + | + | + | + | + |
| E0394 | ϕ52237-like | + | + | + | + | + |
| 1026b | ϕ52237-like | + | + | + | + | + |
| 708a | ϕ52237-like | + | + | + | + | - | + |
| 2618a | P2L-A | + | + | + | - | - | + |
| 2661a | P2L-A | + | + | + | - | - | + |
| 2692a | P2L-A | + | + | + | - | - | + |
| 2717a | P2L-A | + | + | + | - | - | + |
| E0021 | P2L-A | + | + | + | - | - | + |
| E0235 | P2L-A | + | + | + | - | - | + |
| E0279 | P2L-A | + | + | + | - | - | + |
| E0345 | P2L-A | + | + | + | - | - | + |
| E0384 | P2L-A | + | + | + | - | - | + |
| E0386 | P2L-A | + | + | + | - | - | + |
| K96243 | P2L-A | + | + | + | - | - | + |
| S13 | P2L-A | + | + | + | - | - | + |
| 2698a | P2L | + | + | - | - | - | - |
| 2704a | P2L | + | + | - | - | - | - |
| E0342 | P2L | + | + | - | - | - | - |
| E0366 | P2L | + | + | - | - | - | - |
| E0377 | P2L | + | + | - | - | - | - |
| 2613a | - | - | - | - | - | ND |
| 2667a | - | - | - | - | - | ND |
| 2673a | - | - | - | - | - | ND |
| 2682a | - | - | - | - | - | ND |
| 2769a | - | - | - | - | - | ND |
| E0016 | - | - | - | - | - | ND |
| E0034 | - | - | - | - | - | ND |
| E0241 | - | - | - | - | - | ND |
| E0356 | - | - | - | - | - | ND |
| E0411 | - | - | - | - | - | ND |
| MSHR305 | - | - | - | - | - | ND |

### Strains with low ϕX216 plaquing efficiency

| Strains with low ϕX216 plaquing efficiency | P2-like prophage PCR probe results | Multiplex |
|--------------------------------------------|-----------------------------------|-----------|
| Strains with low ϕX216 plaquing efficiency | P2-like 1 | P2-like 2 | P2-like group A | ϕX216 scrnA | ϕX216 scrnB | GI2 |
| 2625a | ϕ52237-like | + | + | + | + | + |
| 2670a | P2L-A | + | + | + | - | - | + |
| E0037 | P2L-A | + | + | + | - | - | + |
| E0380 | P2L-A | + | + | + | - | - | + |
| 2637a | - | - | - | - | - | ND |
| 2650a | - | - | - | - | - | ND |
| 2660a | - | - | - | - | - | ND |
Table 1 *B. pseudomallei* P2-like prophage distribution screen (Continued)

| Strain   | P2L-A | P2L | ϕX216 | GI2 | ND  |
|----------|-------|-----|-------|-----|-----|
| 2685a    | -     | -   | -     | -   | ND  |
| 2708a    | -     | -   | -     | -   | ND  |
| 2719a    | -     | -   | -     | -   | ND  |
| 2764b    | -     | -   | -     | -   | ND  |
| E0024    | -     | -   | -     | -   | ND  |
| E0031    | -     | -   | -     | -   | ND  |
| E0181    | -     | -   | -     | -   | ND  |
| E0378    | -     | -   | -     | -   | ND  |
| E0383    | -     | -   | -     | -   | ND  |
| E0393    | -     | -   | -     | -   | ND  |
| 1710a    | -     | -   | -     | -   | ND  |
| 1710b    | -     | -   | -     | -   | -   |
| 1106b    | -     | -   | -     | -   | ND  |
| 406e     | -     | -   | -     | -   | ND  |

Non ϕX216 plaquing strains 25.0% (4/16), P2-like prophage candidate positive strains

| Strain   | P2L-A | P2L | ϕX216 | GI2 | ND  |
|----------|-------|-----|-------|-----|-----|
| 2671a    | +     | +   | +     | -   | -   |
| 2674a    | +     | +   | +     | -   | -   |
| 2677a    | +     | +   | +     | -   | -   |
| Pasteur 6068 | + | + | + | - | + |
| 2614a    | -     | -   | -     | -   | -   |
| 2617a    | -     | -   | -     | -   | ND  |
| 2640a    | -     | -   | -     | -   | ND  |
| 2665a    | -     | -   | -     | -   | ND  |
| 2689b    | -     | -   | -     | -   | ND  |
| 2694a    | -     | -   | -     | -   | ND  |
| E0008    | -     | -   | -     | -   | ND  |
| E0183    | -     | -   | -     | -   | ND  |
| E0350    | -     | -   | -     | -   | ND  |
| E0396    | -     | -   | -     | -   | ND  |
| 1106a    | -     | -   | -     | -   | ND  |
| MSH-R668 | -     | -   | -     | -   | ND  |

* ϕ52237-like assignment; positive PCR amplicons from multiplex probes P2-like 1, P2-like 2, P2-like group A, and individual PCR probes ϕX216 scrnA, ϕX216 scrnB and GI2. P2L-A assignment; positive PCR amplicons from multiplex probes P2-like 1, P2-like 2, P2-like group A and individual PCR probe GI2. P2L assignment; positive PCR amplicons from multiplex probes P2-like 1, P2-like 2.
* PSI216 source strain.
* 1026b ϕ52237-like prophage is split into two segments and likely non-functional [15].
* P2-like prophage group cannot be determined based on PCR results. May be P2L-A or ϕ52237-like.
* ϕ96243 prophage (group P2-A) located at GI2 [9].
* Encodes the predicted prophage PI-S13-1 (group P2-A) [88].
* P2-like prophage group cannot be assigned based on PCR results. May be P2L or ϕ52237-like.
* ND, GI2 probe results not determined.
* Non-confluent lysis / individual plaques when spot tested with ~106 pfu ϕX216.
* The strain 1710b genome does not contain a P2-like prophage or prophage insertion at GI2.

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including E0237, produced PCR results indicative of a close relationship with \( \varphi 522237/\varphi X216 \). \( B. \text{pseudomallei} \) 1710b, K96243, S13 and 1026b each produced PCR results that match sequence-based predictions for the presence of prophages \[7,8,15\]. Whereas strain 1710b is negative for a P2-like prophage, K96243 and S13 are both positive for subgroup A prophages (Table 1). Furthermore, 1026b is predicted to carry a \( \varphi 522237 \)-like prophage that is split into two fragments located in different regions of chromosome I (GenBank:CP002833.1, Locus # BP1026B_I0126-10172 and BP1026B_I3339-I3345). It is important to note that a positive hit for a subgroup A prophage does not exclude the possibility of a strain possessing multiple subgroup A prophages or more distantly related P2-like prophages. For instance, \( B. \text{pseudomallei} \) K96243 encodes both the \( \varphi K96243 \) subgroup A prophage in genomic island 2, as well as the predicted subgroup B prophage GI15 on chromosome II, but the subgroup A PCR results hide the presence of the subgroup B GI15 prophage due to the fact that the GI15 probe amplicons are identical in size to those from the \( \varphi K96243 \) prophage. The PCR probe results also do not indicate whether the candidate prophages can release viable phage progeny or are defective, as observed with the 1026b split \( \varphi 522237 \)-like prophage. The 30 strains that produced positive hits for P2-like prophages were additionally screened with the GI2 PCR probe. Strain 1710b was used as a P2-like-minus negative control. The 25 subgroup A candidate strains all produced positive PCR results for prophage integration into the 3’ end of the tRNA-Phe gene resulting in the formation of genomic island 2. The five candidates that failed to produce a positive GI2 PCR result were categorized as P2-like only. While our results do not definitively identify these five P2-like candidates as subgroup B members, subgroup B phages are predicted to use a different \( \text{attB} \) site and integration mechanism \[8\].

There is a strong correlation between P2-like prophage-positive \( B. \text{pseudomallei} \) strains and high efficiency plaquing by \( \varphi X216 \) on those strains (specificity 79.5%, positive predictive value 73.3%). In other words, it seems as though many \( B. \text{pseudomallei} \) strains that can be efficiently infected by \( \varphi X216 \) have been previously infected by one of its P2-like relatives and, strictly speaking, have been converted into lysogens.

Conclusions

Phage \( \varphi X216 \) has one of the highest strain infectivity rates reported among the \( B. \text{pseudomallei} \) phages characterized to date. Our results indicate that in contrast to previously isolated phages, \( \varphi X216 \) infects and propagates only on strains belonging to the \( B. \text{pseudomallei} \) clade. This is a desirable diagnostic trait and we believe \( \varphi X216 \) represents a good candidate platform for the development of phage-based \( B. \text{pseudomallei} \) diagnostic tools. Although \( \varphi X216 \) infects both \( B. \text{pseudomallei} \) and \( B. \text{mallei} \), these two species can be distinguished using \( \varphi 1026b \) which is \( B. \text{mallei} \)-specific \[10\]. The independent isolation of nearly identical \( \varphi X216 \) and \( \varphi 522237 \) phages from Thai and Vietnamese isolates, respectively, combined with the apparent broad distribution of P2-like prophage elements in \( B. \text{pseudomallei} \) highlights the success of this closely-related clade of lysogenic phages at infection and spread among a diverse spectrum of \( B. \text{pseudomallei} \) strains \[16\].

Methods

Bacterial growth and preparation of phage lysates

\( Burkholderia \) sp. used in this study are listed in Additional file 1. \( Burkholderia \) sp. and \( Escherichia coli \) strains were grown at 37°C with aeration in Lennox LB media as previously described \[17\]. For growth of \( B. \text{mallei} \), LB was supplemented with 2-4% glycerol. Growth media for Bp82 and its derivatives were augmented with 80 \( \mu \)g/mL adenine \[18\]. All procedures involving \( B. \text{pseudomallei} \) and \( B. \text{mallei} \) were performed in Select Agent approved Biosafety Level 3 (BSL3) facilities in the Rocky Mountain Regional Biosafety Laboratory (CSU) and the United States Army Medical Research Institute of Infectious Diseases using Select Agent compliant procedures and protocols. Phage plaque plates were prepared by adding 200 \( \mu \)l of a \( Burkholderia \) sp. overnight culture to 4 mL of molten top agar (0.6% agar, 0.1% glycerol and 2 mM CaCl\(_2\)) at 55°C followed by gentle mixing and pouring of the mixture onto LB agar plates. For the use of four-sectored 100 mL petri plates, volumes were adjusted to 100 \( \mu \)L of overnight culture and 2 mL molten top agar per sector. Phage lysates were
either added to top agar prior to pouring onto an LB agar plate or were spotted onto solidified top agar containing bacteria and allowed to dry prior to incubation at 37°C. Phage lysates were diluted in either Phage buffer [PB; 50 mM Tris–HCl (pH 7.4), 10 mM MgSO₄, 2 mM CaCl₂, 75 mM NaCl] or SM buffer [50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 8 mM MgSO₄, 0.002% gelatin] [19].

**Phage isolation and enumeration**

qx216 was plaque-purified twice from spontaneously formed plaques by released phage on *B. pseudomallei* E0237 using small scale liquid lysates using *B. pseudomallei* 2698a as a host strain. Plate lysates were prepared by flooding inverted plates with 5 mL of PB followed by incubation for either 3 h at 37°C or overnight at 4°C without agitation. The liquid was recovered from plates and bacteria pelleted by centrifugation at 16,000xg for 1 min at room temperature. Supernatants were combined and sterilized with a 0.2 μm disposable syringe filter (DISMIC-25AS Life Science Products, Inc., Frederick, CO). To create adapted lysates, plate lysates were used sequentially to infect a host strain followed by lysis recovery and reinfection for two to four cycles. For liquid lysates, 1 mL of a *B. mallei* ATCC23344 overnight culture, 1 mL phage lystate at approximately 10⁶ pfu/mL, 1 mL 10 mM CaCl₂ and 10 mM MgCl₂ were combined and incubated without agitation at 37°C for 15 min for initial phage attachment. 1.5 mL each of these mixtures were inoculated into 2 × 250 mL of pre-warmed LB with 2% glycerol in two 1 L disposable fretted Erlenmeyer flasks (Corning, Elmira, NY) and incubated overnight at 37°C with aeration. After overnight incubation, lysates were sometimes treated with 1% chloroform although better results were obtained when this step was omitted. Lysates were centrifuged at 4,000xg for 20 min at 4°C. Supernatants were combined with 25 mL 1 M Tris–HCl (pH 7.4) to a final concentration of 50 mM Tris–HCl, pre-filtered through a 0.2 μm disposable vacuum filtration unit and then filtered through a 0.2 μm disposable vacuum filtration unit to achieve sterility (Nalgene, Rochester, NY). Lysates were stored at 4°C in the dark. To determine phage titers, lysates were serially diluted in PB and 10 μL aliquots spotted onto top agar plates with appropriate *Burkholderia* sp. tester strains. Isolated plaques were counted and titers (pfu/mL) calculated.

**Burst size determination**

Phage burst sizes were determined by generation of one-step growth curves as previously described [19]. Briefly, a *B. mallei* ATCC23344 liquid lystate was inoculated using the same procedure described above for a single 250 mL volume. After the initial attachment mixture was incubated for 15 min and inoculated into a 1 L flask, triplicate 200 μL samples were recovered to produce *T₀* plaque plates using *B. mallei* ATCC23344 as the indicator strain. Triplicate samples (200 μL at 60 min, 100 μL at 80 min, and 50 μL 100 min through 180 min) were collected at 20 min intervals until 180 min post-inoculation to generate plaque plates. Plaques were counted and titers determined for each time point. One-step growth curves were repeated three times with similar results. Burst size was determined as the average fold increase in final pfu counts versus input pfu after one cycle of phage replication. Input pfu values were determined by averaging pfu/mL values taken at *T₀* and *T₁*.

**Determination of phage infectivity**

100 mm or four-sectored plaque plates were prepared as described above using each of the *Burkholderia* sp. strains listed in Additional file 1. Each sector was spotted with 20 μL each of *B. mallei* ATCC23344 liquid lystate, equating to approximately 10⁶ and 10⁴ pfu. For *φ2237*, sectors were additionally spotted with approximately 10³ pfu, a titer that was not obtained with qx216. Strains were considered positive for infection if they produced distinct plaques with either 10⁶ or 10⁴ pfu aliquots in multiple independent trials. *B. mallei* were considered positive for infection if plaques were observed when 10² pfu were mixed with the *B. mallei* indicator strain in LB top agar (0.6% agar). *B. pseudomallei* O-antigen mutants were tested simultaneously using both spotting and mixing methods.

**Recombinant DNA techniques**

DNA Restriction enzymes, T4 DNA ligase and *Taq* polymerase were purchased from NEB (Ipswich, MA) and used according to recommended protocols. Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) and are listed in Additional file 2. Plasmid DNA was purified using the GeneJet Plasmid Miniprep Kit from Fermentas (Glen Burnie, MD).

**PCR screening of candidate P2-like lysogens**

Primer sets were designed to amplify regions that were either conserved or unique to subsets of six previously described P2-like *Burkholderia* phage genomes deposited in Genbank, (GenBank:BX571965, GenBank:BX571966, GenBank:DQ087285, GenBank:CP000623, GenBank:CP000624, GenBank:CP000085) [8]. The genomic island 2 primer set was designed to span the tRNA-Phe gene (BURPS1710b_0354) and the primers were designed to anneal to highly conserved bacterial and phage genome regions [8]. Multiplex primers were designed to have calculated Tₘ values within 1°C of one another and to
amplify products separated in size by approximately 100 bp. Purified bacterial genomic DNA was used as a PCR template.

**Lysogen isolation**
A top agar plate of the *B. pseudomallei* 1710b derivative Bp516 was spotted with approximately 10^6 pfu/mL of 1710b-adapted φX216 plate lysate [20]. Bacteria were recovered from turbid zones of lysis and streaked to isolation. Isolated colonies were assessed for φX216 infectability and screened by PCR for the presence of the φX216 prophage at genomic island 2 and with other φX216 primer sets.

**B. pseudomallei O-antigen mutant strain construction**
DNA fragments corresponding to the 470-bp 5′ and 608-bp 3′ regions of the *wbiE* gene of Bp1026b were PCR amplified from genomic DNA using *Taq* polymerase with primers P2348 & P2349 and P2350 & P2351, respectively, and joined by overlap extension PCR [21]. The resulting 1,068-bp product was digested with *EcoRI* and ligated with *EcoRI* digested pEXGm5B [20] DNA to yield pPS2882. The 1.4-kb *FRT*-Km*-FRT* cassette of pFKm4 [20] as released by digestion with *XmaI* and ligated between the partially digested chromosomal DNA fragments contained in pPS2882 to create pPS2896. The pPS2896 plasmid was used to delete the *wbiE* region from Bp82 by allelic exchange employing previous published procedures [20,22]. This yielded the Δ*wbiE* mutant Bp82.39 and the presence of the correct mutant allele was confirmed by PCR amplification of the deletion region using primers P2368 and P2369. Sequence-defined *B. pseudomallei* 1026 *wbi*:T24 transposon insertion mutants were obtained through an ongoing project.

**Genomic DNA purification**
Bacterial genomic DNA was purified with the Qiagen Gentra Puregene Gram negative Bacteria kit according to the manufacturer’s recommendations (Qiagen, Valencia, CA). Phage particles were semi-purified by polyethylene glycol precipitation as previously described [23]. Briefly, 30 g NaCl was added to 500 mL of sterile filtered *B. mallei* ATCC23344 liquid lysate (10^8 pfu/mL) and stirred continuously on ice while 50 g of polyethylene glycol 8000 (PEG) was slowly added. The mixture was then stirred continuously overnight at 4°C. PEG-precipitated lysates were pelleted by centrifugation at 11,000×g for 15 min at 4°C and the supernatant discarded. Pellets were suspended in 8 mL SM buffer, combined with 8 mL chloroform, vortexed vigorously for 30 s and centrifuged at 4,000×g for 15 min at 4°C. Aqueous layers were retained and extracted two additional times with chloroform to remove any remaining PEG. This concentrated phage particles approximately 10-fold. Phage DNA was purified using a modification of the protocol described by Kaslow [24]. To 3 mL total concentrated lysate, 15 μL DNase I (1 mg/mL) and 30 μL RNase A (10 mg/mL) were added and incubated at 37°C for 30 min. Then 150 μL 10% SDS, 125 μL 0.5 M EDTA (pH 8.0), and 250 μL STEP buffer [0.1% SDS, 10 mM Tris–HCL (pH 7.4), 80 mM EDTA, 1 mg/mL proteinase K] were added, and the mixture incubated for 30 min at 65°C. Genomic DNA from enzymatically treated lysates was phenol + chloroform extracted. 3.5 mL TE-saturated phenol was added to enzymatically treated lysates, mixed by inversion, and centrifuged at 800×g for 5 min at room temperature. The aqueous phase was retained and extracted twice with 3.5 mL phenol + chloroform (1:1) and once with 3.5 mL chloroform. Phage genomic DNA was ethanol precipitated by adding 1.2 mL 7.5 M NH4-acetate and 4.5 mL –20°C Ethanol (96%), followed by 15 min incubation on ice. Phage genomic DNA was spooled onto a sealed Pasteur pipette, transferred to a fresh 1.5 mL microfuge tube, air dried briefly and suspended in 200 μL TE buffer resulting in a DNA concentration of approximately 1 μg/μL.

**Sequencing and annotation**
Random and φ52237-sequence guided φX216 genome fragment clones were constructed by restriction digest of purified φX216 genomic DNA with *EcoRI*, *EcoRI* + *HindIII* or *Agel* and ligation with *EcoRI*, *EcoRI* + *HindIII* or *Smal* digested pUC19 DNA [25], respectively, followed by transformation of *E. coli* DH5α or GBE180 [26] using standard transformation protocols [27] and recovery of white colonies on LB plates containing 100 μg/mL ampicillin and 50 μg/mL 5-bromo-4-chloro-3-indolyl-B-D-galactopyranoside (X-gal). φ52237-sequence-guided PCR amplicons were designed to close gaps and confirm fragment clone borders. Sequencing was accomplished using M13F and M13R primers, as well as φ52237-sequence guided primer walking of fragment clones and PCR amplicons using an ABI 3130xL Genetic Analyzer (Applied Biosystems, Carlsbad, CA) at the Colorado State University Proteomics and Metabolomics Facility. φX216 Illumina sequencing libraries were prepared using the TruSeq DNA Sample Preparation Kit v2, (Illumina, San Diego, CA), following the manufacturer’s instructions. Phage DNA was fragmented to a range of 300–400 bp using a Covaris acoustic shearing device, (Covaris Inc., Woburn, MA) followed by 3’ adenylation and adapter ligation. Ligation products were purified on an agarose gel and the DNA fragments enriched via PCR. Fragmented Phage DNA was sequenced by high-throughput Illumina parallel sequencing using 100 bp mate-pair Illumina HiSeq 2000 reversible terminator
chemistry. The library was run on 15% of a single lane. Reads were trimmed for quality and de novo short-read genome assembly was performed using the Velvet 1.1.05 sequence assembler algorithm with a hash length of 99 and a final graph with 3 nodes and n50 of 37412 nt [28]. Open reading frames were identified with GeneMark gene prediction software using a viral-optimized Heuristic approach [29]. Putative gene identification was conducted by sequence alignment with pfam2377 (GenBank: DQ087285.2) [8] and individual open reading frames queried using the NCBI Basic Alignment Search Tool (BLAST). Genome annotation, mapping, sequence alignments, and comparative analyses were conducted using Gene Construction Kit v3.0 and Geneious Pro 5.4.6 bioinformatics software. The annotation map was created using Adobe Illustrator CS5. The final qx216 genome sequence has been deposited in GenBank under accession # JX681814.

Additional files

Additional file 1: qx216 host range, word document, Host range of qx216. Table of qx216 host range for 72 B. pseudomallei strains and other Burkholderia species.

Additional file 2: Oligonucleotides, word document, Oligonucleotides and probe regions. Table of oligonucleotides and probe regions designed for this study.

Competing interests

The authors declare no competing interests.

Authors’ contributions

BHK, CRC, DD, KV, and HPS conceived and designed the experiments. BHK conducted experiments with B. pseudomallei and other Burkholderia strains. DD conducted host range tests with B. mallei strains. BHK, CRC and SLJ conducted genome sequencing and annotation. BHK, CRC DD, and HPS wrote the manuscript. All authors read and approved the final manuscript.

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