**Characterization of *Erwinia tracheiphila* bacteriophage FBB1 isolated from
spotted cucumber beetles that vector *E. tracheiphila***

Benzhong Fu, Yingyan Zhai[§], Mark Gleason, Gwyn A. Beattie*

Department of Plant Pathology & Microbiology, Iowa State University, Ames, IA 50011, U.S.A.

***Corresponding author:** Gwyn A. Beattie, E-mail: gbeattie@iastate.edu, 1344 Advanced
Teaching & Research Building, 2213 Pammel Dr, Ames, IA 50011-1101

[§]Current address: Bio-Agriculture Institute of Shaanxi Province, Xi'an 710043, China

Abstract: *Erwinia tracheiphila*, the causal pathogen of bacterial wilt of cucurbit crops, is disseminated by cucumber beetles. A bacteriophage, designated FBB1, was isolated from spotted cucumber beetles (*Diabrotica undecimpunctata*) that were collected from a field where *E. tracheiphila* is endemic. FBB1 was classified into the *Myoviridae* family based on its morphology, which includes an elongated icosahedral head (106×82 nm) and a putatively contractile tail (120 nm). FBB1 infected all 62 *E. tracheiphila* strains examined and also three *Pantoea* spp. strains. FBB1 virions were stable at 55°C for 1 h and tolerated a pH range from 3 to 12. FBB1 has a genome of 175,994 bp with 316 predicted coding sequences and a GC content of 36.5%. The genome contains genes for a major bacterial outer-membrane protein, a putative exopolysaccharide depolymerase, and 22 predicted tRNAs. The morphology and genome indicate that FBB1 is a T4-like virus and thus in the *Tevenvirinae* subfamily. FBB1 is the first virulent phage of *E. tracheiphila* to be reported, and to date, is one of only two bacteriophages to be isolated from insect vectors of phytopathogens. Collectively, the results support FBB1 as a promising candidate for biocontrol of *E. tracheiphila* based on its virulent (lytic) rather than lysogenic lifestyle, its infection of all *E. tracheiphila* strains examined to date, and its infection of a few non-pathogenic bacteria that could be used to support phage populations when pathogen numbers are low.

Keywords: Bacterial Pathogens, Biological Control, Ecology, Genomics, Microbe-genome Sequencing, Molecular, Virology

INTRODUCTION

Erwinia tracheiphila is the causal agent of bacterial wilt of cucurbits, a disease that involves bacterial multiplication in the plant xylem leading to rapid wilt and often plant death (Saalau Rojas et al. 2015). The disease is spread by insect vectors, mainly spotted and striped cucumber beetles (*Diabrotica undecimpunctata* and *Acalymma vittatum*, respectively). Bacterial wilt of cucurbits is particularly damaging to cucumbers, melons, and squash. Due to the lack of resistance in these crops, this disease is usually managed by applying insecticides to suppress the cucumber beetle populations (Saalau Rojas et al. 2015). Biological control strategies that include bacteriophages (also called phages) could provide an additional option for disease management.

As concerns about bacterial resistance to antibiotics have continued to escalate, phage therapy has been increasingly evaluated as an option for managing infectious bacteria, including human pathogens (Dedrick et al. 2019), zoonotic pathogens (Gigante and Atterbury 2019), and food-borne pathogens (O'Sullivan et al. 2019). These efforts have generally employed virulent (lytic) phages rather than temperate (lysogenic) phages. Some of the potential advantages of deploying phages for biocontrol include their specificity to target pathogen strains and species, their suitability for use as phage mixtures to expand their host range, and the ability of some phages to disrupt the bacterial biofilms that often develop during bacterial infection of plant and animal hosts (Luong et al. 2020).

Phage therapy has been explored for many plant pathogens. A recent review (Sieiro et al. 2020) documented over 25 examples of the effective use of phage therapy in plant agriculture, including for control of the major bacterial diseases of potatoes (soft rot, blackleg, bacterial wilt, and common scab of potato), tomatoes (bacterial spot and bacterial wilt), fruit trees (fire blight of apple and pear), and vines (cankers of kiwi, and Pierce's disease of grapes). These studies

commonly employed phage mixtures, called cocktails, to broaden the range of pathogen strains that can be managed, and included phages that recognize distinct receptors on the pathogens to reduce the probability of resistance arising. These studies demonstrated that phages can reduce both the incidence and severity of many phyto-bacterial diseases. Collectively, these studies have illustrated the potential for phage therapy against pathogens causing surface lesions, as in foliar and fruit spots and rots, as well as wilts in which the pathogens are in the xylem.

The *Myoviridae* are a major family of bacteriophages that include tailed double-stranded DNA (dsDNA) phages that often have a lytic lifestyle (Lavigne and Ceyssens 2012; Petrov et al. 2010). A major model for our current understanding of these virulent phages is the *Escherichia coli* phage T4, which emerged as a model after a half century of focus on the three T-even phages T2, T4 and T6 (Petrov et al. 2010). These T4-like viruses are now classified into the subfamily *Tevenvirinae*. These phages generally have large genomes (160-260 kb), elongated icosahedral heads, contractile tails, six long tail fibers that serve to recognize bacterial surface receptors, and short tail fibers that promote irreversible attachment (Comeau et al. 2007). In brief, the T4 infection cycle includes binding to the host cell, injecting the dsDNA genome, inhibiting host metabolism, degrading the host chromosome, and chemically modifying the resulting nucleotides for phage DNA synthesis. During the synthesis of T4 virions, the DNA is packaged into the head and the tail and tail fibers are assembled before being joined together to form complete virions. The virions are released through the action of proteins (e.g., lysozyme, holins) that help degrade the host cell wall.

Here, we report on the isolation and characterization of the first virulent bacteriophage of the cucurbit wilt pathogen *E. tracheiphila*, and thus the first candidate phage for biocontrol of this pathogen. The lysogenic *E. tracheiphila* bacteriophage EtG was previously isolated from an

infected cucumber plant and its 30-kb genome sequence was reported (Andrade-Domínguez et al. 2018). Among related phytopathogens, *E. amylovora* has been the subject of phage-based management studies, with many *E. amylovora* bacteriophages isolated from soil and tree tissues and several phages developed into commercial products for fire blight management (Nagy et al. 2012). *E. amylovora* phages vary in their host specificity, with some specific to individual strains and others infecting additional species such as *Pantoea* spp. (Nagy et al. 2012; Park et al. 2018). Currently, genome sequences are available for dozens of *E. amylovora* phages as well as phages that infect the pathogen *Erwinia pyrifoliae* and the nonpathogen *Erwinia tasmaniensis*. In this study, we looked for *E. tracheiphila* phage in soils from fields with diseased cucurbits, from asymptomatic plant tissues, and from both spotted and striped cucumber beetles. We obtained phages only from spotted cucumber beetles. To the best of our knowledge, this is only the second report of the isolation of a plant pathogen-infecting phage from the pathogen's insect vector; the first report was for a phage infecting *Pantoea stewartii* from the corn flea beetle (*Chaetocnema pulicaria*) (Woods et al. 1981).

MATERIALS AND METHODS

Bacterial strains and growth conditions

The bacterial strains used in this study are shown in Table 1. All bacteria except *Escherchia coli* were grown at 30°C in King's B (KB) medium (King et al. 1954) with shaking or on KB plates (1.5% agar). *E. coli* was grown in Luria-Bertani (LB) medium at 37°C. Bacteria were stored at -70°C.

Phage isolation and purification

Environmental samples were collected on July 29, 2019, from field plots at the Iowa State University Horticultural Research Farm in Gilbert, Iowa. Bacterial wilt of cucurbits is endemic at this field site. Samples included flowers and leaves from asymptomatic muskmelon plants (*Cucumis melo* cultivar Athena), soils from beneath the muskmelon plants, spotted cucumber beetles, and striped cucumber beetles. All samples were stored at 4°C until processing. Extracts were collected from four independent soil samples, one set of pooled flowers, and one set of pooled leaves, with each sample having 3 to 5 g of soil or tissue. They were also collected from two sets of striped cucumber beetles (one with three beetles and one with eight) and three sets of spotted cucumber beetles (one each with three, seven and nine beetles). The beetles remained alive at 4°C until processing. Samples were placed in 5 ml of 1/10-strength KB for each 1 g of sample. The 1/10-strength KB served as a phage buffer. The soil samples were placed on a rotating shaker for 2 h, and the plant and insect tissues were manually macerated. The resulting slurries were centrifuged at $8,000 \times g$ for 5 min, and the supernatants were filtered using 0.2- μ m filters.

The filtrates were tested for phages using a soft agar overlay plaque assay. This assay was conducted by combining an aliquot of the filtrate, 200 μ l of *E. tracheiphila* BHKY cells grown in KB broth to an optical density at 600 nm (OD_{600}) of 0.5 ($\sim 10^8$ cells/ml), and KB medium with 0.4% agar (top agar) that had been prewarmed in a 50°C water bath. This mixture was poured uniformly onto KB medium with 1.5% agar (bottom agar), and the plates were incubated at 30°C for 2 days. The filtrates were also enriched for phages by combining them with a culture of strain BHKY grown in KB broth at 30°C at a starting density of approximately 10^7 cells/ml. Mixtures were incubated with shaking for 48 h, and then either the mixtures were centrifuged and filtered

as above, or aliquots of the mixtures were transferred to a fresh culture of BHKY cells for a serial enrichment.

Phages were purified by performing at least five sequential plaque assays, and generally many more than five, each on a single selected plaque. After phage amplification on plates exhibiting confluent lysis, bacteria and cell debris were removed by centrifugation and filtration. Phage stocks were maintained at 4°C for short-term storage and at -70°C with 30% glycerol for long-term storage.

Host range determination

The phage host range was assayed by the spot assay method. Briefly, fresh bacterial cultures were adjusted to an OD₆₀₀ of 0.5. Phage infectivity of a given host was evaluated by dropping 20 µl of the cell suspension onto a KB plate, letting it soak in, then placing 5 µl of phage stock (~10⁸ PFU/ml) onto the bacterial spot. Phage buffer (5 µl) and kanamycin (50 µg/ml) (1 µl) were put on plates for reference. Plates were incubated at 30°C for 2 days. Complete host lysis was scored as a positive reaction. Phage hosts were verified in at least three independent spot assays, and, for many hosts, further confirmed using the plaque assay.

Transmission electron microscopy (TEM) of phage

Phage were prepared by amplifying phage from two independent plaques from a phage stock and creating two replicate suspensions with >10¹¹ plaque-forming units (PFU)/ml in each. The morphology of the phage in the suspensions was observed by transmission electron microscopy (200kV JEOL 2100) at the Iowa State University Roy J. Carver High-Resolution Microscopy Facility. One 10-µl drop of each suspension was independently visualized using a 2% (wt/vol) uranyl acetate negative stain. The phage particles in multiple images representing the original phage stock were consistent in their morphology.

One-step growth curve and host strain-killing curve

A one-step growth curve was generated to determine the latent period and burst size. A culture of *E. tracheiphila* strain BHKY, which was grown in KB medium to 10^8 cells/ml, was combined with a phage stock at a multiplicity of infection (MOI) of 0.01. After allowing the phages to adsorb for 15 min at 30°C, the mixture was centrifuged at $8,000 \times g$ for 10 min to remove non-adsorbed phage particles. The pellet of bacteria-phage complexes was diluted 10^4 -fold with KB medium, incubated at 30°C, and aliquots were removed every 10 min to determine the phage titer by a plaque assay. We evaluated two independently-generated growth curves, each with three replicate BHKY cultures. The burst size was calculated by dividing the mean phage count estimated during the plateau stage by the mean phage count estimated during the latent stage.

A killing curve was generated for the host strain BHKY to evaluate the dynamics and extent of host killing. A BHKY culture (10^8 cells/ml) was mixed with phage stocks at MOIs of 0.01 and 0.1. The rate of killing of the host cells was determined by monitoring the OD₆₀₀ every 30 to 60 min until the culture was clear.

Phage stability tests

The impact of temperature, pH, and UV radiation on phage stability was evaluated. Phage stocks ($\sim 10^6$ PFU/ml) were incubated at -20, 4, 25, 37, 55, 65, and 80°C for 1 h, or were diluted 10^3 -fold with phage buffer that had been adjusted to pH 1 to pH 14 and incubated at 25°C for 1 h. Phage stocks (500 μ L of $\sim 10^6$ PFU/ml) were also placed in 1.5 ml centrifugation tubes and, with the lids opened and the tubes vertical under the UV lamp, the phages were exposed to a UV-C light (Philips TUV G30T8, UV dose 50 mJ/cm² at 254 nm and 30 cm distance from the lamp) for 5, 10, 15 and 30 min, or to a UV-A light (LIGHTFE Black light UV Flashlight,

approximately 10 $\mu\text{mol}/\text{m}^2/\text{sec}$ at 15 cm distance from the lamp) for 5, 10, 15, 20, 25 and 30 min. Phage titers were determined before and after incubation using the plaque assay. All tests involved three replications. The relative stability to temperature was determined using 25°C as a reference point, the relative stability to pH was determined using pH 7.0 as a reference point, and the stability to UV was determined using 0 min (just prior to UV treatment) as a reference point.

Genome sequence and assembly

Genomic DNA was extracted from a purified phage stock using a phage DNA extraction kit (Norgen-Biotek, Canada), visualized on an agarose gel, quantified with a Qubit Fluorometer (Invitrogen), and subjected to sequencing at the Iowa State University DNA Facility using the Nextera DNA Flex Library Prep kit (Illumina, San Diego) and the Illumina HiSeq 3000 platform with $2 \times 100\text{-bp}$ paired-end reads. A total of 1,703,547 reads were generated. These were trimmed of the adaptors with Trimmomatic software (v0.39) (Bolger et al. 2014) and subjected to quality control using FastQC (v0.11.9) (available online from Babraham Bioinformatics). The paired-end reads were assembled *de novo* using SPAdes v3.14.0 (Bankevich et al. 2012), and the assembly was analyzed using QUAST (Gurevich et al. 2013) .

Genome annotation and analysis

Genes were predicted and annotated using RASTtk IRIS (Brettin et al. 2015) and PHANOTATE (McNair et al. 2019). tRNAs were detected using tRNAscan-SE 2.0.5 (Chan and Lowe 2019) and ARAGORN (Laslett and Canback 2004). Genome comparisons were made using LAST (Kielbasa et al. 2011). Pairwise comparisons of the complete genome sequence to selected phage genomes were performed using the Virus Classification and Tree Building Online Resource (VICTOR) (Meier-Kolthoff and Göker 2017). A map comparing closely related genomes was drawn using BLAST Ring Image Generator (BRIG) (Alikhan et al. 2011).

Statistics

When appropriate, values were expressed as the mean $\pm$ standard deviation. Values reflecting the proportion of the relative activity were transformed using an arcsine (square root) transformation to achieve normality, and the values were subjected to analysis of variance followed by Fisher's Least Significant Difference test for multiple comparisons.

Nucleotide sequence accession number

The complete phage genome sequence of FBB1 was deposited in NCBI with the GenBank Accession Number MT920315.

RESULTS AND DISCUSSION

A phage putatively in the *Myoviridae* family was isolated from spotted cucumber beetles.

We failed to isolate *E. tracheiphila*-infecting phages from four soil samples from field plots where the pathogen is endemic, even after three serial enrichments on *E. tracheiphila*. We similarly failed to isolate phages from tissues of asymptomatic muskmelon plants or from two independent sets of striped cucumber beetles after each of three enrichment cycles on *E. tracheiphila*. However, we successfully isolated phages from all three independent sets of spotted cucumber beetles; these phages were isolated from beetle homogenates prior to the enrichments. We purified and characterized one of these phages, which we designated FBB1 (*Fu-Beattie-Beetle-1*). Phage FBB1 formed sharply delimited clear plaques with a diameter of 3.9 ± 0.7 mm ($n = 54$) when grown on strain BHKY on KB medium at 30°C (Figure 1A).

The morphology of phage FBB1 virions observed by TEM suggests that the phage belongs to the *Caudovirales* order and *Myoviridae* family (Figure 1B). We designated the phage

vB_EtrM_FBB1 in accordance with phage nomenclature recommendations (Kropinski et al. 2009). The phage is 226.0 ± 12.5 nm in length, with an icosahedral and moderately elongated head that is 106.0 ± 12.5 nm $\times$ 82.0 ± 4.2 nm, and a tail that is 120 ± 9.7 nm $\times$ 21 ± 3.9 nm ($n = 20$). The size and shape of FBB1 virions are similar to virions in the *Tevenvirinae* subfamily, which generally have heads and tails that are around 110 nm and 114 nm in length, respectively, and have a base plate with short spikes and a collar (Lavigne et al. 2009). The spikes on the base plate of FBB1 were visible, although the collar was not (Figure 1B).

Although many bacterial plant pathogens are disseminated by insect vectors, only one phage of a plant pathogen has previously been isolated from an insect vector. In 1981, Woods *et al.* (1981) isolated a phage infecting *Pantoea (Erwinia) stewartii* from a corn flea beetle (*Chaetocnema pulicaria* Melsheimer). The morphology of phage FBB1 is distinct from the bacteriophage of *P. stewartii*, which had an octahedral head with a diameter of only 58 nm, a short neck, and a non-contractile tail. The genome sequence of the *P. stewartia* phage is not available. Few bacteriophages have been isolated and characterized from insects, but two phage groups that infect endosymbionts in arthropods are known. The APSE (*Acyrtosiphon pisum* secondary endosymbiont) phages are temperate phages that infect the endosymbiont *Hamiltonella defensa* in aphids and other hemipterans (Rouil et al. 2020); these phages have short tails characteristic of the *Podoviridae* family and can carry toxin genes that protect aphids against parasitoid wasps (Oliver et al. 2009). Similarly, the WO phages are temperate phages that infect *Wolbachia*, a widespread endosymbiont among arthropods and nematodes (Metcalf and Bordenstein 2012); these phages carry genes that influence the reproductive strategies of their hosts (LePage et al. 2017).

The host range of phage FBB1 includes *E. tracheiphila* and some *Pantoea* spp.

We evaluated the host range of phage FBB1 using 62 *E. tracheiphila* strains that were collected from the midwestern and northeastern regions of the U.S. from 2008 to 2010. These strains were isolated from four crop species, muskmelon (*Cucumis melo*), cucumber (*C. sativus*), and squash (*Cucurbita pepo* and *C. moschata*). We also examined infectivity of other Gamma-Proteobacteria, including other phytopathogens. FBB1 was able to infect and lyse all 62 *E. tracheiphila* strains tested (Table 1). This contrasts with the bacteriophage of *P. stewartii* isolated from the corn flea beetle (Woods et al. 1981), which infected only eight of the 13 *P. stewartii* strains tested. The ability of FBB1 to infect all of the *E. tracheiphila* strains tested, which collectively represented the entire geographic range of the pathogen, makes it a promising candidate for use as a biocontrol agent.

The phage FBB1 also infected *P. stewartii* strain ESRif-9A and *Pantoea ananatis* (previously *P. agglomerans*) strains BRT98 and BRT175, but did not infect *Pantoea eucalypti*. In contrast to the known pathogenicity of *P. stewartia* ESRif-9A (Menelas et al. 2006), strains BRT98 and BRT175 are not thought to be pathogens (Sabaratnam and Beattie 2003). A previous study found similar cross-reactivity across these host genera for bacteriophage phiEa2809 on *E. amylovora* and *Pantoea agglomerans* (Lagonenko et al. 2015). FBB1 did not infect the phytopathogens *E. amylovora*, *Pectobacterium carotovorum* or *Pseudomonas syringae* nor did it infect the nonpathogens *Erwinia* spp. EhWHL9, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas putida* R20 or *Pseudomonas fluorescens* A506. The ability of FBB1 to infect at least two nonpathogenic *Pantoea* strains could enable these strains to be used as carrier bacteria that amplify FBB1 numbers after co-application to plants. The application of bacteria that do not harm the plant but serve as an alternative host to phages is a strategy that has proven successful

273 to reduce diseases caused by *E. amylovora* (Boulé et al. 2011) and *Ralstonia solanacearum*
274 (Tanaka et al. 1990).

275 **Phage FBB1 is stable to a broad temperature and pH range.**

276 A one-step growth curve of FBB1 using the *E. tracheiphila* strain BHKY in KB medium
277 showed that FBB1 had a latent period of 50 min, with phage release reaching a maximum around
278 70 min (Figure 2A). The latent period of FBB1 was similar to that of the phage of *P. stewartii*
279 isolated from the corn flea beetle, which was 55 min. The burst size of FBB1 was low, only 15.8,
280 whereas the *P. stewartii* phage had a burst size of 236 (Woods et al. 1981).

281 The bacteriolytic activity of FBB1 on BHKY in KB medium was examined at two MOI
282 levels (Figure 2B). An MOI of 0.1 prevented detectable growth, whereas an MOI of 0.01 did not
283 (Figure 2B). For both MOI levels, the eventual reduction in the OD₆₀₀ indicating complete or
284 near complete lysis.

285 The ability of phage to tolerate a breadth of environmental conditions is an important
286 consideration for biocontrol potential in the field. We tested the tolerance of phage FBB1 to
287 temperatures from -20 to 80°C. As compared to the number of FBB1 virions that were active
288 after a 1-h exposure to 25°C, over 75% of the FBB1 virions were infectious after a 1-h exposure
289 to 37 and 55°C, and most remained infectious after exposure to 4°C (Figure 3A). These results
290 indicate that FBB1 is stable over the full range of temperatures typically encountered by crops
291 such as cucurbits in temperate regions. Exposure to 65°C or higher for 1-h inactivated FBB1,
292 whereas exposure to -20°C resulted in high variation in infectivity. The latter suggests that FBB1
293 titer would likely decline during cryopreservation in the absence of a cryoprotectant. The range
294 of temperatures tolerated by phage FBB1 was similar to that of the lytic phage phiEaP-8, which
295 infects *E. amylovora* and *E. pyrifoliae* (Park et al. 2018).

We also tested the stability of FBB1 to environmental acidity, as the pH of plant tissues such as the apoplast and xylem sap are generally acidic but vary among species (e.g., Gloser et al. 2016). FBB1 was stable over a broad range of pH values, with over 70% of the virions that were lytic at pH 7 also showing lytic activity after 1-h exposure to each pH value tested from pH 3 to 12 (Figure 3B). FBB1 exhibited the highest stability in the range of pH 4 to 7, which is the range most common for bacterial environments on and in plants. The pH range that FBB1 tolerated was broader than that tolerated by the *E. amylovora* and *E. pyrifoliae* phage phiEaP-8, which did not tolerate pH < 5 or pH > 10 (Park et al. 2018).

Ultraviolet radiation (UVR) can be toxic to phage. Of the solar radiation reaching the Earth's surface, less than 10% is generally UVR, and of this UVR, over 95% is UV-A (315-400 nm) with the remainder being mostly UV-B (290-315 nm). Although little UV-C (100-280 nm) reaches the Earth's surface, UV-C is generally strongly toxic to phage. We confirmed this UV-C toxicity to FBB1 during exposure to 254 nm (Figure 3C). FBB1 was more tolerant to exposure to 365 nm than 254 nm (Figure 3D), as expected given the lower energy of this UV-A wavelength; however, FBB1 was more sensitive to UV-A than were phages of *E. amylovora* and *E. pyrifoliae*, which were almost completely stable when exposed to 365 nm for 1 h (Park et al. 2018). A variety of strategies have been developed to enhance the UV tolerance of phage used for biocontrol, including altering the timing of application to lessen exposure, using formulations with protectants, and deriving protection from non-target carrier bacteria (Jones et al. 2012), such as the nonpathogenic *Pantoea* strains that are hosts to FBB1.

Whole-genome sequence-based phylogeny indicates FBB1 is in an unclassified genus in the *Tevenvirinae* subfamily.

318 The FBB1 genome is 175,944 bp with a GC content of 36.5% based on paired-end reads.
319 The sequencing coverage was 575-fold. The lower GC content of FBB1 than of its bacterial
320 hosts, *E. tracheiphila* (50.5%), *P. stewartii* (53.5%) and *P. ananatis* (53.7%), is consistent with
321 previous reports of lower GC content for virulent phage than their hosts (Bailly-Bechet et al.
322 2007). The FBB1 genome was predicted to contain 316 coding sequences (CDS), of which
323 41.5% (131) had predicted functions and 58.5% (185) were annotated as hypothetical proteins.
324 The average CDS was 552 bp, with the total CDS accounting for 94.1% of the genome length.

325 A whole-genome comparison performed using BLASTn supported classification of
326 FBB1 into the *Myoviridae* family and *Tevenvirinae* subfamily. This family is known to have a
327 circularly permuted genome with redundant termini, as derived from packaging slightly more
328 than one genome into a phage head using random initiation points. The closest relative shared
329 less than 80% identity, indicating that FBB1 does not fit into a known species when using a 95%
330 sequence identity threshold as a classifier for phage species (Adriaenssens and Brister 2017).
331 FBB1 was most closely related to the *Erwinia* phage Cronus (GenBank accession MH059636.2)
332 (Figure 4), sharing 77% nucleotide identity with 29% coverage. Cronus was isolated from
333 organic waste in Denmark, infects the host bacterium *E. amylovora*, and has a linear genome of
334 175,774 bp and a GC content of 42.0%. Based on the taxonomy browser at NCBI, the *Erwinia*
335 phage Cronus and *Pantoea* phage Phynn (Accession MN038175) are the only phage in the
336 *Tevenvirinae* family that have been sequenced from these bacterial genera, and neither has been
337 classified into a genus.

338 The *Tevenvirinae* subfamily currently has 11 defined genera in the International
339 Committee on Taxonomy of Viruses (ICTV) Master List (available online from the ICTV). We
340 used the Virus Classification and Tree Building Online Resource (VICTOR) to evaluate the

relatedness of FBB1 and Cronus to representative phage of these 11 genera, along with five of the *Tevenvirinae* phage on the ICTV Master Species List that have not yet been classified into genera. The resulting phylogenetic tree shows that FBB1 and Cronus form a distinct branch from the other genera, suggesting classification into a new genus (Figure 5). FBB1 and Cronus exhibit a genome structure that is similar to other phages in the *Tevenvirinae* subfamily (Figure 6).

FBB1 is rich in tRNA genes.

The FBB1 and Cronus genomes are particularly divergent from the other phages in a locus containing clustered tRNA genes (Figure 6). tRNA genes can increase the phage translation efficiency by providing additional tRNAs for codons that are particularly abundant in the phage genome. Phage with large genomes commonly have all of their tRNA genes in a cluster, with the tRNA-Met-CAT generally present in the highest copy number in these clusters (Morgado and Vicente 2019). In contrast to the 3 to 8 tRNA genes identified in phage CC31, JS98 and T4, 22 tRNA genes were predicted in the FBB1 genome, with the same tRNAs predicted by tRNAscan-SE and ARAGORN (tRNA-Gly-TCC, tRNA-Trp-CCA, tRNA-Thr-TGT, tRNA-Arg-TCT, tRNA-Met-CAT, tRNA-Met-CAT, tRNA-Leu-TAA, tRNA-Phe-GAA, tRNA-Glu-TTC, tRNA-Ser-TGA, tRNA-Tyr-GTA, tRNA-Leu-TAG, tRNA-Ala-TGC, tRNA-Pro-TGG, tRNA-Ile-GAT, tRNA-Lys-TTT, tRNA-Asp-GTC, tRNA-Asn-GTT, tRNA-Ser-GGA, tRNA-Ser-GCT, tRNA-His-GTG, and tRNA-Gln-TTG). The Cronus genome has the same tRNAs and in the same order, but has an additional tRNA for Phe (predicted as tRNA-Phe-AAA or tRNA-Phe-GAA). Both genomes have two tRNA-Met-CAT genes.

The FBB1 genome likely contains non-glycosylated, modified cytosines.

FBB1 has homologs of most proteins known to contribute to nucleotide metabolism in phage T4, with a few exceptions (Supplemental Table 1). Whereas many *Tevenvirinae* have both

aerobic and anaerobic ribonucleotide reductases, FBB1 and Cronus have only the aerobic form, which is comprised of the NrdA and NrdB subunits. Like other T4-like phages, FBB1 and Cronus both have a dCMP hydroxymethylase for converting deoxycytosine monophosphate (dCMP) to 5-hydroxymethylcytosine (^{hm5}dCMP), which helps protect phage DNA from degradation by phage nucleases. However, following incorporation into DNA, T4 further modifies ^{hm5}dCMP by glucosylation to protect it from host nucleases; FBB1 lacks this predicted function. In particular, glucosylation requires at least one of two glucosyltransferases (GTs), an α -GT or a β -GT. T4 has both types of GT, whereas other T4-like phages have none or only one (Figure 6). Cronus has an α -GT, suggesting that it glucosylates its DNA. The potential impact of a lack of glycosylation on FBB1 susceptibility to host nucleases is unknown.

The FBB1 genome contains genes for a putative exopolysaccharide depolymerase and a major bacterial outer-membrane protein.

Many phages that infect the plant pathogen *E. amylovora* encode a depolymerase that can break down amylovoran, the major extracellular polysaccharide (EPS) of this pathogen (Born et al. 2014; Knecht et al. 2018). FBB1 has a gene encoding a putative EPS depolymerase (FBp200, Supplemental Table 1). This protein shares 30% identity with a known amylovoran depolymerase (Accession YP_007005466.1) (Born et al. 2014), 40% identity with the homologous protein in Cronus, and 58% identity with a protein in *Pantoea* phage Phynn (Accession QDH49038.1). This protein may enhance the sensitivity of *E. tracheiphila* and *P. agglomerans* to FBB1, as was observed with *E. amylovora* to phage L1 (Born et al. 2014).

Among the many FBB1 genes with unknown functions, several are found primarily in FBB1 and Cronus. These include a 361-aa protein (FBp084) specific to FBB1 and a 245-aa protein (FBp244) found only in FBB1 and Cronus. Among the FBB1 genes with predicted

functions, a gene encoding a homolog of Lpp (FBp144) was unique to FBB1 among the *Tevenvirinae*; it is nestled between the two tRNA-Met-CAT genes in the tRNA locus. Interestingly, Lpp is one of the major outer membrane proteins found in the *Enterobacteriaceae*. The FBB1 protein shares 52% identity with Lpp of *Erwinia tracheiphila* and other *Erwiniaceae*, and about 55% identity with the Lpp lipoprotein of a large number of *Salmonella* strains. The *lpp* gene is not present in Cronus but is present in three *Salmonella* phages, PVPSE1, SSE121 and GEC_vB_MG, all of which are in a distinct subfamily of the *Myoviridae*, namely the *Vequintavirinae*. We do not yet know if this gene is expressed, functional, or relevant to FBB1 biology. As a major bacterial outer-membrane protein, however, it would be a candidate receptor for phage and thus is an unusual protein to find within the genome of a virus, particularly one lacking an envelope.

FBB1 exhibits polymorphisms from other phage in its tail fiber proteins.

The tail fiber proteins are among the most variable proteins within the *Tevenvirinae* (Comeau et al. 2007), reflecting their role in binding to host-specific receptors and their importance in determining phage host range. The FBB1 genes encoding the short tail fiber protein (homolog of *gp12* in T4) and fibritin neck whisker protein (homolog of *wac* in T4) were particularly divergent among the *Tevenvirinae* examined, as seen with other T4-like phages (Comeau et al. 2007). The sequence variation for both proteins was most pronounced at the C-terminal ends (Figure 7). For the short tail fibers, this is the domain involved in host receptor recognition. For fibritin neck whisker proteins, this is the domain that interacts with the tail fibers (Comeau et al. 2007; Letarov and Krisch 2013). FBB1 also exhibits polymorphisms at the loci for the long tail fiber protein components; these include the proximal subunit (*gp34* homolog), and the hinge connector (*gp35* and *gp36* homologs) (Figure 7). FBB1 and Cronus

both lack a homolog to the *gp37* gene, which encodes an adhesin at the tip of the long tail fiber in T4. At least one of these proteins, the *gp36* homolog, is closely related to a *Pantoea* phage protein (Accession QDH49178), supporting the possibility that FBB1 utilizes the same receptor on both its *E. tracheiphila* and *Pantoea spp.* hosts.

FBB1 exhibits a few other notable differences in gene content from related phages. FBB1 lacks mobile elements such as *seg* and *mob* genes, which encode distinct classes of homing endonucleases and are especially numerous in the T4 genome. FBB1 also has two adjacent genes for capsid vertex proteins (Figure 6), which are proteins that form the capsid attachment site for the DNA packaging complex and later the tail. These may have arisen from a duplication event based their similarity (57% identity). The *Tevenvirinae* vary in the number of copies of this gene (Figure 7), which is perhaps related to their location at the ends of divergent transcripts.

FBB1 as a potential biocontrol agent.

We have identified a phage that shows promise for use in the biocontrol of the pathogen *E. tracheiphila* based on (1) its lifestyle as a virulent rather than lysogenic phage, as reflected in its strong relatedness to T4, a model virulent phage, (2) its ability to infect all of the *E. tracheiphila* strains examined, which collectively comprise the majority of *E. tracheiphila* strains that are currently in laboratory collections, (3) its ability to infect related bacteria that could be used as carrier bacteria in mixed inocula, (4) its tolerance to environmental stresses and thus potential fitness following application to plants, (5) its genetic potential to produce EPS depolymerase, which could enhance susceptibility of the pathogen in the xylem where *E. tracheiphila* is widely believed to produce EPS as a factor that promotes wilt, and (6) its apparent adaptation toward translational efficiency based on its high number of tRNA genes, which could increase its fitness in the field.

This work also highlights some challenges for biocontrol with FBB1. One challenge is the relatively low burst size in *E. tracheiphila*. This low burst size could limit its efficacy when applied alone, but co-administration with carrier bacteria could potentially amplify the phage following inoculation. A second challenge is the method of delivery, as the introduced phages likely need to reach the xylem for effective control, unless they are targeted for action within the pathogen vector, cucumber beetles. Strategies for targeting bacteriophages to the xylem of grapes have been developed (Das et al. 2015), but phage targeting to the xylem of vegetable crops or to insect vectors has not yet been explored.

Lastly, this work highlights interesting questions regarding the ecology of the phages, pathogens, and beetles. For example, in our sampling in July, we found phages in spotted cucumber beetles (*D. undecimpunctata*) but not in striped cucumber beetles (*A. vittatum*). In subsequent work in which we collected samples in September, however, we isolated at least one phage from a striped cucumber beetle (data not published). Do these beetle species exhibit inherent or seasonal differences in their acquisition of the pathogen, phage or their conduciveness to pathogen or phage multiplication or survival? Cells of *E. tracheiphila* have been detected in the foregut and at the junction of the midgut and hindgut of the beetles (Saalau Rojas et al. 2015) , but where do the phage localize within the beetles? How are phage acquired, how wide is their distribution among cucumber beetles, and what is the typical titer of phage in individual beetles? Do aspects of the biology of the pathogen, such as a transition from a planktonic to a biofilm state in the xylem, influence its susceptibility to phage? Do aspects of the pathogen biology influence the acquisition of phages or bacteria by beetles? The identification and characterization of FBB1 in this study provides a much-needed foundation to begin to

address these ecological questions and explore phage therapy for managing bacterial wilt of cucurbits.

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Table 1. Bacterial strains used in this study and susceptibility to infection by phage FBB1.

Genus and species	Strains	Lytic activity ^a	Ref ^b
<i>Erwinia tracheiphila</i> (<i>Et-melo</i> clade) ^c	BoCa4-1b, BoCu1-2, BoCu2-1, BoCu3-1b, BontCu, Cuke1IN, FCa2-3, FCu1-3, FCu3-3, FishCu1-5, FishCu3-1, GHM3-1, GrinCu, GuthCu, HCu, HCu1-4, HFCu, HFMusk, HM2-2, KYMusk, LamMusk1, LamMusk2, LIMusk2, LIMusk3, LIMusk1, MaMax, MBrut1, MBrut3, MBrut4, MBrut6, MBrut7, MCa1-1, MCa4-2, McM1-1, McM2-4, MDCuke, Musk1IN, OKDH1, OKMusk1, OKMusk2, OkMusk3, TedCu(10), TPINCu1, UnisCa1-5, UnisCu1-1, ZimmMusk	+	(all strains) 1
<i>Erwinia tracheiphila</i> (<i>Et-melo</i> clade) ^c	HCa1-5	+	2
<i>Erwinia tracheiphila</i> (<i>Et-melo</i> clade) ^c	SCR3	+	3
<i>Erwinia tracheiphila</i> (<i>Et-CI</i> clade) ^c	BHKY, BuffGH, GZ4, LICuke2, LISumSq1, MISpSq, NYAcSq1, NYAcSq2, NYZuch1, NYZuch2, PPHow1, ppHow2, ZittCuke1, ZittCuke2	+	(all strains) 1
<i>Erwinia amylovora</i>	Ea8R	--	4
<i>Erwinia</i> spp.	EhWHL9	--	5
<i>Enterobacter cloacae</i>	JL1157	--	6
<i>Escherichia coli</i>	MC4100	--	7
<i>Pantoea stewartii</i>	ESRif-9A	+	8
<i>Pantoea ananatis</i>	BRT98, BRT175	+	(both strains) 5
<i>Pantoea eucalypti</i>	299R	--	5
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	MIPM1	--	9
<i>Pseudomonas syringae</i> pv. <i>tomato</i>	DC3000	--	10
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	B728a	--	11
<i>Pseudomonas putida</i>	R20	--	12
<i>Pseudomonas fluorescens</i>	A506	--	4

^a The ability (+) or inability (--) of phage FBB1 to lyse the indicated bacterial strain(s).
^b 1, (Saalau Rojas et al. 2013); 2, (Nazareno and Dumenyo 2015); 3, (Saalau Rojas and Gleason 2012); 4, (Wilson and Lindow 1993); 5, (Nadarasah and Stavrinides 2014); 6, (Loper et al. 1993); 7, (Silhavy et al. 1984); 8, (Menelas et al. 2006); 9, GA Beattie; 10, (Moore et al. 1989); 11, (Loper and Lindow 1987); 12, (Colbert et al. 1993)
^c The clades of *E. tracheiphila* strains were defined by Shapiro et al. (2018)

Figure Captions

Figure 1. Morphology of the (A) plaques and (B) virion of the bacteriophage FBB1. For (A) *E. tracheiphila* strain BHKY was grown on KB medium. For (B) the phage was visualized using a 2% uranyl acetate negative stain and transmission electron microscopy. The scale bar represents 100nm.

Figure 2. One-step growth curve (A) and bacterial growth reduction curve (B) of FBB1 on *E. tracheiphila* stain BHKY in KB medium. For (A), the curve is representative of two replicate experiments. Values are the mean $\pm$ standard deviation (SD) ($n = 3$). For (B), BHKY cells were mixed with FBB1 at a multiplicity of infection (MOI) value of 0.01 and 0.1 and growth was monitored spectroscopically.

Figure 3. Effect of temperature (A), pH (B), and exposure to UV-C (C) and UV-A (D) on the stability of phage FBB1. Phage stocks (10^6 PFU/ml) were incubated for 1 h at the indicated temperatures (A) and pH value (B), or were exposed to UV-C (254 nm) or UV-A (365 nm) for the indicated times (C, D). The values shown are the mean $\pm$ SD of the phage titer when expressed relative to the phage titer at 25°C (A), pH 7 (B), and 0 min of exposure (C, D), with three replicate phage stocks examined for each condition.

Figure 4. A comparison of the genomes of *E. tracheiphila* phage FBB1 and its closest relative, the *E. amylovora* phage Cronus. The Cronus genome is the reference genome (innermost circle). The second and tracks represent the GC content (black) and GC skew (purple/green) of Cronus, respectively. The outerblue ring represents FBB1, with the color intensity proportional to the

BLASTn identity to Cronus (white is most dissimilar; blue is most similar). tRNA genes are shown in red.

Figure 5. Phylogenetic tree of FBB1 and Cronus phages relative to representative phages of the 11 named genera and unclassified phage in the *Tevenvirinae* subfamily of the *Myoviridae*. The tree was derived using the Genome-BLAST distance phylogeny method as implemented by VICTOR (Meier-Kolthoff and Göker 2017), with the results from the D6 formula within VICTOR shown.

Figure 6. Mauve alignment of selected *Tevenvirinae* phage genomes with highlighted differential features. The ProgressiveMauve alignment was performed with default seed size (15), with colors indicating locally collinear blocks (LCBs), and LCBs below the center line for a phage genome indicating an orientation in the inverse direction. Similarity profiles are shown within the LCBs, where the height of the color indicates the average level of conservation in that region across the five phages. The white boxes with black borders under the LCBs indicate the features annotated in GenBank, with boxes below the line representing genes transcribed from the reverse strand (primarily genes involved in host DNA degradation, nucleotide synthesis and modification, and DNA synthesis and repair), and those above the line transcribed in the forward direction (primarily structural genes for forming the head, neck, tail, and tail fibers). The highlighted features include red bars, which show positional and size differences in the tRNA loci, and triangles, which indicate the presence (solid) or absence (open) a gene. The indicated tail fiber genes are present in all of the phage but are polymorphic.

Figure 7. Variation in the genes and encoded proteins in the (A) short tail fiber and (B) long tail fiber loci. The numbers indicate % identity in amino sequence to the T4 homologs, with the % identity shared by FBB1 and Cronus shown in parentheses. Solid colors indicate regions of high sequence similarity among similarly colored genes, with patterns indicating regions of high similarity shared only by subsets of homologs, and open regions indicating sequences unique among the organisms shown. The genes are labeled using T4 gene names: *gp10* and *gp11*, baseplate wedge subunit and tail pin; *gp12*, short tail fiber protein; *wac*, fibritin neck whisker protein; *gp13*, neck protein; *gp34*, proximal subunit of long tail fiber; *gp35*, proximal hinge connector of long tail fiber; *gp36*, distal hinge connector of long tail fiber; *gp37*, distal subunit of long tail fiber (missing in FBB1 and Cronus); *gp38*, assembly catalyst of distal long tail fiber in T4 (only the CC31 and JS98 proteins encoded by genes in this position appear to be homologs); holin.

e-Xtra Table Titles

Supplemental Table 1. Annotation of FBB1 genome.

Figure 1. Morphology of the (A) plaques and (B) virion of the bacteriophage FBB1. For (A) *E. tracheiphila* strain BHKY was grown on KB medium. For (B) the phage was visualized using a 2% uranyl acetate negative stain and transmission electron microscopy. The scale bar represents 100nm.

82x40mm (300 x 300 DPI)

Figure 2. One-step growth curve (A) and bacterial growth reduction curve (B) of FBB1 on *E. tracheiphila* strain BHKY in KB medium. For (A), the curve is representative of two replicate experiments. Values are the mean $\pm$ standard deviation (SD) ($n = 3$). For (B), BHKY cells were mixed with FBB1 at a multiplicity of infection (MOI) value of 0.01 and 0.1 and growth was monitored spectroscopically.

165x46mm (300 x 300 DPI)

Figure 3. Effect of temperature (A), pH (B), and UV exposure to UV-C (C) and UV-A (D) on the stability of phage FBB1. Phage stocks (106 PFU/ml) were incubated for 1 h at the indicated temperatures (A) and pH value (B), or were exposed to UV-C (at 254 nm) or UV-A (365 nm) for the indicated times (C, D). The values shown are the mean $\pm$ SD of the phage titer when expressed relative to the phage titer at 25°C (A), pH 7 (B), and 0 min of exposure (C, D), with three replicate phage stocks examined for each condition.

127x95mm (300 x 300 DPI)

Figure 4. A comparison of the genomes of *E. tracheiphila* phage FBB1 and its closest relative, the *E. amylovora* phage Cronus. The Cronus genome is the reference genome (innermost circle). The second and third tracks represent the GC content (black) and GC skew (purple/green) of Cronus, respectively. The outermost blue ring represents FBB1, with the color intensity proportional to the BLASTn identity to Cronus (white is most dissimilar; blue is most similar). tRNA genes are shown in red.

106x99mm (300 x 300 DPI)

Figure 5. Phylogenetic tree of FBB1 and Cronus phages relative to representative phages of the 11 named genera and unclassified phage in the Tevenvirinae subfamily of the Myoviridae. The tree was derived using the Genome-BLAST distance phylogeny method as implemented by VICTOR (Meier-Kolthoff and Göker 2017), with the results from the D6 formula within VICTOR shown.

177x85mm (300 x 300 DPI)

Figure 6. Mauve alignment of selected Tevenvirinae phage genomes with highlighted differential features. The ProgressiveMauve alignment was performed with default seed size (15), with colors indicating locally collinear blocks (LCBs), and LCBs below the center line for a phage genome indicating an orientation in the inverse direction. Similarity profiles are shown within the LCBs, where the height of the color indicates the average level of conservation in that region across the five phages. The white boxes with black borders under the LCBs indicate the features annotated in GenBank, with boxes below the line representing genes transcribed from the reverse strand (primarily genes involved in host DNA degradation, nucleotide synthesis and modification, and DNA synthesis and repair), and those above the line transcribed in the forward direction (primarily structural genes for forming the head, neck, tail, and tail fibers). The highlighted features include red bars, which show positional and size differences in the tRNA loci, and triangles, which indicate the presence (solid) or absence (open) a gene. The indicated tail fiber genes are present in all of the phage but are polymorphic.

177x132mm (300 x 300 DPI)

Figure 7. Variation in the genes and encoded proteins in the (A) short tail fiber and (B) long tail fiber loci. The numbers indicate % identity in amino sequence to the T4 homologs, with the % identity shared by FBB1 and Cronus shown in parentheses. Solid colors indicate regions of high sequence similarity among similarly colored genes, with patterns indicating regions of high similarity shared only by subsets of homologs, and open regions indicating sequences unique among the organisms shown. The genes are labeled using T4 gene names: gp10 and gp11, baseplate wedge subunit and tail pin; gp12, short tail fiber protein; wac, fibritin neck whisker protein; gp13, neck protein; gp34, proximal subunit of long tail fiber; gp35, proximal hinge connector of long tail fiber; gp36, distal hinge connector of long tail fiber; g37, distal subunit of long tail fiber (missing in FBB1 and Cronus); gp38, assembly catalyst of distal long tail fiber in T4 (only the CC31 and JS98 proteins encoded by genes in this position appear to be homologs); holin.

177x43mm (300 x 300 DPI)

Supplemental Table 1. Annotation of FBB1 genome.

Locus tag	Start	Stop	Length	Strand	Product	T4 gene
FBp001	2142	13	2130	-	rIIA protector from prophage-induced early lysis	<i>rIIA</i>
FBp002	2363	2151	213	-	Hypothetical protein	
FBp003	2625	2419	207	-	Hypothetical protein	
FBp004	4470	2644	1827	-	DNA topoisomerase II large subunit	<i>gp39</i>
FBp005	4920	4540	381	-	Hypothetical protein	
FBp006	5213	4917	297	-	Hypothetical protein	
FBp007	5681	5286	396	-	Hypothetical protein	
FBp008	5865	5689	177	-	Hypothetical protein	
FBp009	6311	5874	438	-	Hypothetical protein	
FBp010	6514	6308	207	-	Hypothetical protein	
FBp011	7050	6616	435	-	Hypothetical protein	
FBp012	7219	7052	168	-	Hypothetical protein	
FBp013	7905	7219	687	-	Exonuclease	<i>dexA</i>
FBp014	8132	7902	231	-	Hypothetical protein	
FBp015	9470	8142	1329	-	DNA helicase	<i>dda</i>
FBp016	9766	9467	300	-	Hypothetical protein	
FBp017	10622	9768	855	-	Putative anti-sigma factor	<i>srd</i>
FBp018	11299	10622	678	-	Putative RNA polymerase ADP-ribosylase	<i>modA</i>
FBp019	11536	11345	192	-	Hypothetical protein	
FBp020	11658	11533	126	-	Hypothetical protein	
FBp021	11929	11705	225	-	Small outer capsid	<i>soc</i>
FBp022	12274	11957	318	-	Hypothetical protein	
FBp023	12797	12276	522	-	dCTP pyrophosphatase	<i>gp56</i>
FBp024	13143	12829	315	-	Hypothetical protein	
FBp025	14161	13145	1017	-	DNA primase	<i>gp61</i>
FBp026	14665	14198	468	-	Hypothetical protein	
FBp027	14858	14688	171	-	Hypothetical protein	
FBp028	15036	14869	168	-	Hypothetical protein	
FBp029	15448	15029	420	-	Hypothetical protein	
FBp030	15711	15445	267	-	Hypothetical protein	
FBp031	15992	15708	285	-	Hypothetical protein	
FBp032	16252	15989	264	-	Hypothetical protein	
FBp033	16611	16249	363	-	Hypothetical protein	
FBp034	16905	16690	216	-	Hypothetical protein	
FBp035	18352	16913	1440	-	DNA helicase	<i>gp41</i>
FBp036	18700	18362	339	-	Head vertex assembly chaperone	<i>gp40</i>
FBp037	19868	18690	1179	-	RecA-like recombination protein	<i>UvsX</i>
FBp038	20685	19942	744	-	dCMP hydroxymethylase	<i>gp42</i>
FBp039	20905	20699	207	-	Hypothetical protein	

FBp040	21355	20930	426	-	Hypothetical protein	
FBp041	21539	21363	177	-	Hypothetical protein	
FBp042	24310	21599	2712	-	DNA polymerase	<i>gp43</i>
FBp043	24601	24401	201	-	Hypothetical protein	
FBp044	24974	24603	372	-	Translational repressor of early genes	<i>regA</i>
FBp045	25552	24977	576	-	Clamp loader small subunit	<i>gp62</i>
FBp046	26511	25549	963	-	DNA polymerase clamp loader subunit	<i>gp44</i>
FBp047	27247	26552	696	-	DNA polymerase sliding clamp accessory protein	<i>gp45</i>
FBp048	27658	27290	369	-	RNA polymerase binding protein	<i>rpbA</i>
FBp049	27859	27662	198	-	Hypothetical protein	
FBp050	29567	27879	1689	-	Recombination-related endonuclease	<i>gp46</i>
FBp051	30583	29564	1020	-	Recombination-related endonuclease	<i>gp47</i>
FBp052	31175	30570	606	-	Hypothetical protein	
FBp053	31441	31310	132	-	Hypothetical protein	
FBp054	31566	31438	129	-	Hypothetical protein	
FBp055	31854	31669	186	-	Hypothetical protein	
FBp056	32045	31848	198	-	Hypothetical protein	
FBp057	32398	32042	357	-	Hypothetical protein	
FBp058	32627	32400	228	-	Hypothetical protein	
FBp059	33159	32617	543	-	RNA polymerase sigma factor for late transcription	<i>gp55</i>
FBp060	34696	33251	1446	-	Hypothetical protein	
FBp061	34820	34686	135	-	Hypothetical protein	
FBp062	35071	34820	252	-	Hypothetical protein	
FBp063	35367	35068	300	-	Hypothetical protein	
FBp064	35698	35345	354	-	Hypothetical protein	
FBp065	35859	35695	165	-	Hypothetical protein	
FBp066	36053	35856	198	-	Hypothetical protein	
FBp067	36379	36050	330	-	Hypothetical protein	
FBp068	36722	36438	285	-	Hypothetical protein	
FBp069	37067	36777	291	-	Hypothetical protein	
FBp070	37392	37138	255	-	Hypothetical protein	
FBp071	37721	37410	312	-	Glutaredoxin	<i>nrdH</i>
FBp072	37902	37708	195	-	Hypothetical protein	
FBp073	38000	37899	102	-	Hypothetical protein	
FBp074	38467	37997	471	-	Recombination endonuclease VII	<i>gp49</i>
FBp075	39001	38504	498	-	Hypothetical protein	
FBp076	39493	39122	372	-	Hypothetical protein	
FBp077	39642	39493	150	-	Hypothetical protein	
FBp078	39910	39626	285	-	Hypothetical protein	
FBp079	40194	39997	198	-	Hypothetical protein	
FBp080	40423	40181	243	-	Hypothetical protein	

FBp081	40735	40457	279	-	Thioredoxin	<i>nrdC</i>
FBp082	41253	40945	309	-	Hypothetical protein	
FBp083	41750	41250	501	-	Hypothetical protein	
FBp084	42829	41747	1083	-	Hypothetical protein (unique to FBB1)	
FBp085	43215	42961	255	-	Hypothetical protein	
FBp086	43916	43215	702	-	Hypothetical protein	
FBp087	44229	43909	321	-	Hypothetical protein	
FBp088	44472	44176	297	-	Hypothetical protein	
FBp089	44674	44453	222	-	Hypothetical protein	
FBp090	45511	44735	777	-	Hypothetical protein	
FBp091	46557	45574	984	-	Thioredoxin	
FBp092	46803	46630	174	-	Hypothetical protein	
FBp093	47142	46915	228	-	Hypothetical protein	
FBp094	48148	47144	1005	-	Thioredoxin	
FBp095	48369	48148	222	-	Hypothetical protein	
FBp096	48550	48362	189	-	Hypothetical protein	
FBp097	49094	48543	552	-	Hypothetical protein	
FBp098	49309	49091	219	-	Hypothetical protein	
FBp099	49772	49296	477	-	Hypothetical protein	
FBp100	49957	49772	186	-	Hypothetical protein	
FBp101	50265	49987	279	-	Hypothetical protein	
FBp102	50688	50269	420	-	Hypothetical protein	
FBp103	50844	50692	153	-	Hypothetical protein	
FBp104	51223	50939	285	-	Lysis inhibition regulator	<i>rI</i>
FBp105	51444	51232	213	-	Hypothetical protein	
FBp106	51814	51476	339	-	Hypothetical protein	
FBp107	52208	51846	363	-	Hypothetical protein	
FBp108	52920	52333	588	-	Thymidine kinase	<i>tk</i>
FBp109	53090	52920	171	-	Hypothetical protein	
FBp110	53266	53090	177	-	Hypothetical protein	
FBp111	53475	53263	213	-	Hypothetical protein	
FBp112	54032	53472	561	-	Hypothetical protein	
FBp113	54427	54029	399	-	Hypothetical protein	
FBp114	54741	54424	318	-	Hypothetical protein	
FBp115	55298	54738	561	-	Hypothetical protein	
FBp116	55767	55306	462	-	Site-specific RNA endonuclease	<i>regB</i>
FBp117	55945	55829	117	-	Hypothetical protein	
FBp118	56346	55942	405	-	Hypothetical protein	
FBp119	56606	56481	126	-	Hypothetical protein	
FBp120	57136	56606	531	-	Hypothetical protein	
FBp121	57694	57287	408	-	Endonuclease V, N-glycosylase UV repair enzyme	<i>denV</i>

FBp122	58241	57753	489	-	Tail-associated lysozyme
FBp123	58752	58288	465	-	Hypothetical protein
FBp124	59063	58749	315	-	Hypothetical protein
FBp125	59344	59060	285	-	Hypothetical protein
FBp126	59652	59341	312	-	Hypothetical protein
FBp127	60358	59675	684	-	Hypothetical protein
FBp128	60680	60345	336	-	Hypothetical protein
FBp129	60981	60673	309	-	Hypothetical protein
FBp130	61408	60974	435	-	Hypothetical protein
FBp131	61961	61395	567	-	Hypothetical protein
FBp132	62598	62002	597	-	Hypothetical protein
FBp133	62922	62662	261	-	Hypothetical protein
FBp134	63134	62922	213	-	Hypothetical protein
FBp135	63512	63144	369	-	Hypothetical protein
FBp136	64159	63905	255	-	Hypothetical protein
FBt001	64276	64205	72	-	tRNA-Gly-TCC
FBt002	64448	64378	71	-	tRNA-Trp-CCA
FBt003	64529	64457	73	-	tRNA-Thr-TGT
FBt004	64609	64537	73	-	tRNA-Arg-TCT
FBp137	65026	64646	381	-	Hypothetical protein
FBp138	65412	65026	387	-	Hypothetical protein
FBp139	65644	65414	231	-	Hypothetical protein
FBp140	66081	65641	441	-	Hypothetical protein
FBp141	66299	66081	219	-	Hypothetical protein
FBp142	66537	66310	228	-	Hypothetical protein
FBp143	66767	66534	234	-	Hypothetical protein
FBt005	66992	66921	72	-	tRNA-Met-CAT
FBp144	67471	67235	237	-	Major outer membrane lipoprotein
FBt006	67628	67557	72	-	tRNA-Met-CAT
FBp145	67972	67631	342	-	Hypothetical protein
FBt007	68073	67990	84	-	tRNA-Leu-TAA
FBt008	68208	68136	73	-	tRNA-Phe-GAA
FBp146	68456	68208	249	-	Hypothetical protein
FBt009	68626	68554	73	-	tRNA-Glu-TTC
FBt010	68810	68722	89	-	tRNA-Ser-TGA
FBt011	68904	68819	86	-	tRNA-Tyr-GTA
FBt012	68994	68913	82	-	tRNA-Leu-TAG
FBt013	69266	69191	76	-	tRNA-Ala-TGC
FBp147	69868	69266	603	-	Hypothetical protein
FBt014	69935	69862	74	-	tRNA-Pro-TGG
FBt015	70017	69945	73	-	tRNA-Ile-GAT
FBt016	70160	70087	74	-	tRNA-Lys-TTT
FBp148	70495	70208	288	-	Hypothetical protein

FBt017	70770	70698	73	-	tRNA-Asp-GTC	
FBt018	71011	70930	82	-	tRNA-Asn-GTT	
FBp149	71398	71195	204	-	Hypothetical protein	
FBt019	71809	71719	89	-	tRNA-Ser-GGA	
FBp150	71881	71456	426	-	Hypothetical protein	
FBt020	71975	71889	87	-	tRNA-Ser-GCT	
FBt021	72710	72638	73	-	tRNA-His-GTG	
FBt022	73034	72963	72	-	tRNA-Gln-TTG	
FBp151	73239	73063	177	-	Putative exported protein	
FBp152	73532	73311	222	-	Hypothetical protein	
FBp153	73926	73573	354	-	Hypothetical protein	
FBp154	74111	73923	189	-	Hypothetical protein	
FBp155	74296	74108	189	-	Hypothetical protein	
FBp156	74704	74402	303	-	Hypothetical protein	
FBp157	75175	74717	459	-	Hypothetical protein	
FBp158	75426	75175	252	-	Hypothetical protein	
FBp159	76130	75423	708	-	Deoxynucleoside monophosphate (dNMP) kinase	<i>gp1</i>
FBp160	76726	76133	594	-	Tail completion protein	<i>gp3</i>
FBp161	77667	76843	825	-	DNA end protector protein	<i>gp2</i>
FBp162	78116	77667	450	-	Head completion protein	<i>gp4</i>
FBp163	78163	78738	576	+	Baseplate wedge subunit	<i>gp53</i>
FBp164	78738	80489	1752	+	Baseplate hub structural protein and lysozyme R	<i>gp5</i>
FBp165	80482	80973	492	+	Hypothetical protein	<i>gp5.1</i>
FBp166	80974	81267	294	+	Hypothetical protein	<i>gp5.2</i>
FBp167	81267	83210	1944	+	Baseplate wedge subunit	<i>gp6</i>
FBp168	83207	86305	3099	+	Baseplate wedge initiator	<i>gp7</i>
FBp169	86298	87302	1005	+	Baseplate wedge subunit	<i>gp8</i>
FBp170	87373	88233	861	+	Baseplate wedge tail fiber connector	<i>gp9</i>
FBp171	88233	90047	1815	+	Baseplate wedge subunit and tail pin	<i>gp10</i>
FBp172	90047	90715	669	+	Baseplate wedge subunit and tail pin	<i>gp11</i>
FBp173	90715	92043	1329	+	Short tail fiber protein	<i>gp12</i>
FBp174	92053	93792	1740	+	Fibritin neck whisker protein	<i>wac</i>
FBp175	93822	94781	960	+	Neck protein	<i>gp13</i>
FBp176	94778	95548	771	+	Neck protein	<i>gp14</i>
FBp177	95585	96418	834	+	Tail sheath stabilizer and completion protein	<i>gp15</i>
FBp178	96420	96923	504	+	Terminase, small subunit	<i>gp16</i>
FBp179	96901	98724	1824	+	Terminase, large subunit	<i>gp17</i>
FBp180	98756	100741	1986	+	Tail sheath subunit	<i>gp18</i>
FBp181	100859	101350	492	+	Tail fibers protein	<i>gp19</i>
FBp182	101419	102987	1569	+	Portal vertex protein	<i>gp20</i>
FBp183	102987	103268	282	+	Prohead core protein	<i>gp67</i>

FBp184	103268	103693	426	+	Prohead core (capsid and scaffold) protein	<i>gp68</i>
FBp185	103693	104349	657	+	Prohead core scaffolding protein and protease	<i>gp21</i>
FBp186	104382	105200	819	+	Prohead assembly (scaffolding) protein	<i>gp22</i>
FBp187	105220	106773	1554	+	Major capsid protein	<i>gp23</i>
FBp188	106853	108139	1287	+	Capsid vertex protein	<i>gp24</i>
FBp189	108139	109422	1284	+	Capsid vertex protein	
FBp190	109462	110460	999	+	Tail fiber protein	
FBp191	110453	111292	840	+	Head outer capsid protein	<i>hoc</i>
FBp192	111854	111306	549	-	Hypothetical protein	
FBp193	113215	112187	1029	-	RNA ligase	<i>rnlB</i>
FBp194	113718	113314	405	-	Hypothetical protein	
FBp195	113959	113702	258	-	Hypothetical protein	
FBp196	114230	113961	270	-	Hypothetical protein	
FBp197	114411	114223	189	-	Hypothetical protein	
FBp198	114608	114408	201	-	Hypothetical protein	
FBp199	114925	114608	318	-	Hypothetical protein	
FBp200	117620	115020	2601	-	EPS depolymerase	
FBp201	118158	117631	528	-	Hypothetical protein	
FBp202	118898	118191	708	-	Inhibitor of gp21 prohead protease	<i>inh</i>
FBp203	118960	120465	1506	+	DNA helicase	<i>uvsW</i>
FBp204	120468	120842	375	+	Hypothetical protein	
FBp205	120829	121056	228	+	DNA helicase	
FBp206	121278	121111	168	-	Hypothetical protein	
FBp207	121527	121312	216	-	Hypothetical protein	
FBp208	121769	121524	246	-	Hypothetical protein	
FBp209	122178	121771	408	-	Recombination, repair and ssDNA-binding protein	<i>uvsY</i>
FBp210	122283	122185	99	-	Hypothetical protein	
FBp211	122781	122386	396	-	Baseplate wedge subunit	<i>gp25</i>
FBp212	123404	122778	627	-	Baseplate hub subunit	<i>gp26</i>
FBp213	123455	124207	753	+	Baseplate hub assembly catalyst	<i>gp51</i>
FBp214	124204	125361	1158	+	Baseplate hub subunit	<i>gp27</i>
FBp215	125315	125842	528	+	Baseplate hub. distal subunit	<i>gp28</i>
FBp216	125839	127620	1782	+	Baseplate hub subunit, tail length determinator	<i>gp29</i>
FBp217	127630	128679	1050	+	Baseplate tail tube cap	<i>gp48</i>
FBp218	128679	129638	960	+	Baseplate tail tube initiator	<i>gp54</i>
FBp219	130004	129663	342	-	Hypothetical protein	
FBp220	132149	130068	2082	-	ADP-ribosyltransferase	<i>alt</i>
FBp221	132398	132210	189	-	Hypothetical protein	
FBp222	133879	132398	1482	-	DNA ligase	<i>gp30</i>

FBp223	134177	133908	270	-	Hypothetical protein	
FBp224	135094	134180	915	-	Hypothetical protein	
FBp225	135317	135084	234	-	Hypothetical protein	
FBp226	135955	135557	399	-	Hypothetical protein	
FBp227	136164	135955	210	-	Hypothetical protein	
FBp228	136379	136161	219	-	Hypothetical protein	
FBp229	136543	136379	165	-	Hypothetical protein	
FBp230	136922	136554	369	-	Hypothetical protein	
FBp231	137348	136998	351	-	Hypothetical protein	
FBp232	137663	137466	198	-	Hypothetical protein	
FBp233	138020	137772	249	-	rIII lysis inhibition accessory protein	<i>rIII</i>
FBp234	138471	138142	330	-	Head assembly chaperone protein	<i>gp31</i>
FBp235	138830	138528	303	-	Hypothetical protein	
FBp236	139420	138854	567	-	dCMP deaminase	<i>cd</i>
FBp237	139743	139420	324	-	Hypothetical protein	
FBp238	139958	139743	216	-	Hypothetical protein	
FBp239	140251	139955	297	-	Hypothetical protein	
FBp240	140471	140259	213	-	Hypothetical protein	
FBp241	141447	140554	894	-	3'-phosphatase, 5'-polynucleotide kinase	<i>pseT</i>
FBp242	141667	141449	219	-	Hypothetical protein	
FBp243	141867	141664	204	-	Hypothetical protein	
FBp244	142598	141864	735	-	Hypothetical protein (specific to FBB1 and <i>Erwinia</i> phage Cronus)	
FBp245	142777	142595	183	-	Hypothetical protein	
FBp246	143095	142802	294	-	Hypothetical protein	
FBp247	143448	143092	357	-	Hypothetical protein	
FBp248	143942	143436	507	-	Inhibitor of host transcription	<i>alc</i>
FBp249	145159	144005	1155	-	RNA ligase A	<i>rnla</i>
FBp250	145344	145156	189	-	Hypothetical protein	
FBp251	145559	145341	219	-	Hypothetical protein	
FBp252	145972	145556	417	-	Endonuclease II	<i>denA</i>
FBp253	147144	146005	1140	-	Ribonucleotide reductase (aerobic), beta subunit	<i>nrdB</i>
FBp254	149424	147181	2244	-	Ribonucleotide reductase (aerobic), alpha subunit	<i>nrdA</i>
FBp255	149723	149412	312	-	Hypothetical protein	
FBp256	149952	149725	228	-	Hypothetical protein	
FBp257	150806	149949	858	-	Thymidylate synthase	<i>td</i>
FBp258	151105	150806	300	-	Hypothetical protein	
FBp259	151538	151083	456	-	Hypothetical protein	
FBp260	152079	151531	549	-	Dihydrofolate reductase	<i>frd</i>
FBp261	152471	152124	348	-	Hypothetical protein	
FBp262	152759	152538	222	-	Hypothetical protein	

FBp263	152911	152756	156	-	Hypothetical protein	
FBp264	153141	152914	228	-	Hypothetical protein	
FBp265	153416	153141	276	-	Hypothetical protein	
FBp266	153761	153477	285	-	Hypothetical protein	
FBp267	154788	153871	918	-	ssDNA binding protein	<i>gp32</i>
FBp268	155496	154840	657	-	DNA helicase loader	<i>gp59</i>
FBp269	155795	155493	303	-	Late promoter transcription accessory protein	<i>gp33</i>
FBp270	156048	155773	276	-	dsDNA binding protein, late transcriptional regulation	<i>dsbA</i>
FBp271	156230	156051	180	-	Hypothetical protein	
FBp272	157147	156230	918	-	Ribonuclease H	<i>rnh</i>
FBp273	157216	160965	3750	+	Long tail fiber (proximal subunit)	<i>gp34</i>
FBp274	160973	162103	1131	+	Hinge connector of long tail fiber (proximal connector)	<i>gp35</i>
FBp275	162204	164153	1950	+	Hinge connector of long tail fiber (distal connector)	<i>gp36</i>
FBp276	164187	164636	450	+	Long tail fiber assembly catalyst (distal)	<i>gp38</i>
FBp277	164669	165328	660	+	Holin lysis mediator	<i>t</i>
FBp278	165616	165341	276	-	Anti-sigma 70 factor	<i>asiA</i>
FBp279	165959	165690	270	-	Hypothetical protein	
FBp280	166523	166074	450	-	Hypothetical protein	
FBp281	166714	166523	192	-	Hypothetical protein	
FBp282	167046	166711	336	-	Hypothetical protein	
FBp283	167249	167043	207	-	Hypothetical protein	
FBp284	168403	167342	1062	-	Hypothetical protein	
FBp285	169467	168403	1065	-	UDP-galactopyranose mutase	
FBp286	170180	169548	633	-	Transcriptional activator of middle period transcription	<i>motA</i>
FBp287	170572	170306	267	-	Hypothetical protein	
FBp288	170954	170727	228	-	Hypothetical protein	
FBp289	172318	170951	1368	-	DNA topoisomerase	<i>gp52</i>
FBp290	172546	172349	198	-	Hypothetical protein	
FBp291	173007	172558	450	-	Nucleoid disruption protein	<i>npp</i>
FBp292	173483	173073	411	-	Hypothetical protein	
FBp293	173803	173480	324	-	Hypothetical protein	
FBp294	173913	173800	114	-	Hypothetical protein	
FBp295	174168	173971	198	-	Hypothetical protein	
FBp296	174912	174232	681	-	Hypothetical protein	
FBp297	175887	174952	936	-	rIIB protector from prophage-induced early lysis	<i>rIIB</i>