Isolation and Characterization of vB_PagP-SK1, a T7-Like Phage Infecting *Pantoea agglomerans*

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Abstract

Background: Pantoea is a genus within the *Enterobacterales* whose members encompass free-living and host-associated lifestyles. Despite our growing understanding of the role of mobile genetic elements in the biology, ecology, and evolution of this bacterial group, few *Pantoea* bacteriophages have been identified and characterized.

Materials and Methods: A bacteriophage that could infect *Pantoea agglomerans* was isolated from barnyard soil. We used electron microscopy and complete genome sequencing to identify the viral family, and evaluated its host range across 10 different *Pantoea* species groups using both bacterial lawn and phage lawn assays. The latter assays were carried out using a scalable microplate assay to increase throughput and enable spectrophotometric quantitation. We also performed a phylogenetic analysis to determine the closest relatives of our phage.

Results: Phage vB_PagP-SK1 belongs to the genus *Teseptimavirus* of the Podoviridae family in the order Caudovirales. The 39,938 bp genome has a modular structure with early, middle, and late genes, along with the characteristic direct terminal repeats of 172 bp. Genome composition and synteny were similar to that of the *Erwinia amylovora* phage, vB_EamP-L1, with the exception of a few loci that are most similar to genes of phage infecting other members of the *Enterobacteriaceae*. A total of 94 *Pantoea* strains were surveyed and vB_PagP-SK1 was found to infect 15 *Pantoea* strains across three species, predominantly *P. agglomerans*, along with one *Erwinia billingiae* strain.

Conclusions: vB_PagP-SK1 belongs to the *Teseptimavirus* genus and has a host range that spans *multiple species* groups, and is most closely related to the *E. amylovora* phage, vB_EamP-L1. The presence of xenologous genes in its genome indicates that the genome is a mosaic of multiple *Teseptimavirus* phages that infect members of the *Enterobacteriaceae*.

Keywords: *Teseptimavirus*, *Pantoea*, Podoviridae, host range, *Erwinia*, lytic

Introduction

PANTOEA IS A genus within the *Enterobacterales* whose members frequently form associations with plants and animals, often leading to disease in plant and animal hosts, as well as opportunistic infections in humans.[1–3] Strains of *Pantoea* have also been harnessed for a variety of biotechnological applications, including biocontrol, bioremediation, and therapeutic products.[4] Many of the genetic factors contributing to these capabilities, including specific genetic determinants as well as plasmids, have been identified and characterized.[5–7] Bacteriophages capable of infecting *Pantoea*, however, remain underexplored despite the importance of these mobile genetic elements in shaping the general biology, ecology, and evolution of bacteria.[8,9]

To date, very few bacteriophages capable of infecting species of *Pantoea* have been described and characterized. LIMEzero and LIMElight were isolated using *Pantoea agglomerans* as the host, and were assigned to the genus *Phikmvvirus* (PhiKMV-like viruses) in the Podoviridae family using both imaging approaches and genome analysis.[10] Host range assays of these phages using a selection of

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Pantoea and *Erwinia* strains showed that LIMEzero was only able to infect the *P. agglomerans* strain from which it was isolated, while LIMElight also infected a second *P. agglomerans* strain, LMG 2660.[10] No plaque formation was observed for either phage on overlays of *Erwinia amylovora* strain GBBC 403, *Erwinia mallotivora* strain LMG 1271, and *Pantoea stewartii* strains LMG 2717 and LMG 2719; however, some *Erwinia* phages have been reported to infect strains of *Pantoea*. Phage L1 and S2 (Podoviridae), identified as *E. amylovora* phage, are able to also infect representative strains of *P. agglomerans* and *Pantoea ananatis*.[11] The *E. amylovora* phage S10 and M7 (*Myoviridae*) infected only *P. ananatis* or *P. agglomerans*, respectively, while phage S7 (Podoviridae) exhibited an even broader host range, infecting not only *E. amylovora* but also *Erwinia billingiae*, *P. agglomerans*, *Pantoea vagans*, and *P. ananatis*.[11]

Here we report the isolation, characterization, and complete genome sequence of vB_PagP-SK1; a lytic bacteriophage that was identified as a member of the genus *Teseptimavirus* of the Podoviridae family in the order Caudovirales. vB_PagP-SK1 has a genome size of 39,938 bp and is most closely related to the *E. amylovora* phage vB_EamP-L1 despite having been isolated on a strain of *P. agglomerans*. Our host range assays revealed that vB_PagP-SK1 is capable of infecting 15 strains of *Pantoea* across three species groups along with one *E. billingiae* strain, suggesting that vB_PagP-SK1 is a broad host range phage.

Materials and Methods

Bacterial strains and culturing conditions

Bacterial strains (Table 1) were revived from −80°C glycerol stocks and cultured on lysogeny broth (LB) agar plates. Plates were incubated aerobically at 30°C for 24–48 h after which they were transferred to a 4°C fridge for storage. Log-phase liquid cultures were prepared by inoculating 5 mL LB tubes with a single colony and placing in a 220 rpm shaking incubator at 30°C for 12–18 h.

Phage isolation, amplification, and visualization

A 50 g soil sample taken from a barnyard near Craven, Saskatchewan, Canada, was gently agitated with 150 mL of deionized water for 30 min at room temperature. The mixture was then vacuum filtered using a Buchner funnel fitted with a glass fiber filter (934AH; Whatman, Reeve Angel). The filtered sample was transferred to sterile 50 mL conical tubes and centrifuged at 4000 *g* in a Sorvall ST16R centrifuge with a 3655-swinging bucket rotor for 20 min. The supernatant was filtered through a 0.22 *μ*m bottle filter (Nalgene) and 1 mL of the sample was spread onto an LB agar plate with a top agar overlay containing 100 *μ*L *P. agglomerans* SN01121 (OD$_{600nm}$ = 0.6), 1 mL of 1×LB, and 4 mL of 0.5% molten agar. The spread plate was incubated at 30°C for 24–72 h before plaque formation was scored. A single plaque was purified as per Sambrook and Russell,[12] and suspended in 1 mL of phage buffer (10 mM Tris-HCl pH 8, 10 mM MgSO$_4$, 150 mM NaCl) with 50 *μ*L of CHCl$_3$ and stored overnight at 4°C. The single plaque suspension was then diluted in 100-fold steps from 10^0 to 10^{-6} and standard top agar overlays were prepared with 10 *μ*L of phage suspension to determine which dilution reached 80–90% plaque confluence for amplification.

The isolated phage was amplified by preparing 30 top agar plates using the appropriate phage dilution. Plates were incubated at 30°C for 24 h and 5 mL of phage buffer was pipetted onto the surface of the plates. Plates were shaken gently for 1 h at room temperature to allow the phage to diffuse into the buffer. Phage buffer was then transferred from the plates to sterile 50 mL conical tubes and centrifuged at 4000 *g* for 20 min. Supernatant was then cleared with chloroform in accordance with Sambrook and Russell,[12] filtered through a 0.22 *μ*m polyethersulfone syringe filter (VWR International), and phage lysate titered. Aliquots of the high titer lysate were frozen with glycerol at −20°C and −80°C, and the remainder was stored at 4°C. Phage lysate was negatively stained by first applying a small drop of lysate onto carbonized formvar-coated grids (#FF300-CU-50; Electron Microscopy Sciences), and removing the excess liquid by blotting with filter paper. Staining was then performed by adding 2% (wt/vol) phosphotungstic acid (pH 6.8) containing ∼0.01% bovine albumin, and after a 10-s incubation, blotting to remove excess liquid. The phage was then imaged with a JEOL JEM-1011 transmission electron microscope using a Gatan-ES1000W Digital Camera at the Roy Romanow Provincial Laboratory.

DNA extraction

Phage genomic DNA was extracted using a modified zinc chloride phage precipitation protocol described by Santos.[13] High titer lysate (>1.0×10^9 PFU/mL) was cleared with chloroform as per Sambrook and Russell,[12] and 1.5 mL was added to a sterile 2 mL conical tube. DNAse I and RNAse I were added to final concentrations of 100 *μ*g/mL followed by incubation at 37°C for 30 min. Then, 30 *μ*L of sterile 2.0 M ZnCl$_2$ was added to the reaction and the mixture was incubated at 20°C for 5 min followed by centrifugation at 21,000 *g* on a Sorvall Legend Micro 21R centrifuge for 1 min. Supernatant was discarded, and the pellet was resuspended in 500 *μ*L of TES solution (0.1 M Tris-HCl pH 8, 0.1 M EDTA, 0.3% SDS) and incubated at 68°C for 20 min.

Subsequently, 90 *μ*L of 3 M potassium acetate pH 4.8 was added and the mixture was vortexed gently for 30 s followed by incubation on ice for 20 min. The debris was pelleted by centrifugation at 21,000 *g* for 1 min. Supernatant was transferred to a sterile 1.5 mL microfuge tube and an equal volume of absolute isopropanol was added. The solution was gently vortexed for 10 s followed by incubation on ice for 5 min. DNA was pelleted by centrifugation at 21,000 *g* for 10 min, and the pellet washed twice with 70% ethanol and allowed to air dry. DNA was resuspended in deionized water. Resuspended DNA was further purified by the use of an Omega Bio-tek E.Z.N.A. Cycle-Pure PCR cleanup kit.

Genome sequencing and in silico analysis

Library preparation was performed using the NEBNext Fast DNA Library Prep Set as per the manufacturer's recommended protocols. The phage sequencing library was then sequenced on an Ion PGM (Life Technologies) with 200 bp reads on an Ion 314 v2 chip. Ion Torrent average sequence coverage for vB_PagP-SK1 was 369-fold. The genome was assembled using the MIRA software suite (v3.9) on the Ion PGM server. Putative genes were identified using GeneMark.hmm[14] and genome maps generated with

TABLE 1. SUSCEPTIBILITY OF BACTERIAL STRAINS TO vB_PagP-SK1 USING BOTH THE BACTERIAL AND PHAGE LAWN METHODS

Genus/species	Isolate	Host/locale[a]	Location	Source	Bacterial lawn[b]	Phage lawn[c]	Control (OD600)	Phage (OD600)	Difference
Aeromonas sp.	SM02150	Lake water	Regina, SK, Canada	[1]	–	–	1.17	1.17	0.00
Erwinia amylovora	EA321	Hawthorn			–	na	Na	na	na
Erwinia billingiae	EhWHF18	Unidentified		George Sundin, Michigan State	+	+	1.25	0.48	–0.61
Escherichia coli	B/r	B strain, UV resistance		Gwyn Beattie, Iowa State	–	na	na	na	na
E. coli	OP50	B strain, uracil auxotroph			–	na	na	na	na
Kosakonia sp.	12202	Melon		ICMP	–	na	na	na	na
Mixta calida	B021323	Human, 28-year-old female, urine midstream	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	–	–	1.34	1.35	0.01
M. calida	BB957621-A1	Human, male, CAPD dialysate, peritonitis	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.47	1.34	–0.09
M. calida	BB957621-A2	Human, male, CAPD dialysate, peritonitis	Winnipeg, Canada	St. Boniface General Hospital	–	–	0.82	0.86	0.05
M. calida	BB957621-B1	Human, male, CAPD dialysate, peritonitis	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.37	1.44	0.05
M. calida	BB957621-B2	Human, male, CAPD dialysate, peritonitis	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.49	1.45	–0.02
M. calida	BB957621-C1	Human, male, CAPD dialysate, peritonitis	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.37	1.37	0.00
M. calida	BB957621-C2	Human, male, CAPD dialysate, peritonitis	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.58	1.40	–0.12
Pantoea agglomerans	240R	Pear flower	California, USA	Steven Lindow, UC Berkeley	–	–	0.54	0.48	–0.10
P. agglomerans	308R	Pear flower	California, USA	Steven Lindow, UC Berkeley	–	–	0.64	0.61	–0.04
P. agglomerans	3-770398	Human, female, blood	Toronto, Canada	Sunnybrook Hospital	+	+	1.47	0.62	–0.58
P. agglomerans	H42501	Human, male, blood	Toronto, Canada	Sunnybrook Hospital	–	–	1.41	0.85	–0.40
P. agglomerans	B015092	Human, 9-year-old female, urine midstream	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	–	–	0.83	1.23	0.49
P. agglomerans	B016395	Human, 83-year-old female superficial wound	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	–	–	0.47	0.47	0.00
P. agglomerans	B025670	Human, 13-year-old male, superficial wound	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	–	–	0.53	0.52	–0.04
P. agglomerans	B026440	Human, female, sputum, aortic aneurysm	Winnipeg, Canada	St. Boniface General Hospital	+	–	1.23	0.82	–0.34
P. agglomerans	Eh318	Apple leaf	Regina, SK, Canada	Brion Duffy	–	–	0.47	0.52	0.10
P. agglomerans	G4032547	Human, ear	Regina, SK, Canada	Regina General Hospital	+	+	1.41	0.66	–0.53
P. agglomerans	SN01080	Slug	Regina, SK, Canada	[1]	–	–	1.21	1.05	–0.13
P. agglomerans	SN01121	Bee	Regina, SK, Canada	[1]	+	+	1.27	0.68	–0.47
P. agglomerans	SN01122	Bee	Regina, SK, Canada	[1]	–	–	0.64	0.78	0.22
P. agglomerans	SN01170	Caterpillar	Regina, SK, Canada	[1]	–	–	1.32	0.83	–0.37

(continued)

TABLE 1. (CONTINUED)

Genus/species	Isolate	Host/locale[a]	Location	Source	Bacterial lawn[b]	Phage lawn[c]	Control (OD600)	Phage (OD600)	Difference
P. agglomerans	SP00202	Apple	Regina, SK, Canada	—	—	—	0.79	0.78	−0.01
P. agglomerans	SP00303	Raspberry	Regina, SK, Canada	—	+	+	1.28	0.72	−0.44
P. agglomerans	SP01202	Strawberry leaf and stem	Regina, SK, Canada	—	—	+	1.42	0.59	−0.58
P. agglomerans	SP01230	Virginia creeper leaves and stem	Regina, SK, Canada	—	—	—	1.41	0.84	−0.41
P. agglomerans	SP02022	Thistle	Regina, SK, Canada	—	+	—	0.50	0.46	−0.07
P. agglomerans	SP02230	Diseased tree leaf	Regina, SK, Canada	—	+	—	0.60	0.55	−0.08
P. agglomerans	SP02243	Tree	Regina, SK, Canada	—	—	+	1.12	0.62	−0.45
P. agglomerans	SP03310	Diseased maize leaf	Regina, SK, Canada	—	—	+	1.18	0.66	−0.44
P. agglomerans	SP03383	Diseased maize leaf	Regina, SK, Canada	—	—	—	1.32	0.89	−0.33
P. agglomerans	SP04011	Tomato leaf	Regina, SK, Canada	—	—	—	1.56	0.99	−0.36
P. agglomerans	SP04021	Tomato leaf	Regina, SK, Canada	—	—	—	1.32	1.17	−0.11
P. agglomerans	SP04022	Tomato leaf	Regina, SK, Canada	—	—	+	1.71	0.79	−0.54
P. agglomerans	SP05051	Tomato leaf	Regina, SK, Canada	—	+	+	0.93	0.35	−0.62
P. agglomerans	SP05052	Tomato leaf	Regina, SK, Canada	—	+	—	0.84	0.76	−0.09
P. agglomerans	SP05092	Tomato leaf	Regina, SK, Canada	—	—	—	0.58	0.53	−0.09
P. agglomerans	SP05120	Diseased maize leaf	Regina, SK, Canada	—	—	—	1.37	0.86	−0.38
P. agglomerans	SS02010	Soil, ground squirrel burrow	Regina, SK, Canada	—	—	—	0.48	0.44	−0.07
P. agglomerans	TX10	Human, sputum	Houston, Texas	Texas Children's Hospital	—	—	1.34	1.30	−0.03
P. agglomerans	DB522094	Human, elbow sore	Winnipeg, Canada	St. Boniface General Hospital	—	—	0.50	0.47	−0.06
P. agglomerans	M41864	Human, female, blood	Toronto, Canada	Sunnybrook Hospital	—	—	0.71	0.72	0.01
P. agglomerans	SM03214	Goose feces	Regina, SK, Canada	—	—	—	0.54	0.48	−0.12
P. agglomerans	BC594466A	Subhepatic abscess	Winnipeg, Canada	St. Boniface General Hospital	—	—	0.95	0.91	−0.04
P. agglomerans	BE528629	Human, peritoneal dialysis	Winnipeg, Canada	St. Boniface General Hospital	—	—	0.72	0.76	0.05
P. agglomerans	G0063668	Clinical	Regina, SK, Canada	Regina General Hospital	—	—	0.74	0.96	0.29
P. agglomerans	BB834250	Human, female, sputum, aortic aneurysm	Winnipeg, Canada	St. Boniface General Hospital	—	—	0.72	0.75	0.05
P. agglomerans	13301	Golden delicious apple	New Zealand	ICMP	+	—	0.44	0.43	−0.02
P. agglomerans	SP03412	Diseased bean leaf	Regina, SK, Canada	—	+	—	0.75	0.59	−0.21
P. agglomerans	BB350028B	Human, female, blood culture, fever	Winnipeg, Canada	St. Boniface General Hospital	+	—	0.67	0.59	−0.12
P. agglomerans	7373	Onion	South Africa	ICMP	+	—	0.48	0.39	−0.20
P. agglomerans	DC432	Maize		David Coplin, Ohio State	—	—	0.50	0.46	−0.08
P. agglomerans	DC556	Gypsophila (baby's breath)		David Coplin, Ohio State	—	—	0.57	0.46	−0.18
P. agglomerans	1574	Unidentified	Netherlands	ICMP	—	—	0.66	0.58	−0.12
P. agglomerans	12531	Gypsophila (baby's breath)	Netherlands	ICMP	—	—	0.77	0.55	−0.28
P. agglomerans	17124	Olive	Italy	ICMP	—	—	0.79	0.63	−0.20
P. agglomerans	83	Wheat	Italy	ICMP	—	—	0.79	0.71	−0.10

(continued)

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TABLE 1. (CONTINUED)

Genus/species	Isolate	Host/locale[a]	Location	Source	Bacterial lawn[b]	Phage lawn[c]	Control (OD600)	Phage (OD600)	Difference
P. agglomerans	5565	Soybean	New Zealand	ICMP	−	−	0.63	0.56	−0.10
P. agglomerans	1512	Green bean		ICMP	−	−	0.50	0.50	0.00
P. agglomerans	1373	Balsam	India	ICMP	−	−	0.65	0.52	−0.19
P. agglomerans	SP05130	Diseased maize stamen	Regina, SK, Canada[1]		−	−	0.86	0.71	−0.18
P. agglomerans	SP03190	Healthy tree leaf	Regina, SK, Canada[1]		−	−	0.94	0.93	−0.02
P. agglomerans	SP03392	Maize	Regina, SK, Canada		−	−	1.04	0.99	−0.05
P. agglomerans	09-1957-a	Human, renal failure	Winnipeg, Canada	St. Boniface General Hospital	−	−	0.45	0.49	0.10
Pantoea ananatis	15320	Rice	Australia	ICMP	−	−	na	na	na
P. ananatis	B7	Maize, rifR derivative of M232A	Wisconsin, USA	Steven Lindow, UC Berkeley	−	−	na	na	na
P. ananatis	BRT175	Strawberry	Brentwood, California	Gwyn Beattie, Iowa State	−	−	0.68	0.68	0.00
P. ananatis	BRT98	Strawberry	Brentwood, California	Gwyn Beattie, Iowa State	−	−	0.57	0.55	−0.03
P. ananatis	DC434	Maize		David Coplin, Ohio State	−	−	na	na	na
P. ananatis	17671	Rice	Cambodia	ICMP	−	−	0.68	0.64	−0.07
P. ananatis	LMG5342	Human, wound	Georgia	Teresa Coutinho, University of Pretoria	−	−	0.66	0.70	0.07
P. ananatis	LMG20103	Eucalyptus	South Africa	Teresa Coutinho, University of Pretoria	−	−	0.91	0.59	−0.35
P. ananatis	Cit30-11R	Naval orange leaf	California, USA	Steven Lindow, UC Berkeley	−	−	0.48	0.46	−0.04
P. ananatis	M232A	Maize	Wisconsin, USA	Steven Lindow, UC Berkeley	−	−	0.61	0.54	−0.12
Pantoea brenneri	09-1151	Human, posthemicholectomy	Winnipeg, Canada	St. Boniface General Hospital	−	−	1.32	1.30	−0.02
P. brenneri	B014130	Human, 11-year-old male superficial wound	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	+	+	1.28	0.63	−0.50
P. brenneri	B024858	Human, 26-year-old female, breast abscess	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	−	−	0.59	0.53	−0.10
Pantoea conspicua	B011017	Human, 11-year-old female superficial wound	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	−	−	0.68	0.63	−0.07
Pantoea dispersa	2-M11657A	Human, male, blood	Toronto, Canada	Sunnybrook Hospital	−	−	0.67	0.67	0.01
P. dispersa	2-M11657B	Human, male, blood	Toronto, Canada	Sunnybrook Hospital	−	−	0.45	0.46	0.02
P. dispersa	625	Sorghum	India	ICMP	−	−	0.48	0.49	0.02
Pantoea eucalypti	299R	Pear flower	California, USA	Steven Lindow, UC Berkeley	−	−	na	na	na
P. eucalypti	5-F9026	Human, male, blood	Toronto, Canada	Sunnybrook Hospital	−	−	0.83	0.89	0.07
P. eucalypti	B011489	Human, 52-year-old female, superficial wound	Saskatchewan, Canada	Roy Romanow Provincial Laboratory	−	−	0.57	0.59	0.03
P. eucalypti	SP02021	Thistle leaf	Regina, SK, Canada[1]		−	−	1.34	0.83	−0.38

(continued)

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TABLE 1. (CONTINUED)

Genus/species	Isolate	Host/locale[a]	Location	Source	Bacterial lawn[b]	Phage lawn[c]	Control (OD600)	Phage (OD600)	Difference
P. eucalypti	SP03372	Diseased maize leaf	Regina, SK, Canada	¹	–	–	1.39	1.37	–0.01
P. eucalypti	SP03391	Diseased bean leaf	Regina, SK, Canada	¹	–	–	0.54	0.54	0.01
P. eucalypti	SP04013	Tomato leaf	Regina, SK, Canada	¹	–	+	1.38	0.76	–0.45
Pantoea eucrina	06-868	Human	Winnipeg, Canada	St. Boniface General Hospital	–	–	0.91	0.92	0.01
P. eucrina	TX5	Human, blood	Houston, Texas	Texas Children's Hospital	–	–	0.76	0.93	0.22
P. eucrina	TX6	Human, blood	Houston, Texas	Texas Children's Hospital	–	–	0.73	0.89	0.21
Pantoea latae	TX3	Human, blood	Houston, Texas	Texas Children's Hospital	–	–	0.58	0.46	–0.20
Pantoea septica	06-2465-a	Human, cerebellar (stroke)	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.23	1.09	–0.11
P. septica	06-2465-b	Human, cerebellar (stroke)	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.35	1.30	–0.03
P. septica	10-1150	Human	Winnipeg, Canada	St. Boniface General Hospital	–	–	1.28	1.16	–0.09
P. septica	1-X44686	Human, female, blood	Toronto, Canada	Sunnybrook Hospital	–	–	1.35	1.38	0.02
P. septica	M1517	Human, female, blood	Toronto, Canada	Sunnybrook Hospital	–	–	0.69	0.66	–0.04
P. septica	B016375	Human, female, finger	Regina, SK, Canada	Roy Romanow Provincial Laboratory	–	–	1.31	1.05	–0.20
P. septica	BB350028A	Human, female, blood culture, fever	Winnipeg, Canada	St. Boniface General Hospital	–	–	0.99	0.86	–0.13
P. septica	G2291404	Human	Regina, SK, Canada	Regina General Hospital	+	–	1.35	1.37	0.02
P. septica	G4071105	Human, urine	Regina, SK, Canada	Regina General Hospital	–	–	0.86	0.63	–0.26
Pantoea stewartii	626	Maize	India	ICMP	–	–	0.73	0.73	0.01
Pseudomonas syringae	UnB647	Kidney bean		David Guttman, University of Toronto	–	na	na	na	na
P. syringae	TLP2	Nonpathogenic, healthy potato leaf		David Guttman, University of Toronto	–	na	na	na	na
Staphylococcus aureus	K1-7			Chris Yost, University of Regina	–	na	na	na	na
Streptococcus mutans	UAIS9:wt			Heather Dietz, University of Regina	–	na	na	na	na

[a]CAPD.
[b]Scored as susceptible (+) if plaque formation was observed on bacterial lawn.
[c]Scored as susceptible (+) if % change OD_{600} between Control (no phage) and +Phage was -2σ (less than –0.413).
CAPD, continuous ambulatory peritoneal dialysis.

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FIG. 1. **(A)** Top agar overlay showing plaque morphology of vB_PagP-SK1 on *Pantoea agglomerans* SN01121. Scale bar represents 1 cm. **(B)** Transmission electron micrographs of negatively stained vB_PagP-SK1. Scale bar represents 100 nm.
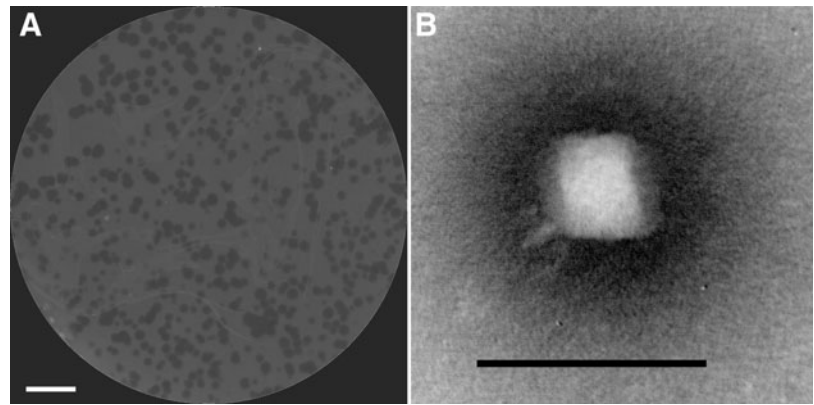
VectorNTI Advance v10 (Thermo Fisher). Sequences were compared with the PHASTER prophage and virus database[15] using standalone BLASTp, and to the NCBI nr database using BLASTx. Genome comparison was performed using progressiveMauve with default parameters.[16] Multi-locus sequence alignment (MLSA) was performed using the nucleotide sequence of four core genes; RNA polymerase, DNA polymerase, head-to-tail joining protein, and terminase large subunit. Bacteriophage phiKMV was used as an outgroup. The MLSA was performed with ClustalX2 using iteration after each alignment step.[17] A maximum likelihood tree was created in MEGAX using the maximum composite likelihood algorithm with complete gap deletion, 8 gamma rate categories, and 500 bootstrap replicates.[18] Pairwise alignments and dot plots were performed with the NCBI BLAST bl2seq tool. The full genome sequence of phage vB_PagP-SK1 has been deposited in GenBank under accession number MN450150.

Host range assays

Plaque formation was assessed using bacterial lawn overlays for each of the 94 strains of *Pantoea* and 17 strains from other genera. Diluted phage lysate (10 μL) that had been cleared with chloroform was mixed with 100 μL of log phase bacterial culture in 1 mL of 1 $\times$ LB and 5 mL of overlay agar at 40°C before being poured onto LB agar plates that had been held at room temperature. The overlay plates were incubated at 30°C for 24 h before plaque formation was scored. Phage lawn overlays were carried out in Greiner flat-bottomed 96-well microplates. Each well contained 250 μL of SM agar (1 L: 10 g glucose, 10 g peptone, 1 g yeast extract, 0.5 g $MgSO_4$ monohydrate, 1.9 g KH_2PO_4, 0.6 g K_2HPO_4, and 20 g agar). Once solidified, 3.6 μL of 1.87×10^8 PFU/mL phage lysate was added to each well and excess moisture was allowed to evaporate. The plates were inoculated with 5 μL of liquid bacterial culture at an optical density (OD) 600 nm of 0.6 ($\sim 1.0 \times 10^9$ cfu/mL). Controls for each isolate consisted of 3.6 μL of sterile phage buffer instead of phage lysate. Plates were incubated for 24 h at 30°C and then refrigerated for 24–48 h at 4°C. OD for each well on the microplates was read using a Biotek Gen5 microplate reader using endpoint scan at a wavelength of 600 nm, and each reading was standardized using the average OD value from the wells of a 96-well plate containing 250 μL of SM agar only. Strains showing a reduction in OD of $\geq 2\sigma$ relative to control were scored as susceptible.

Results

Phage isolation and morphology

Filtered supernatants taken from washed barnyard soil were mixed with a single target strain, *P. agglomerans* SN01121 (SN01121), in a standard bacterial lawn overlay assay. A single plaque was then isolated, amplified, and retested on a lawn of SN01121, resulting in the formation of 2 mm clear plaques with no halos (Fig. 1A). Imaging of the phage lysate by transmission electron microscopy revealed a phage that appeared to have an isometric icosahedral capsid ~ 60 nm in diameter, a short noncontractile tail, and multiple tail fibers, consistent with the members of the Podoviridae family (Fig. 1B). The phage was named vB_PagP-SK1.

Genome analysis

DNA sequencing of vB_PagP-SK1 revealed a genome of 39,938 bp with 44 predicted open reading frames flanked by direct terminal repeats of 172 bp (Table 2). The organization of putative early, middle, and late genes was consistent with that of other members of *Teseptimavirus*[11] (Fig. 2). The early genomic region consists of genes required to initiate an infection,[19] and includes an *S*-adenosyl-L-methionine hydrolase (SAMase), protein kinase, phage RNA polymerase, and phage DNA ligase, which are followed by a predicted T7 early transcription terminator. The middle genomic region consists of bacterial RNA polymerase inhibitor, DNA metabolism genes, and phage DNA replication genes. The late genomic region consists of phage structural proteins, DNA packaging genes, and the holin and endopeptidase lysis-associated genes. Several hypothetical genes are predicted throughout the genome, which have weak hits to phage from other species, including *Citrobacter*, *Cronobacter*, *Pseudomonas*, and *Stenotrophomonas* (Table 2). vB_PagP-SK1 shares 88% sequence coverage and 94% identity with vB_EamP-L1 at the nucleotide level. A MAUVE comparison of vB_PagP-SK1 and vB_EamP-L1 highlights this high-sequence identity between these phages, with the exception of the SAMase, gp0.65, protein kinase, type II holin, the carboxyl-terminal domain of gp17 (tail fiber/EPS depolymerase), and several of the predicted hypothetical genes that are less than 300 bp (Fig. 2 and Table 2).

A phylogenetic analysis was carried out on vB_PagP-SK1 and related *Teseptimavirus* genomes (Table 3) using the concatenated amino acid sequences of the RNA polymerase,

TABLE 2. ANNOTATION OF PREDICTED GENES OF *PANTOEA* PHAGE vB_PAGP-SK1

| Gene[a]	CDS position	Strand	Function	Best blast/PHASTER hit	Accession number	E-value
MF01	1..172		5′ Direct terminal repeat			
1	928..1401	(+)	*S*-adenosyl-L-methionine hydrolase	*Klebsiella* phage K5	NC_028800	1.50E-42
2	1794..1976	(+)	gp0.65	*Erwinia* phage vB_EamP-L1	NC_019510	1.00E-16
3	2101..3306	(+)	Protein kinase	*Stenotrophomonas* phage IME15	YP_006990206.1	4.16E-68
4	3368..6037	(+)	RNA polymerase	*Erwinia* phage vB_EamP-L1	YP_007005430.1	0
5	6843..7000	(+)	Hypothetical head protein	EBPR podovirus 2	AEI70915.1	1.00E+00
6	7070..8083	(+)	DNA ligase	*Erwinia* phage vB_EamP-L1	YP_007005433.1	0
MF02	8093..8120		T7 early terminator			
7	8106..8243	(+)	Hypothetical phage protein	*Citrobacter* phage CR8	CDM21618.1	1.20E-01
8	8773..9093	(+)	gp1.65	*Erwinia* phage vB_EamP-L1	YP_007005436.1	6.78E-57
9	9093..9209	(+)	Hypothetical protein GAP227 28	*Cronobacter* phage Dev_CD_23823	NC_029070	5.47E-05
10	9206..9361	(+)	Bacterial RNAP inhibitor	*Erwinia* phage vB_EamP-L1	YP_007005437.1	1.12E-17
11	9444..10154	(+)	ssDNA binding protein	*Erwinia* phage vB_EamP-L1	YP_007005438.1	2.61E-129
12	10154..10606	(+)	Endonuclease	*Erwinia* phage vB_EamP-L1	YP_007005439.1	4.73E-106
13	10606..11055	(+)	Lysozyme	*Erwinia* phage vB_EamP-L1	YP_007005440.1	1.41E-106
14	11247..12836	(+)	DNA primase/helicase	*Erwinia* phage vB_EamP-L1	YP_007005441.1	0
15	12948..13160	(+)	gp4.3	*Erwinia* phage vB_EamP-L1	YP_007005444.1	3.94E-38
16	13265..13450	(+)	gp4.5	*Erwinia* phage vB_EamP-L1	YP_007005445.1	1.62E-27
17	13504..15630	(+)	DNA polymerase	*Erwinia* phage vB_EamP-L1	YP_007005446.1	0
18	15640..15861	(+)	Hypothetical protein	*Cronobacter* phage Dev2	CDM12546.1	4.00E-04
19	15851..16171	(+)	Hypothetical protein gp5.5	*Klebsiella* phage vB_KpnP_KpV289	NC_028977	6.71E-30
20	16234..16377	(+)	gp5.7	*Erwinia* phage vB_EamP-L1	YP_007005448.1	7.01E-22
21	16386..16664	(−)	Hypothetical protein I7C 035c	*Pseudomonas* phage MR299-2	AFD10713.1	4.70E+00
22	16744..17652	(+)	Exonuclease	*Erwinia* phage vB_EamP-L1	YP_007005450.1	0
23	17649..17753	(+)	gp6.3	*Erwinia* phage vB_EamP-L1	YP_007005451.1	5.29E-07
24	17852..18097	(+)	gp6.5	*Erwinia* phage vB_EamP-L1	YP_007005452.1	1.01E-54
25	18102..18338	(+)	gp6.7	*Erwinia* phage vB_EamP-L1	YP_007005453.1	9.26E-50
26	18325..18597	(+)	gp7.3	*Erwinia* phage vB_EamP-L1	YP_007005454.1	1.46E-55
27	18611..20221	(+)	Head-to-tail joining protein	*Erwinia* phage vB_EamP-L1	YP_007005455.1	0
28	20273..21238	(+)	Capsid assembly protein	*Erwinia* phage vB_EamP-L1	YP_007005456.1	0
29	21423..22463	(+)	Capsid protein	*Erwinia* phage vB_EamP-L1	YP_007005458.1	0
30	22481..22588	(+)	Hypothetical protein	*Stenotrophomonas* phage IME15	YP_006990234.1	8.00E-06
31	22751..23335	(+)	Tail tubular protein A	*Erwinia* phage vB_EamP-L1	YP_007005459.1	4.23E-142
32	23354..25753	(+)	Tail tubular protein B	*Erwinia* phage vB_EamP-L1	YP_007005461.1	0
33	25823..26236	(+)	Tail internal virion protein A	*Erwinia* phage vB_EamP-L1	YP_007005462.1	4.51E-100
34	26248..26829	(+)	Tail internal virion protein B	*Erwinia* phage vB_EamP-L1	YP_0070705463.1	5.20E-135
35	26841..29102	(+)	Tail internal virion protein C	*Erwinia* phage vB_EamP-L1	YP_007005464.1	0
36	29117..33115	(+)	Tail internal virion protein D	*Erwinia* phage vB_EamP-L1	YP_007005465.1	0
37	33177..35675	(+)	gp17 tail fiber - EPS depolymerases	*Erwinia* phage vB_EamP-L1	YP_007005466.1	0
38	35680..35886	(+)	gp17.5 (type II holin)	Enterobacteria phage BA14	YP_002003494.1	5.10E-33
39	35879..36136	(+)	Terminase small subunit	*Erwinia* phage vB_EamP-L1	YP_007005468.1	2.80E-52
40	36239..36703	(+)	Endopeptidase	*Erwinia* phage vB_EamP-L1	YP_007005469.1	2.51E-107
41	36705..37385	(+)	gp18.9	*Erwinia* phage vB_EamP-L1	YP_007005471.1	2.94E-152
42	37397..39157	(+)	Terminase large subunit	*Erwinia* phage vB_EamP-L1	YP_007005472.1	0
43	39419..39565	(+)	gp19.5	*Erwinia* phage vB_EamP-L1	YP_007005475.1	1.20E-26
MF03	39767..39938		3′ Direct terminal repeat			

[a]MF

MF, miscellaneous feature.

DNA polymerase, head-to-tail joining protein, and terminase large subunit (genes 4, 17, 27, 43). The resulting phylogeny, rooted on bacteriophage phiKMV (*Phikmvvirus*, a sister genus to *Teseptimavirus*), places vB_PagP-SK1 and vB_EamP-L1 in their own lineage among phages that infect members of mostly the *Enterobacterales* (Fig. 3).

Host range

The host range of vB_PagP-SK1 was evaluated against 94 strains of *Pantoea* representing 10 known species using a bacterial lawn overlay method. A total of 15 strains were found to be susceptible (Table 1). In addition to the environmental
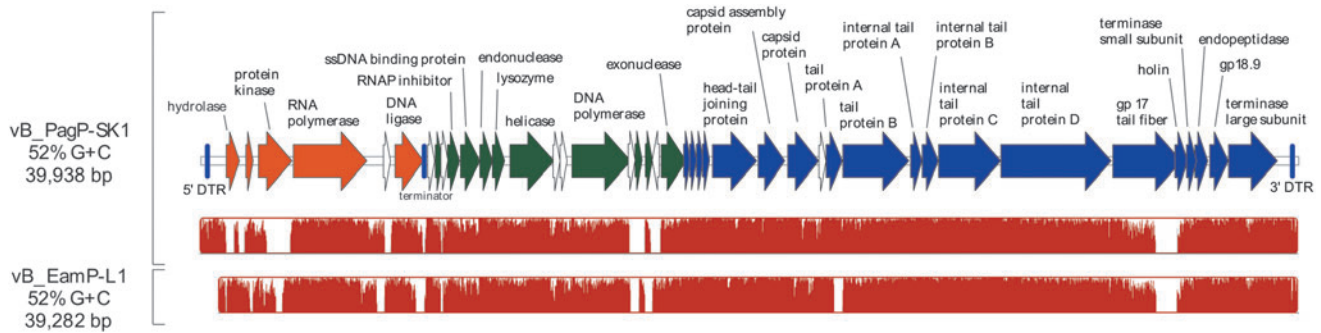
FIG. 2. Genomic organization of vB_PagP-SK1 with predicted early (orange), middle (green), and late (blue) genes. Genes without shading (white) are predicted hypothetical genes with weak hits to other phages, and miscellaneous features are indicated with a purple vertical line. The lower panel shows the results of a Mauve analysis[16] comparing the sequence identity between vB_PagP-SK1 and the *Erwinia amylovora* phage, vB_EamP-L1.

strain SN01121, which was used as the original strain to identify and enrich the phage, 12 other *P. agglomerans* strains were found to be susceptible, including 4 clinical and 8 environmental strains (Table 1). Outside of the *P. agglomerans* group, one of three *Pantoea brenneri* strains and one of nine *P. septica* strains tested were also susceptible. The closely related *E. billingiae* was susceptible, while *E. amylovora* was found to be resistant. All six tested strains of *Mixta calida*, another close relative of *Pantoea*, were resistant (Table 1). Also resistant were the other included enterics,

two *Escherichia coli* strains and a single *Kosakonia cowanii* strain, along with the nonenteric gram-negative bacteria, *Aeromonas* and *Pseudomonas*, and the gram-positive *Streptococcus* and *Staphylococcus* strains (Table 1).
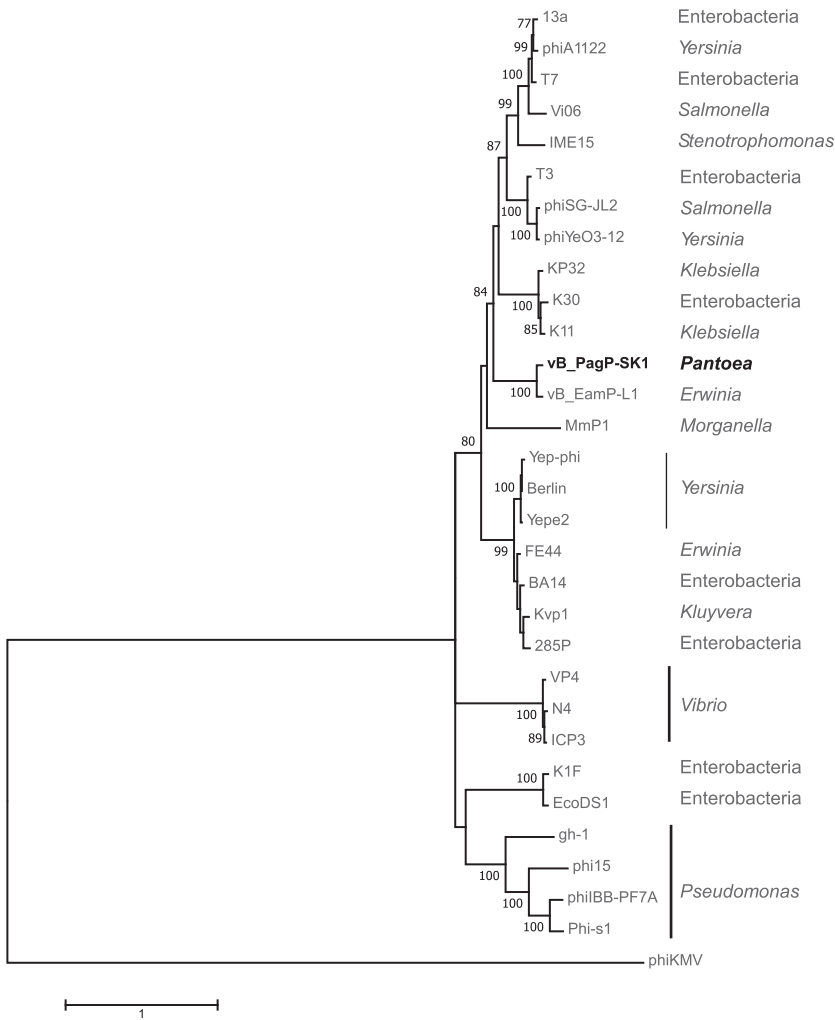
We then evaluated host range using a phage lawn, which was used by Luria and Delbruck[20] to assess the number of resistant bacteria in their populations. This method has the advantage of being more efficient for identifying host range as many strains can be tested simultaneously, and it was expected to recover similar results as the standard bacterial lawn overlay assay. In this assay, we first applied phage to the agar surface in 96-well microplates, and then applied bacteria over the phage lawn. Susceptibility was scored following a spectrophotometric comparison of bacteria with and without phage. Using this assay, 12 strains had a reduction in OD_{600} of more than 0.413 (-2σ) between the no-phage bacterial control and bacteria that had been exposed, and were therefore scored as susceptible (Table 1). Of the 16 strains scored as susceptible by the traditional bacterial lawn method, 7 were also susceptible by the phage lawn method (*E. billingiae*, *P. brenneri* B014130, and *P. agglomerans* strains 3-770398, G4032547, SN01121, SP00303, and SP05051) (Table 1).

Discussion

A T7-like phage, vB_PagP-SK1, capable of infecting *P. agglomerans* SN01121 was isolated from barnyard soil. Imaging using TEM highlighted an icosahedral capsid, short tail, and multiple tail fibers that are characteristic of the members of the Podoviridae (Fig. 1A). Genomic analysis revealed the absence of an integrase or other lysogeny-related genes, suggesting that vB_PagP-SK1 is strictly a lytic phage.[21] Our first host range assay used the bacterial lawn overlay method, which identifies those strains in which vB_PagP-SK1 can successfully initiate infection and produce viral progeny. This approach identified 16 susceptible strains, the majority being *P. agglomerans*, along with 1 *P. brenneri*, *P. septica*, and *E. billingiae* strain (Table 1). Although vB_PagP-SK1 was initially identified as a phage of *P. agglomerans*, it did not infect most *P. agglomerans* strains indicating that vB_PagP-SK1 may not be a strict *P. agglomerans* phage. This is supported by the fact that the genome of vB_PagP-SK1 shared high identity with the

TABLE 3. *TESEPTIMAVIRUS* BACTERIOPHAGE GENOMES USED FOR PHYLOGENETIC AND COMPARATIVE GENOMIC ANALYSES

Phage	Accession number
Enterobacteria phage 13a	NC_011045.1
Enterobacteria phage 285P	NC_015249.1
Enterobacteria phage BA14	NC_011040.1
Enterobacteria phage EcoDS1	NC_011042.1
Enterobacteria phage K1F	NC_007456.1
Enterobacteria phage K30	NC_015719.1
Enterobacteria phage T7	NC_001604.1
Erwinia phage FE44	NC_022744.1
Erwinia phage vB_EamP-L1	NC_019510.1
Klebsiella phage K11	NC_011043.1
Klebsiella phage KP32	NC_013647.1
Kluyvera phage Kvp1	NC_011534.1
Morganella phage MmP1	NC_011085.3
Pseudomonas phage gh-1	NC_004665.1
Pseudomonas phage phi15	NC_015208.1
Pseudomonas phage phiKMV	NC_005045.1
Pseudomonas phage phiIBB-PF7A	NC_015264.1
Pseudomonas phage Phi-S1	NC_021062.1
Salmonella phage phiSG-JL2	NC_010807.1
Salmonella phage Vi06	NC_015271.1
Stenotrophomonas phage IME15	NC_019416.1
Vibrio phage ICP3	NC_015159.1
Vibrio phage N4	NC_013651.1
Vibrio phage VP4	NC_007149.1
Yersinia pestis phage phiA1122	NC_001604.1
Yersinia phage Berlin	NC_008694.1
Yersinia phage phiYeO3-12	NC_001271.1
Yersinia phage Yepe2	NC_011038.1
Yersinia phage Yep-phi	NC_023715.1

FIG. 3. Maximum likelihood MLSA phylogeny of the Teseptimavirus group using concatenated nucleotide sequence of the RNA polymerase, DNA polymerase, head-to-tail joining and terminase genes. The phylogeny was constructed in MEGAX using maximum composite likelihood, 8 gamma categories, and complete gap deletion with 500 bootstrap replicates. MLSA, multilocus sequence alignment.

Erwinia phage, vB_EamP-L1, and the fact that the host range of vB_PagP-SK1 encompassed *E. billingiae*. This suggests that vB_PagP-SK1 is a phage of *Erwinia* that may transiently infect select *Pantoea* strains. The vB_EamP-L1 phage host range had been shown to span a large number of *E. amylovora* strains, although the *E. billingiae* and *Erwinia persicina* strains tested by the authors were resistant.[11] The authors also showed that the host range of vB_EamP-L1 included one *P. agglomerans* and one *P. ananatis* strain, although the *P. vagans* strain was resistant.[11]

The genomes of vB_PagP-SK1 and vB_EamP-L1 shared extensive conservation, but contained multiple variable regions (Fig. 2). Many of these regions corresponded to genes that have been implicated in host specificity and phage–host interactions, including the SAMase, gp0.65, protein kinase, type II holin, and the carboxyl-terminal domain of gp17 (tail fiber/EPS depolymerase).[22–27] SAM hydrolases are responsible for inactivating host restriction enzymes thereby bypassing restriction enzyme-mediated host defence mechanisms.[26] Protein kinases (gp0.7) are responsible for inactivation of host RNAse E that can degrade viral mRNA, and for inactivation of the protein CasB of the host CRISPR defence mechanism.[24,25] Homologues of the gene, gp5.5 (gene 19), have been shown to affect the nucleoid-associated protein

H-NS, which is a bacterial defence mechanism against foreign genetic material, including phage.[22]

By disrupting H-NS, silencing of exogenous DNA is disrupted allowing transcription of phage genes to continue unrestricted.[22] Type II holins (gene 39, gp17.5) are responsible for the timed permeability of the cellular membrane to endopeptidase or other lysis proteins resulting in the degradation of the cell wall and subsequent lysis of the host bacteria.[27] The carboxyl-terminal domain of gp17 is responsible for binding with host lipopolysaccharide.[23] The variability of these specific regions may modify the host range of vB_PagP-SK1 to encompass other species and/or genera.

We also identified several predicted hypothetical genes in vB_PagP-SK1 that were not found in vB_EamP-L1, but have been identified in phage infecting other members of the *Enterobacteriaceae*, *Xanthomonadaceae*, and *Pseudomonadaceae*,[28] including *Cronobacter*, *Citrobacter*, *Pseudomonas*, and *Stenotrophomonas* (Table 1). This suggests that vB_PagP-SK1 is a mosaic of vB_EamP-L1 and other closely related phage species of *Teseptimavirus*, having exchanged specific genetic determinants throughout its genome. The host range of this phage may therefore extend to other members of the *Enterobacteriaceae*. This is consistent with a recent comparative genomics study of 60 *Erwiniaceae* phage

genomes, which found considerable genomic variation and a large proportion of phage proteins with an unknown function.[29]

The plaque morphologies of vB_PagP-SK1 and vB_EamP-L1 were markedly different. The plaques produced by vB_PagP-SK1 lacked secondary halos that had been described for vB_EamP-L1.[11] Halos are usually caused by a diffusible enzyme, such as EPS depolymerase,[30,31] although both vB_PagP-SK1 and vB_EamP-L1 carry a predicted EPS depolymerase/tail fiber protein (gp17). The predicted EPS depolymerase/tail fiber protein (gp17) of the two phages share conservation over the first 60% of their ~2.5 kb nucleotide sequence, with the 3′ end of the gene being divergent. This variability may result in an EPS depolymerase enzyme that has reduced diffusibility or a reduced specificity toward the EPS capsule of the bacterial strains that were evaluated.

We carried out the phage lawn assay of Luria and Delbruck, as carried out in their 1943 landmark article in which they evaluated the number of phage-resistant bacteria in their populations.[20] This method has the advantage of being more efficient for identifying host range as many strains can be tested simultaneously on a single phage lawn, as opposed to using a single plate per strain; however, we found that only 7 of the original 16 strains identified as susceptible by the standard bacterial lawn method were susceptible by the phage lawn method, along with four additional *P. agglomerans* and one *Pantoea eucalypti* strain that were not identified by the bacterial lawn method (Table 1). These discrepancies may be due to lysis of normally resistant bacteria caused by phage-encoded exopolysaccharide depolymerases in phage lysates, or the presence of enzymes, antibiotics, or bacteriocins in phage lysates, which were produced by the original bacterial host.

It is also possible that in some cases, lysis was caused by ''virion-mediated lysis from without,'' a phenomenon through which high concentrations of phage adsorbing to bacterial surfaces can induce sufficient damage to the cell wall, even though there is no successful infection.[32,33] Lysis of resistant bacteria was also reported in spot testing assays with the phage LIMElight on *P. stewartii* LMG 2717, *P. stewartii* LMG 2719, *E. amylovora* GBBC 403, and *E. mallotivora* LMG 1271, all of which were resistant in standard bacterial lawn overlay assays.[10] Given the relatively small proportion of strains that were scored as susceptible with either method, the host range of this phage within *Pantoea* is relatively narrow.

Conclusion

We have characterized the bacteriophage vB_PagP-SK1, which belongs to the *Teseptimavirus* genus and was initially purified as a *P. agglomerans* phage. Our host range analyses suggest that vB_PagP-SK1 is capable of infecting multiple *Pantoea* species along with strains *Erwinia*. Our genomic analysis indicated that vB_PagP-SK1 most closely resembles the *Erwinia* phage vB_EamP-L1, even though the host ranges appear to be slightly different. The presence of xenologous genes in the vB_PagP-SK1 genome originating from phage that infects a breadth of genera indicates that it may be a mosaic of vB_EamP-L1 and other phages that infect members of the *Enterobacteriaceae*, which may be impacting host range.

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