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Isolation and pathogenicity of *Ralstonia solanacearum* in tomato and potential of phage BHDTSo81 against the pathogen

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ABSTRACT: The bacterial wilt disease in tomato caused by *Ralstonia solanacearum* results in harvest losses of up to 90% and significant economic loss to farmers. In this study, 11 *R. solanacearum* strains were isolated in the largest tomato-growing province in Vietnam. These strains belonged to 3 different groups, and 9/11 strains showed different disease rates in experimental tomato plants. A BHDTSo81 phage specific to *R. solanacearum* was isolated from a soil sample. Morphological analysis indicated that BHDTSo81 had podovirus morphology and was classified into the Autographiviridae family. The latent period and burst size of BHDTSo81 were calculated to be approximately 145 min and 8.6 ± 1.8 virions per infected cell, respectively. In a test of 26 bacterial strains, BHDTSo81 infected 7/11 *R. solanacearum* strains, while none of the other bacteria tested were susceptible to the phages. *R. solanacearum* was also challenged *in vitro* and was inactivated by BHDTSo81 for 40 h in broth. The genome of BHDTSo81 is 41,296 bp long with a total GC content of 63% and contains 46 predicted protein-encoding CDSs (coding sequences). No virulence factor or antibiotic resistance gene was found in the genome. Thus, the initial characteristics of phage BHDTSo81 indicate its potential utility as a control agent against *R. solanacearum*.

KEYWORDS: *Ralstonia solanacearum*, bacteriophage, tomato diseases, pathogenicity, bacterial wilt, Vietnam

INTRODUCTION

Tomato is one of the most widely grown vegetable crops in the world. The tomato growing area in Vietnam is approximately 23,000 ha [1]. Tomatoes have become an important and common food that provides high nutritional value and has a positive impact on human health [1, 2]. However, one main factor affecting commercial tomato production in Vietnam is the annual cycle of diseases. During farming, tomatoes are infected by more than 200 different diseases caused by moulds, nematodes, bacteria, and viruses [3]. These diseases not only decrease the yield and quality of tomatoes, but also threaten human health and economic benefits [4].

One of the most common tomato diseases is bacterial wilt caused by *Ralstonia solanacearum* (also known as *Pseudomonas solanacearum*) [5]. The bacterial wilt causes a harvest loss up to 100% in banana, 90% in tomato and potato and a significant economic losses to farmers [6]. The pathogen infects the tomato through root wounds and xylem tissue. It then synthesizes extracellular polysaccharides that inhibit water transportation in the plant and cause wilting or the death of the host [7]. The pathogen has been ranked in second place among the top 10 devastating plant pathogenic bacteria [8]. Previous studies have shown that *R. solanacearum* has 4 phylotypes: Asia I, America II, Africa III, and Indonesia IV [9, 10]. Chemical pesticide usage has been frequently used as a measure of the prevention and treatment of bacterial wilt disease in Vietnam. However, improper usage of chemical pesticides can significantly affect the sustainability of tomato farming and community health [11]. Due to these adverse impacts, it is urgently necessary to adopt an alternative solution to chemical pesticides in the prevention and treatment of bacterial wilt in tomato [12].

The usage of lytic bacteriophages (phages) has obtained serious attention for the prevention and treatment of bacterial diseases in plants [13]. It has shown potential in the control of rhizosphere bacterial targets due to its specificity and safety and economic efficiency [5, 12]. Some previous studies have reported that phages can effectively control bacterial wilt disease in tomatoes [5, 14]. Most studies on *Ralstonia* phages are mainly conducted in Asian countries such as Japan, Thailand, Indonesia, China, Korea [15–19]. The *R. solanacearum* phages are quite diverse in morphologies belonging to the families of Podoviridae, Inoviridae, Siphoviridae, or Myoviridae. Their genome size also has a wide variation with about 160–220 kb of phage such as RSL1 and with about 40 kb of phages such as RSA1 and RSB1 [20]. *R. solanacearum* bacteria

are very diverse in genetic and pathogenicity [9, 10]. Thus, broad host range phages at species level are desired for pathogenic bacteria control [21]. Previous studies by Bhunchoth et al[15] and Addy et al[17] showed *Ralstonia* phages J2, RSB2, and RsoP1IDN had a broad host range. Particularly, all phages belong to the Podoviridae family [15, 17]. However, the capacity of phages to control *R. solanacearum* isolates in Vietnam has not been investigated. In this study, *R. solanacearum* strains were isolated in the largest tomato-growing province in Vietnam. Their diversity of genetics and pathogenicity were then examined. A phage specific to *R. solanacearum* was isolated, and its characteristics such as lytic activity, host range, whole genomic information, and safety were investigated.

MATERIALS AND METHODS

Isolation of *R. solanacearum*

Seventeen tomato plant samples with apparent bacterial wilt symptoms were collected from tomato cultivation areas in Da Loan and Hiep Thanh, Duc Trong district, Lam Dong province, Vietnam. The samples were taken as whole plants, including roots, stems and leaves, then placed in plastic Ziploc bags before being transported to the laboratory. They were stored at 4 °C until used for bacterial isolation.

Semi-selected South Africa agar (SMSA) medium containing 1 g casamino acid, 10 g peptone, 10 ml glycerol, and 17 g agar per litre was sterilised at 121 °C for 15 min. Next, 5 mg crystal violet, 100 mg polymyxin β sulfate, 25 mg bacitracin, 5 mg chloromycetin, 0.5 mg penicillin, and 100 mg cycloheximide were added into 1 liter of sterilised SMSA medium. The parts of the tomato samples with confirmed wilt disease symptoms were used for the isolation step. The infected stem segments (approximately 10 cm from the symptom site) were cut using a sterilised scalpel and washed under running tap water. The samples were placed in beakers and sterilised with 70% ethanol, then rinsed with sterile distilled water. Small stem pieces were put into falcon tubes containing 10 ml of sterile distilled water and stirred in an incubator shaker for 15 min. The ooze solutions were serially 10-fold diluted with sterile distilled water up to 10^{-4}. Then, 100 µl of diluted microbial suspension was spread on the SMSA medium and incubated at 30 °C for 48 h. Single colonies of typical shape and colour were picked and serially streaked onto the SMSA medium for the purification of isolates [22].

The purified isolates were streaked onto casamino acid peptone glucose (CPG) medium (1 g casamino acid, 10 g peptone, 5 g glucose, and 17 g agar per liter) and incubated at 30 °C for 48 h. Gram staining was performed according to the method described by Divya and Yogendra [23]. Bacterial isolates were subjected to colony polymerase chain reaction (PCR) method with a *R. solanacearum*-specific primer pair RS-F-759 (5′-GTCGCCGTCAACTCACTTTCC-3′) and RS-R-760 (5′-GTGCCCGTAGCAATGCGGAATCG-3′) [17]. The PCR program was conducted with initiation at 96 °C for 3 min, followed by 35 cycles at 94 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s, and the final extension at 72 °C for 5 min. The amplified products were separated on a 1% agarose gel for 30 min at 80 V with the gelred 6x DNA stain.

Identification and analysis of phylogenetic tree

Bacterial DNA was extracted from the bacterial colony using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany) as recommended by the manufacturer. The whole 16S rRNA gene sequence region was amplified with specific primer pairs and sequenced using the Sanger method. BLAST software was used to compare reference sequences from the database. The phylogenetic tree of the *R. solanacearum* collection was built using MEGA 11 software using the Neighbor-Joining method and 1,000 bootstrapping replications.

Pathogenicity test of *R. solanacearum* in tomato

Six-week-old Beefsteak F1 tomato plants were used for the toxicity test of *R. solanacearum* strains. The biomass of bacterial strains was collected after 48 h of proliferation by centrifugation at 1,500×*g* for 5 min. The biomass was resuspended, and the density was adjusted to 10^8 CFU/ml before artificial inoculation. Each experimental treatment corresponded to a strain of *R. solanacearum*, and a physiological saline solution was used as a control. Each treatment was conducted in triplicate, with 10 plants each time, and arranged in a completely randomised design (CRD). Plants were artificially inoculated by dipping the point of a surgical blade into the bacterial solution and making a wound 3 mm long, 1 mm deep, and 10 mm from the root flare. After 10 days, the toxicity of the bacterial strains was evaluated through the disease rate index. The disease rate (%) = (Number of diseased plants/Total number of plants in the treatment) × 100.

Phage isolation

Soil samples were obtained from tomato fields in Duc Trong district, Lam Dong province, Vietnam, and transported to the laboratory for phage isolation. Five grams of soil were placed in a falcon tube, to which 5 ml of distilled water and 5% (w/v) chloroform were added. The mixture was vortexed for 5 min. It was then centrifuged at 2,432×*g* for 10 min at room temperature to obtain the supernatant, and the soil and the chloroform layers were discarded. The supernatant was further centrifuged at 9,727×*g* for 5 min at 4 °C to remove the remaining chloroform. The resulting supernatant was filtered using a 0.22-µm pore size filter. A 1 ml filtrate was added to a falcon tube containing 100 µl of log-phase *R. solanacearum* bacterial culture and 9 ml of tryptic soy broth (TSB) medium (17 g

tryptone, 3 g soytone, 2.5 g glucose, 5 g NaCl, and 2.5 g K_2HPO_4 for a liter medium). The mixture was shaken at 150 rpm for 24 h at 30 °C and was then centrifuged at 9,727×*g* for 5 min at 4 °C. The resulting supernatant was passed through a 0.22 µm pore size filter, and the filtrate was subjected to a plaque assay. A mixture of 100 µl of the filtrate and 200 µl of log-phase *R. solanacearum* bacterial culture was added to 3 ml of molten 0.5% tryptic soy agar (TSA) which was made from TSB by adding 17 g agar per liter (maintained at 42 °C) and pouring over a 1.5% Luria–Bertani (LB) agar plate (10 g peptone, 5 g yeast extract, 0.5 g NaCl, and 17 g agar for a liter medium). After incubation overnight at 30 °C, a single transparent plaque was selected from the plate, suspended in SM buffer, incubated overnight at 4 °C, and passed through a 0.22-µm filter. The resulting filtrate was subjected to the above protocol 3 times in succession to purify the phage. The morphologies of bacteriophages were examined using a transmission electron microscope (JEOL JEM-1010, Jeol Ltd., Tokyo, Japan) operating at 80 kV voltage and an instrumental magnification of 25,000–30,000 at the Vietnam National Institute of Hygiene and Epidemiology.

Determination of burst size and latent period of phage

The culture of *R. solanacearum* was incubated at 150 rpm and 30 °C until it reached an OD_{600} of 0.1 (approximately 10^7 CFU/ml). The phage was added at a multiplicity of infection (MOI) of 0.01 (phage:host). This mixture was shaken for 10 min at 150 rpm and 30 °C and then centrifuged at 9,168×*g* for 5 min at 4 °C. The pellet was resuspended in the same volume of TSB and incubated in the same condition. A part of the volume was collected and diluted 100-fold in SM buffer on ice every 5 min. The diluted samples were centrifuged at 9,168×*g* for 5 min at 4 °C, and the supernatant was used for phage concentration determination using the double agar-layer method. The burst size and latent period of the phage were determined according to a previously described method [24]. The experiment was conducted in triplicate.

Host range determination of phage

The host range of the phage was evaluated using various bacterial isolates (Table 1), the susceptibility of which was determined using a drop plaque assay. A 100 µl aliquot of an overnight bacterial culture was mixed with 3.0 ml molten 0.5% (w/v) TSA (maintained at 42 °C) and placed onto a 1.5% (w/v) LB agar plate. After 2 min, each plate received 2 µl of phage stock (approximately 10^9 PFU/ml). The plates were incubated at 30 °C overnight and then examined for clear zones on the bacterial lawn.

Table 1 Pathogenicity test on tomato plants after 10 days of artificial inoculation.

No.	Strain	Disease rate (%)
01	Ps003	96.7^{de}
02	Ps004	43.3^{b}
03	Ps009	0.0^{a}
04	Ps014	70.0^{c}
05	Ps015	0.0^{a}
06	Ps020	46.7^{b}
07	Ps021	100.0^{e}
08	Ps022	83.3^{cd}
09	Ps024	100.0^{e}
10	Ps025	93.3^{de}
11	Ps031	70.0^{c}

Values with different letters are significant different using Duncan's multiple rank test ($p \leqslant 0.05$).

Phage nucleic acid extraction

A volume of phage solution was treated with 1.5 µl DNase I (2,000 U/ml, New England Biolabs, Massachusetts, USA), then incubated at 37 °C for 1 h 30 min. The enzyme was then inactivated by incubating the mixture at 80 °C for 10 min. Next, Proteinase K (Fisher Scientific UK Ltd., Loughborough, UK) was added to a final concentration of 0.5 mg/ml. The mixture was then incubated at 56 °C for 1 h. A solution of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the mixture. The ratio of the additional solution and the starting solution was 1:1. After incubating the mixture for 2 min at room temperature, the solution was centrifuged at 13,000×*g* and 30 °C for 10 min to obtain the supernatant, which was subjected to the phenol:chloroform:isoamyl alcohol (25:24:1) treatment for an additional 2 times. The final supernatant was mixed with 3 M sodium acetate in an amount equal to 1/10 volume of the supernatant, followed by the addition of ice-cold 96% (v/v) ethanol. After incubating overnight at −70 °C, the mixture was centrifuged at 13,000×*g* and 4 °C for 5 min to obtain the pellet. Next, a volume of cold 70% (v/v) ethanol was used to wash the pellet. The mixture was kept on ice for 30 min and then centrifuged at 13,000×*g* and 4 °C for 5 min. Finally, 30 µl TE buffer was used to dissolve the pellet. The final product was stored at −70 °C until used.

Phage genome sequencing and analysis

Library preparation of the phage genome used the NEBnext Ultra II DNA Library Prep Kit for Illumina, and sequencing was conducted on an Illumina NextSeq550 (150-bp paired end) at the KTEST company (Ho Chi Minh City, Vietnam). The nucleotide sequence was submitted to NCBI. The read data in FASTQ format files was evaluated by FASTQC v0.11.9 [25]. *De novo* assembly was conducted using Unicycler v0.5.0 [26] with conservative mode. Evaluation of complete

contig assembly was performed by QUAST v5.0.2 [27]. BLAST [28] search against the NCBI database was used to identify contigs of viral origin. Prediction of open reading frame (ORF) and annotation were performed by RAST [29] with Virus Domain and Genetic code 11. The GenBank file generated by RAST was then manually examined to validate identified ORFs if one of the following conditions was satisfied: matching of a BLAST search with a gene of known function from a curated annotation of the closest viral origin; identification of a Pfam family; and having a domain hit by CDD. The presence of potential antimicrobial resistance determinants was investigated using ResFinder v4.1 [30]. Bacteriophage lifestyle was predicted using BACPHILP [31] to test whether the phage genome was likely to be virulent (lytic) or temperate (lysogenic). Detection of tRNA was performed using tRNAscan-SE v2.0 [32].

Multiple whole-genome alignment of the phage from Vietnam and other viral origins was generated using progressiveMauve and then converted back to FASTA format using the xmfa2fasta.pl script. Single nucleotide polymorphism (SNP) sites were identified using snp-sites v2.5.1 [33] to generate SNP-based alignment. Distances between each pair of phage isolates were calculated using snp-dists (0.8.2). A SNP-based phylogenetic tree of phage isolates was reconstructed using Fasttree v2.1.11 [34] with the GTR+CAT model. A circular representation of the phage genome was created using BRIG v0.95 [35].

In vitro control of phage against *R. solanacearum*

The host bacterial culture in TSB was shaken at 150 rpm and 30 °C until it attained an OD_{600} of 0.1. Next, it was divided into 2 aliquots, one of which was mixed with the phage at the MOI of 1.0, while the other had no phage added (the control). The 2 mixtures were shaken at 150 rpm and 30 °C. The OD_{600} were periodically determined. Each trial was performed in triplicate.

Statistical analysis

In the toxicity test of *R. solanacearum* in tomato, each treatment was conducted in triplicate and on ten plantlets each time. One-way analysis of variance (ANOVA) was used to process the resulting data using Statistical Package for the Social Sciences (SPSS) v.20 software for Mac. Duncan's multiple range test demonstrated a statistically significant difference between treatments at $p \leqslant 0.05$.

RESULTS AND DISCUSSION

A collection of *R. solanacearum*

A total of 31 isolates expected to be *R. solanacearum*, named Ps001 to Ps031, were obtained. Virulent bacterial isolates have large, irregular, fluid, white, or cream borders with pink at the centre, while non-virulent isolates have smaller, round, non-mucoid, and dark red colonies. The results of the PCR with specific primer pair RS-F-759/RS-R-760 indicated that 11/31 isolates (Ps003, Ps004, Ps009, Ps014, Ps015, Ps020, Ps021, Ps022, Ps024, Ps025, and Ps031) resulted in electrophoresis bands with the expected size of 281 bp [24]. These isolates were subjected to a Gram staining test. All isolates belonged to the Gram-negative group with short rod-shapes similar to those described for *R. solanacearum* [9, 36]. Results of the 16S rRNA gene sequencing indicated that all isolates were *R. solanacearum*. Besides, the results of the phylogenetic tree analysis showed that these strains were very diverse when 11 isolates were divided into 3 different groups. Group I included Ps004, Ps014, Ps015, Ps024, and Ps025; group II included Ps003 and Ps020; and group III included Ps009, Ps021, Ps022, and Ps031. Previous studies have shown that *R. solanacearum* has 4 phylotypes, including Asia I, America II, Africa III, and Indonesia IV [9, 10]. The clustering of the bacterial isolates partly showed the diversity of *R. solanacearum* in tomato cultivation areas in Lam Dong province, Vietnam.

Pathogenicity of *R. solanacearum*

After 10 days of artificial inoculation, 9/11 strains showed disease in the experimental tomato plants (about 81.8% of the total strains), including Ps003, Ps004, Ps014, Ps020, Ps021, Ps022, Ps024, Ps025, and Ps031. The plants first showed symptoms of disease on the young leaves (the leaves lacked water and became wilted) and then on the older leaves (Fig. 1). However, the disease rates of these strains differed significantly. While strains Ps004 and Ps020 only had average disease rates of 43.3% and 46.7%, respectively, strains Ps003, Ps021, Ps024, and Ps025 had high disease rates of 96.7%, 100%, 100%, and 93.3%, respectively (Table 1).

The bacterium *R. solanacearum* causes bacterial wilt in tomatoes and other solanaceous plants. Bacteria in soil, crop residues, and weeds are widespread and cause severe losses in agricultural production. The bacteria can be spread through seedlings, wind, water, insects, and tools [16]. At the early stages of the disease, the first visible symptoms of bacterial wilt are usually seen on the foliage of plants. Bacteria easily enter the plants through mechanical wounds or insect stings on roots and stems. After entering the plants, they attack the conduction vessels and move along them, damaging the vascular bundles and preventing the movement of water and nutrients [23]. The rate of infection and disease in plants is rapid; this rate depends on the stage of plant growth, the soil moisture, and environmental temperature. However, not all the strains of *R. solanacearum* are pathogenic or have low pathogenicity, resulting in some strains that could not

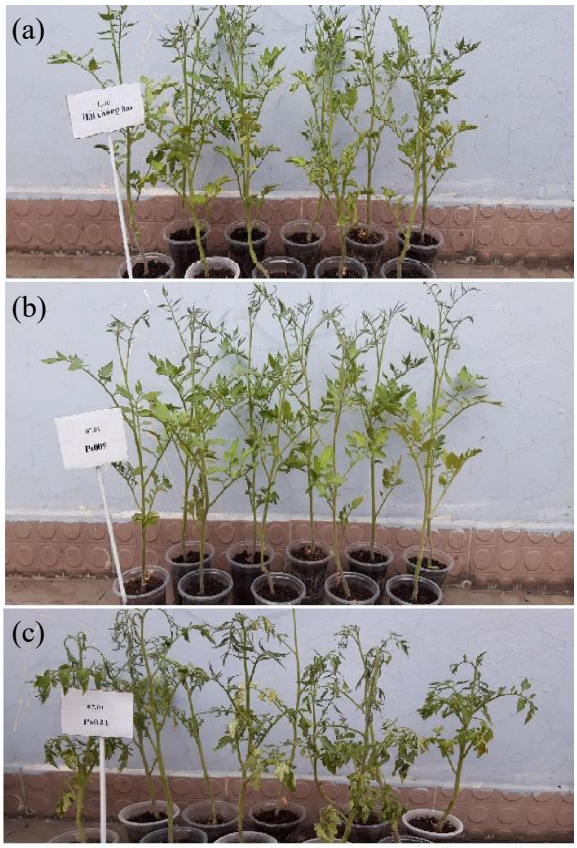

Fig. 1 The results of the toxicity test on tomatoes after 10 days of treatment. (a) Control; (b) Ps009 strain (disease rate of 0%); and (c) Ps021 strain (disease rate of 100%).

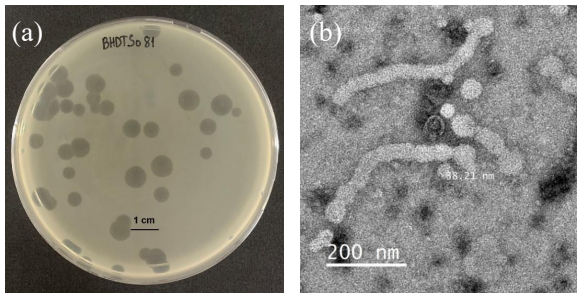

Fig. 2 (a) Top agar overlay showing plaque morphology of phage BHDTSo81; the scale bar indicates 1 cm, (b) electron micrograph of phage BHDTSo81; the bar represents 200 nm.

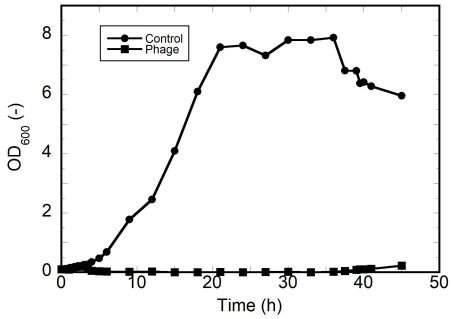

Fig. 3 Changes in optical density at 600 nm (OD_{600}) during inactivation of *R. solanacearum* Ps021 by BHDTSo81 phage in TSB at 30 °C (MOI of 1.0) (closed square). The negative control was without phage (closed circles). Error bars indicating 95% confidence intervals for the averaged values ($n = 3$) are not graphically detectable as the intervals are too narrow.

cause wilting of tomato plants [37]. The virulence of strains depended on the expression of virulence genes. Compared to the phylogenetic tree, the low, medium, and high virulence isolates were randomly distributed in groups I, II, and III. The finding illustrated no association between pathogenicity and the results of genetic diversity analysis based on 16S rRNA gene sequences. The results were consistent with those shown in the previous studies on tomato and tobacco [38, 39].

Lytic activity and morphology of phage

A bacteriophage, named BHDTSo81, was isolated from a soil sample in Bac Hoi village, Duc Trong district, Lam Dong province. The phage was presented as a round and clear plaque approximately 8 mm in diameter (Fig. 2A). The latent period and burst size of BHDTSo81 were approximately 145 min and 8.6 ± 1.8 phages per infected cell, respectively (Fig. S4). From the morphological observation using transmission electron microscopy, morphology of the phage was determined. BHDTSo81 had an icosahedral head approximately 38 nm in diameter (Fig. 2B). It was a podovirus and was classified in the Autographiviridae family [35]. However, the head diameter of BHDTSo81 was smaller than that of RsoP1IDN with 62 nm [17]. Although *R. solanacearum* phages with podovirus morphology were reported in previous studies in Japan, Indonesia, and Thailand [15, 17, 24, 34], the BHDTSo81 was the first lytic phage against *R. solanacearum* with podovirus morphology isolated in Vietnam.

Host range of phage

To evaluate the host range of BHDTSo81, 26 bacterial isolates were used (Table 2). BHDTSo81 created clear zones in cultures of 7/11 strains of *R. solanacearum*. The other bacterial strains of *Xanthomonas* spp. in Table 2 were isolated from tomato and rice fields. None of these bacteria species tested were susceptible to the phage. It indicated that the specificity of host range of BHDTSo81 is high at the species level. It might be beneficial to treat the disease caused by *R. solanacearum*, while does not affect other bacteria in the environment.

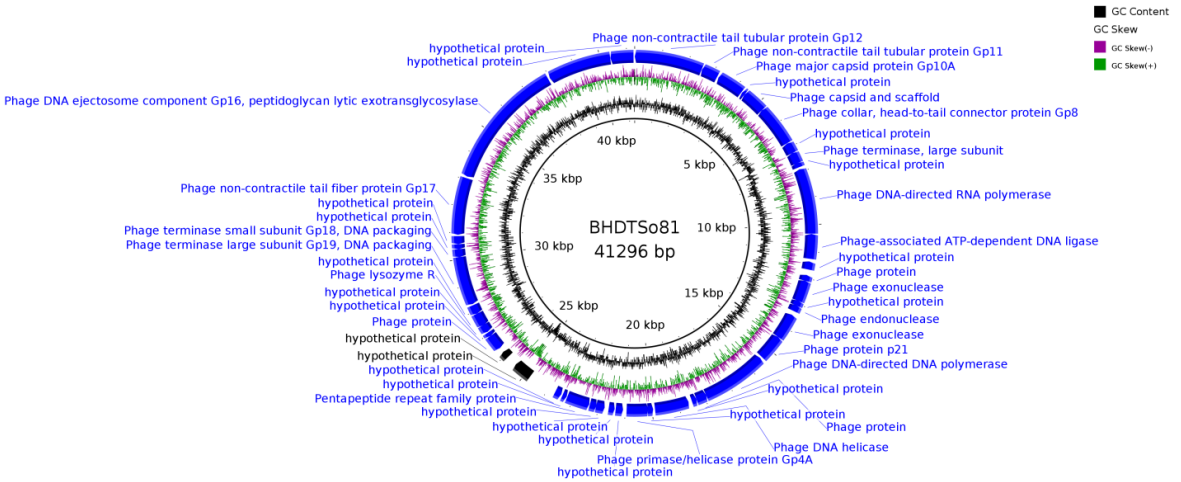

Fig. 4 Genome map of BHDTSo81. The relative orientations of annotated ORFs are indicated as blue and black colours (antisense and sense, respectively).

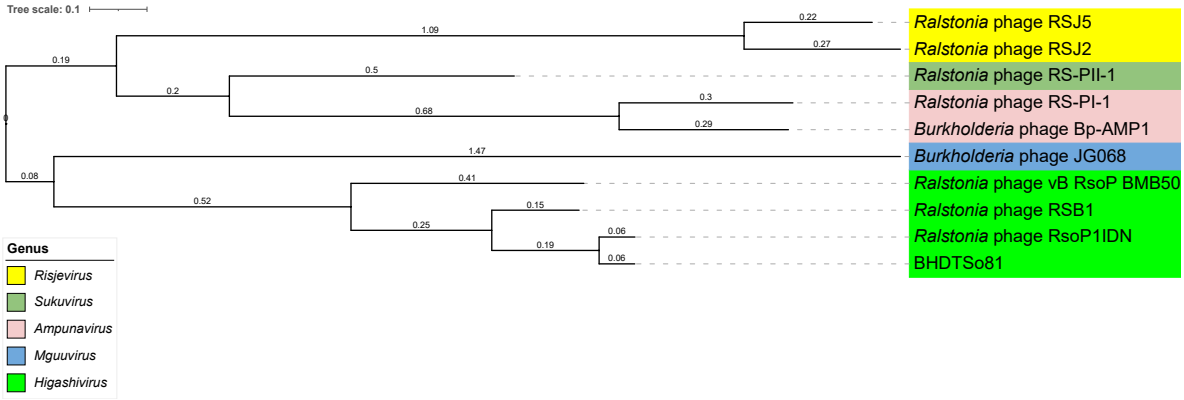

Fig. 5 The SNP-based phylogenetic tree reconstructed from the whole-genome alignment of 10 members of Okabevirinae subfamily. The number on each branch indicates the branch length based on the tree scale. Legend shows different genera belonging to Okabevirinae subfamily.

Inactivation of *R. solanacearum* by BHDTSo81

The capacity of BHDTSo81 to restrict the growth of *R. solanacearum* in TSB was evaluated. As shown in Fig. 3, during the first 2.5 h of incubation, an increased OD_{600} was observed in both treatments (with or without the phage). However, the OD_{600} of the bacterium–phage suspension started to decrease after incubation for 3 h, while that of the control continued to increase. This indicates that a proportion of the host cells had already been infected and lysed by BHDTSo81 by this point. From 3 h to 5 h of incubation, the OD_{600} value of the bacterium–phage continued to decrease, while that of the control strongly increased. The phage was mixed into the bacterial culture at an MOI of 1.0. The lysis of host cells by phages led to the sharp decrease of the suspension OD_{600}. The transparency of the bacterium–phage suspension for approximately 40 h showed the ability of BHDTSo81 to inactivate the host cells, while the turbidity of the control was constantly maintained over the course of the experiment. After 40 h of incubation, the OD_{600} of the bacterium–phage solution increased, indicating the growth of phage-resistant bacteria.

Phage genome analysis

The phage assembly generated one complete contig with estimated read depth of 3078x. The genome of the phage was 41,296 bp long with a total GC content of 63%, containing 46 predicted protein CDSs. The whole genome was annotated with 46 functional proteins (Fig. 4). Genes related to DNA replication and modification enzymes such as polymerase, exonuclease, endonuclease, helicase, primase, and peptidoglycan lytic exotransglycosylase were detected. Genes encoding structural and packaging proteins such as capsid protein, head-to-tail connector, tail fiber, and

Table 2 Host range of BHDTSo81 phage.

Bacterial strain	Plaque formation
Ralstonia solanacearum PS003	+
Ralstonia solanacearum PS004	−
Ralstonia solanacearum PS009	+
Ralstonia solanacearum PS014	−
Ralstonia solanacearum PS015	−
Ralstonia solanacearum PS020	+
Ralstonia solanacearum PS021	+
Ralstonia solanacearum PS022	+
Ralstonia solanacearum PS024	+
Ralstonia solanacearum PS025	+
Ralstonia solanacearum PS031	−
Xanthomonas citri pv. *fuscans* XC06	−
Xanthomonas axonopodis pv. *commiphoreae* XC111	−
Xanthomonas euvesicatoria XC181	−
Xanthomonas citri pv. *fuscans* XC92	−
Xanthomonas axonopodis pv. *commiphoreae* XC141	−
Xanthomonas axonopodis pv. *commiphoreae* XC233	−
Xanthomonas axonopodis XC01	−
Xanthomonas axonopodis pv. *commiphoreae* XC271	−
Xanthomonas axonopodis pv. *commiphoreae* XC264	−
Xanthomonas axonopodis pv. *commiphoreae* XC263	−
Xanthomonas axonopodis pv. *commiphoreae* XC204	−
Xanthomonas axonopodis pv. *commiphoreae* XC205	−
Xanthomonas oryzae pv. *oryzae* LA1+	−
Xanthomonas oryzae pv. *oryzae* L19	−
Xanthomonas oryzae pv. *oryzae* L24	−

(+) susceptible; (−) not susceptible.

tail tubular were also detected. The remaining predicted proteins were considered hypothetical. The phage genome was submitted to NCBI under accession number OP811265.

Genome-wide comparison of phages belonging to the genus *Higashivirus* showed that the most notable difference between the genome of BHDTSo81 from Vietnam and other members of the genus was the putative tail fiber protein. This protein has a length of 711 aa in BHDTSo81, but only 603 aa in RsoP1IDN (locus HOS84_gp34), and only the first 231 aa are conserved with 225/231 (97%) identities; the rest are not similar.

In total, 52,191 SNPs were identified from the whole-genome alignment of 10 members of the Okabevirinae subfamily. The phage closest in origin to BHDTSo81 was *Ralstonia* phage RsoP1IDN with a significant difference of 8,409 SNPs. An SNP-based phylogenetic tree of the whole genomes of the Okabevirinae subfamily indicated that the phage from Vietnam may have emerged from the *Higashivirus* genus (Fig. 5).

No known antibiotic resistant gene or virulence factor was discovered in BHDTSo81. In addition, no known gene associated with lysogeny was detected, e.g. no integrase was found. The prediction of phage lifestyle indicated that BHDTSo81 was likely to be virulent. Temperate phages are not generally accepted for use in phage therapy. Therefore, an important consideration is to classify whether the phage lifestyle is likely to be lytic or lysogenic [40]. In addition, detecting the presence of toxins, virulence factors, or antimicrobial resistance genes is also a vital step. The lytic nature of BHDTSo81 suggests that it might serve as a potential agent to control *R. solanacearum* infection in tomato in Vietnam.

CONCLUSION

The usage of chemical pesticides significantly affects the sustainability of tomato farming and community health in Vietnam. Lytic bacteriophages have attracted serious attention as a tool for the prevention and treatment of bacterial diseases in plants. In this study, 11 *R. solanacearum* strains were isolated from the largest tomato-growing province in Vietnam. Their diversity and toxicity, causing bacterial wilt disease, were then examined in tomato. A phage specific to *R. solanacearum* was isolated. Its characteristics, including lytic activity, host range, whole genomic information, and safety, were investigated. The initial characteristics of phage BHDTSo81 indicated its potential utility as a control agent against *R. solanacearum*.

Appendix A. Supplementary data

The phage genome was submitted to NCBI under accession number OP811265. Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.018.

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Appendix A. Supplementary data

Table S1 The results of PCR, Gram staining, morphology and bacterial morphology.

Isolate	PCR result	Morphology	Gram staining	Colony color
Ps003	+	Rod-shaped	−	Cream border with pink center
Ps004	+	Rod-shaped	−	Cream border with pink center
Ps009	+	Rod-shaped	−	Dark red
Ps014	+	Rod-shaped	−	Cream border with pink center
Ps015	+	Rod-shaped	−	Dark red
Ps020	+	Rod-shaped	−	Cream border with pink center
Ps021	+	Rod-shaped	−	Cream border with pink center
Ps022	+	Rod-shaped	−	Cream border with pink center
Ps024	+	Rod-shaped	−	Cream border with pink center
Ps025	+	Rod-shaped	−	Cream border with pink center
Ps031	+	Rod-shaped	−	Cream border with pink center

"+": Positive result; "−": Negative result.

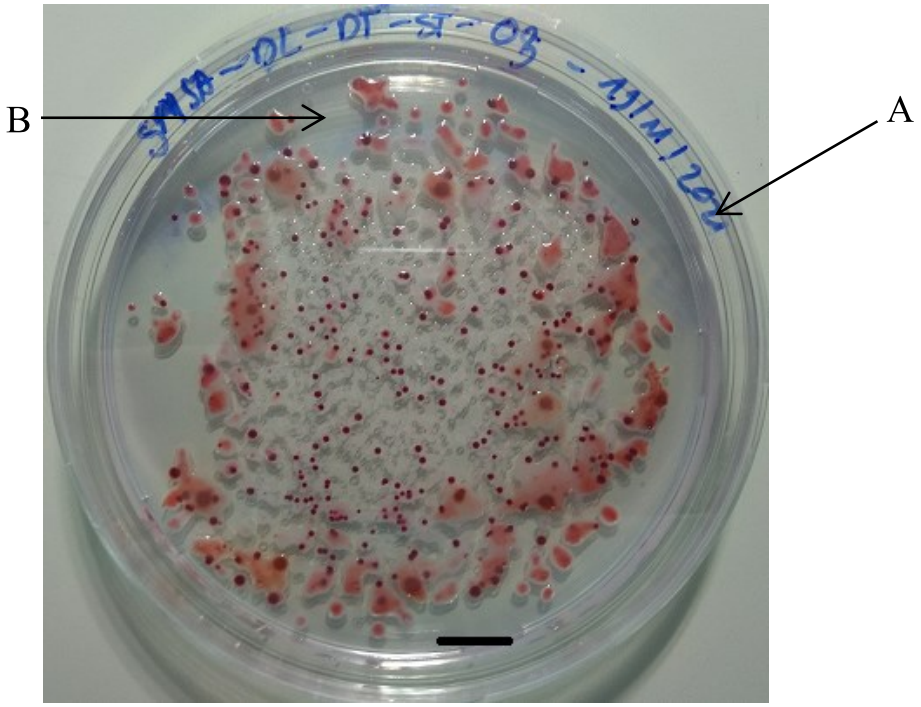

Fig. S1 Bacterial colony morphology on SMSA medium. A: colony of non-virulent isolate; B: colony of virulent isolate.

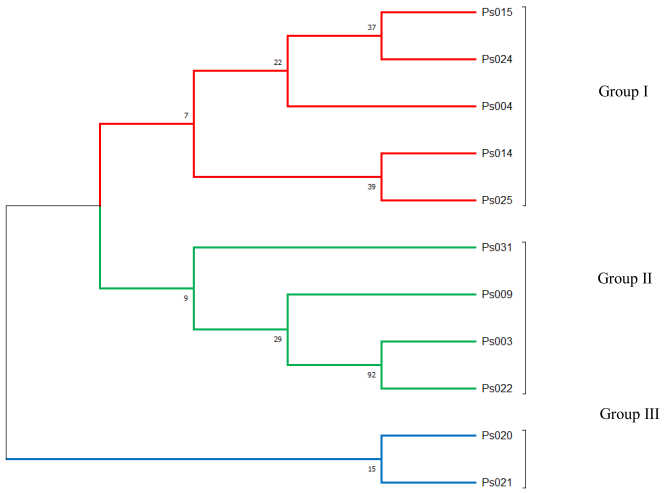

Fig. S2 Phylogenetic tree of 11 *R. solanacearum* isolates.

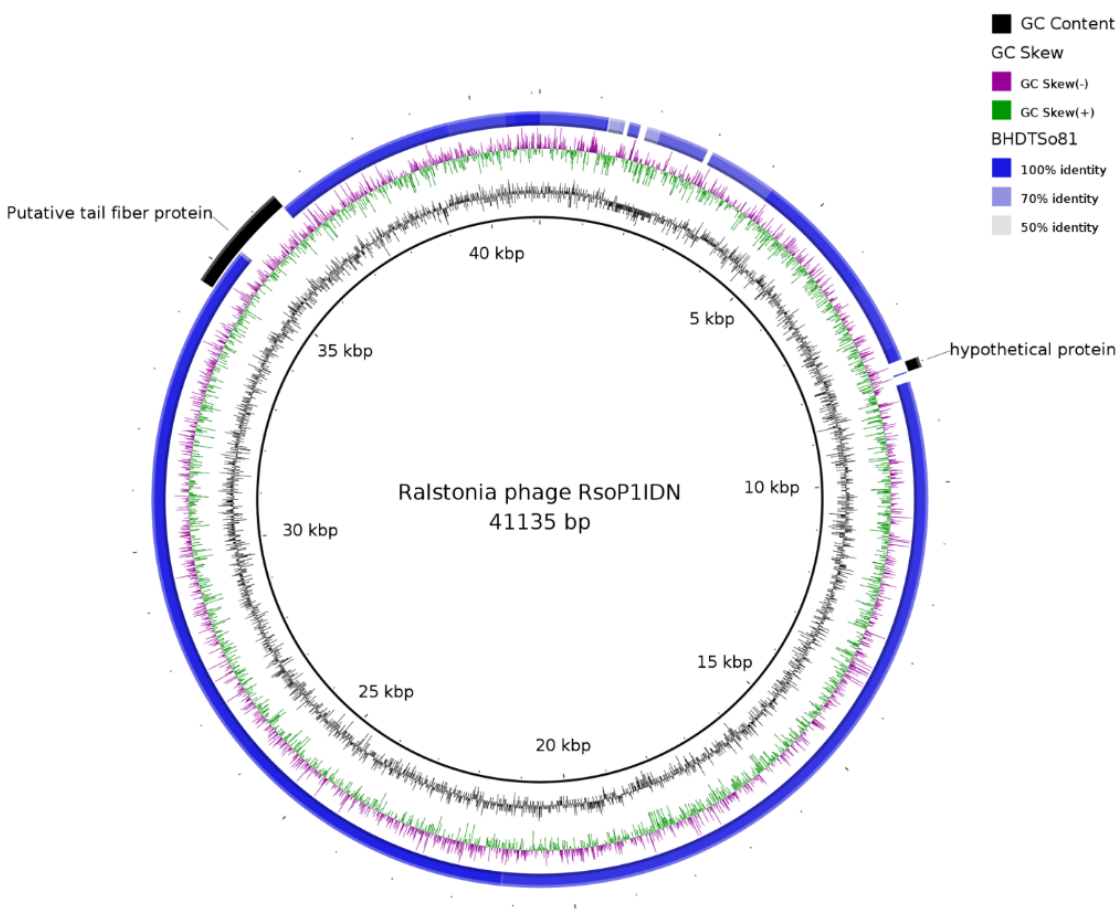

Fig. S3 Comparative genomic analysis of phage BHDTSo81 and the closest origin phage RsoP1IDN belonging to *Higashivirus* genus. The diagram was drawn by BRIG, with *Ralstonia* phage RsoP1IDN as the inner circle reference. The blue ring indicates regions with high pairwise genomic sequence similarity and present on both reference and compared genomes. White gaps show certain sections not present on compared genomes. Black outer ring segments indicate ORFs encoding proteins that are different between BHDTSo81 and RsoP1IDN.

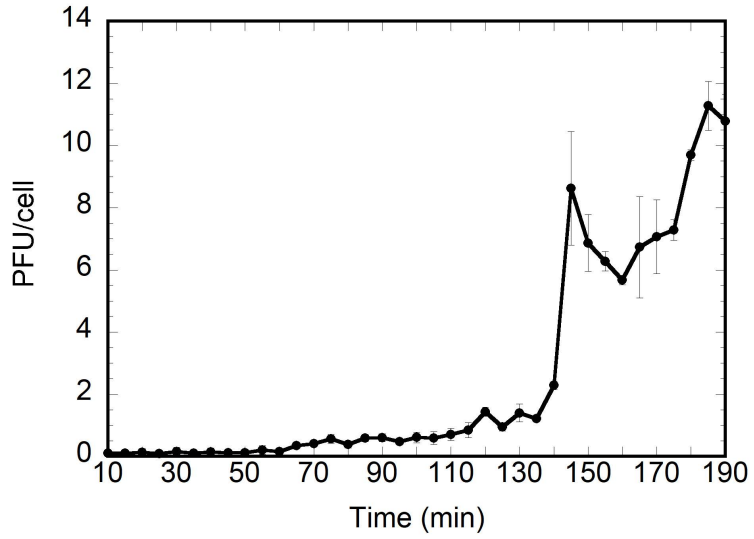

Fig. S4 The one-step growth curve of BHDTSo81 phage.