Two new phages isolated from a urban stream in brazilian midwest. Broad lytic spectrum and one health approach

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ABSTRACT

Tomato (*Solanum lycopersicum L.*) and other economically important crops such as potato, wheat, and soybean are severely affected by bacterial plant diseases, resulting in significant yield losses worldwide and imposing major economic constraints on agricultural production. Among these pathogens, *Klebsiella variicola* and *Enterobacter hormaechei* have gained attention due to their dual role as emerging phytopathogens and opportunistic clinical pathogens, highlighting the need for sustainable and effective control strategies. In this study, we investigated the potential of bacteriophages as biological control agents targeting these bacteria. A novel two lytic bacteriophages, designated SvBr38 and NtBr01, were isolated from an urban stream in Goiânia, Goiás, Brazil, and characterized via transmission electron microscopy. Methodological evaluations included optimal Multiplicity of Infection (MOI), one-step growth assays, and stability tests across temperature, pH, and UV radiation gradients. SvBr38 showed higher replicative efficiency (burst size: 300 PFU/cell), while both phages remained stable between pH 5.0–8.0 and temperatures up to 40°C. Host range analysis revealed a remarkable broad-spectrum activity, lysing not only the primary agricultural targets but also multidrug-resistant (MDR) clinical and environmental isolates of *Pseudomonas aeruginosa* and *Escherichia coli*, while sparing beneficial *Lactobacillus* spp. High-purity genomic DNA was successfully extracted for future sequencing. Phages, SvBr38 and NtBr01 where promising candidates for phage-based biocontrol in agriculture, offering an environmentally friendly alternative for managing phytopathogens within a One Health framework.
KEYWORDS: Bacteriophages, Enterobacter hormaechei, Phytopathogens, Klebsiella variicola

Dois novos fagos isolados de um riacho urbano no Centro-Oeste brasileiro. Amplo espectro lítico e uma abordagem de saúde única

RESUMO

O tomate (*Solanum lycopersicum L.*) e outras culturas economicamente importantes, como a batata, o trigo e a soja, são gravemente afetadas por fitopatógenos, resultando em perdas significativas em todo o mundo e impondo grandes restrições econômicas à produção agrícola. Entre esses patógenos, *Klebsiella variicola* e *Enterobacter hormaechei* ganharam atenção como fitopatógenos emergentes e patógenos clínicos oportunistas, destacando a necessidade de estratégias de controle sustentáveis e eficazes. Neste estudo, investigamos o potencial dos bacteriófagos como agentes de controle biológico direcionados a essas bactérias. Dois novos bacteriófagos líticos, designados SvBr38 e NtBr01, foram isolados de um riacho urbano em Goiânia, Goiás, Brasil, e caracterizados por microscopia eletrônica de transmissão. As avaliações metodológicas incluíram Multiplicidade de Infecção ideal (MOI), ensaios de crescimento em uma etapa e testes de estabilidade de temperatura, pH e radiação UV. SvBr38 apresentou maior eficiência replicativa (tamanho de explosão: 300 PFU/célula), enquanto ambos os fagos permaneceram estáveis entre pH 5,0–8,0 e temperaturas de até 40°C. A análise da gama de hospedeiros revelou uma notável atividade de amplo espectro, lisando não apenas os alvos agrícolas primários, mas também isolados clínicos e ambientais multirresistentes (MDR) de *Pseudomonas aeruginosa* e *Escherichia coli*, não apresentando atividade lítica frente a cepas de *Lactobacillus spp*. DNA genômico de alta pureza foi extraído com sucesso para sequenciamento futuro. Os fagos, SvBr38 e NtBr01 são candidatos promissores para o biocontrole baseado em fagos na agricultura, oferecendo uma alternativa ecológica e sustentável para o manejo de fitopatógenos dentro de uma estrutura de Saúde Única.

PALAVRAS-CHAVE: Bacteriófagos, *Enterobacter hormaechei*, Fitopatógenos, *Klebsiella variicola*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically important horticultural crops worldwide, along with staple and high-value cultivars such as potato (*Solanum tuberosum*), wheat (*Triticum aestivum*), and soybean (*Glycine max*), all of which play a central role in global food security and agricultural sustainability (DIALLO *et al.,* 2024; FAOSTAT, 2025).

Bacterial plant diseases are responsible for substantial yield losses worldwide, contributing to billions of dollars in economic damage annually and highlighting the urgent need for sustainable disease management strategies in modern agriculture (SAVARY *et al*., 2019). *Klebsiella variicola* and *Enterobacter hormaechei* have emerged as particularly challenging agents, while historically recognized for their environmental presence, these species are now identified as important phytopathogens capable of causing systemic damage to plant tissues, while simultaneously gaining prominence as high-priority emerging clinical pathogens (SEKYERE; RETA, 2021).

Current strategies for controlling bacterial phytopathogens rely predominantly on chemical pesticides, copper-based compounds, and, in some regions, antibiotics, which have shown limited long-term efficacy and raised increasing concerns regarding environmental sustainability (LAHLALI *et al*., 2022). The intensive and repeated application of these agrochemicals has been associated with adverse effects on soil health, including disruption of microbial communities, accumulation of toxic residues, and alterations in soil physicochemical properties such as pH, which may negatively impact nutrient availability and crop productivity including the accumulation of toxic residues in soil and water bodies

as well as the elimination of non-target beneficial insects and microorganisms (MEFTAUL *et al*., 2019, SANTANA *et al.,* 2024). Therefore, alternative disease management approaches have gained increasing attention, including the use of resistant cultivars, beneficial microorganisms, and biological control strategies (LAHLALI *et al*., 2022).

Biological control strategies have gained prominence as sustainable alternatives to chemical-based disease management, including the use of beneficial microorganisms such as *Lactobacillus* spp., which can suppress phytopathogens through competitive exclusion and antimicrobial metabolite production, as well as Bacillus thuringiensis, whose Cry toxins have been widely applied for insect pest control in agriculture (BRAVO *et al*., 2007).

Within this biopesticide framework, bacteriophages (phages) offer a unique mechanism of action characterized by two primary life cycles: the lysogenic cycle, where viral DNA integrates into the host genome, and the lytic cycle, which culminates in rapid bacterial cell disruption and the release of new virions, the latter being more indicatred for biological control (RAKHUBA *et al*., 2010; LLOYD, 2012). Despite this potential, the diversity of Brazilian aquatic and soil ecosystems remains an underexplored reservoir for therapeutic microrganismos, such as phages (DANTAS *el tal*., 2024; TACCA *et al*., 2025).

Given the need for sustainable alternatives for the control of bacterial phytopathogens and the limited understanding of the biological and molecular mechanisms involved, it becomes essential to deepen knowledge about potential biocontrol agents. As highlighted by Rivera *et al.* (2024), elucidating these mechanisms is fundamental to interpreting their functionality and enabling future applications. In this context, the investigation of the lytic spectrum, infection dynamics, environmental stability, and genomic potential of recently isolated phages is justified.

The increasing recognition that several phytopathogenic bacteria, including members of the *Klebsiella* and *Enterobacter* genera, also act as opportunistic human pathogens further underscores the complexity of their control and the interconnectedness of agricultural, environmental, and clinical ecosystems (RODRÍGUEZ-MEDINA *et al*., 2019). Therefore, the aim of this study is to explore the potential of a novel bacteriophage as a biological control isolated from an urban stream in Goiânia, Goiás, including isolation, morphological characterization, host range spectrum and evaluation of lityc activie in vitro, agains phitopatogens *Klebsiella variicola* and *Enterobacter hormaechei*, environmental and clinical bacterial isolates.

MATERIALS AND METHODS

BACTERIAL STRAINS AND CULTURE CONDITIONS

Klebsiella variicola and *Enterobacter hormaechei* (Table 1) was Isolated from compostage soil from School of Agronomy of the Federal University of Goiás, gps -16.597339, -49.281713 and identify by Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF), systen Bruker MALDI Biotyper, Execution ID: 231123-1552-1011001425, Instrument ID: 269944.00637, Server version: 4.1.100 (PYTH) 188 2020-04-112_10-35-53. *Lactobacillus* species used in this experiment was are part of the LAMB bacteriotec. All other microorganisms (Table 1) were provided by Dra. Lilian Carla Carneiro, from Institute of Tropical Pathology and Public Health (IPTSP) of the Federal University of Goiás (UFG).

Klebsiella variicola, *Enterobacter hormaechei* and other microorganisms were grow on Nutrient agar medium and incubated overnight in shaker 130 rpm at 30 °C. For *Lactobacillus* strains medium Man, Rogosa and Sharpe (MRS) were used (Universal peptone 10 g/L Meat extract 5 g/L Yeast extract 5 g/L D(+)-Glucose 20 g/L Dipotassium

hydrogen phosphate 2 g/L Triammonium citrate g/L 2 g/L Sodium acetate 5 g/L Magnesium sulfate 0.1 g/L Manganous sulfate monhydrate 0.05 g/L Agar 12 g/L) pH adjustment to 6.5 ± 0.2. *Escherichia coli* was grown using a previously method was described by (PACÍFICO *et al*., (2019).

TABLE 1 Host range analysis of SvBr38 and NtBr01 phages of phytopathogenic bacteria, *Lactobacillus* species and clinical strains.

Bacterial strains	**SvBr38**	**NtBr01**
Klebsiella variicola	+ (Primary host)	+
Pseudomonas aeruginosa 1	+	-
P. aeruginosa 2	+	-
Klebsiella pneumoniae	+	+
Acinetobacter balmannii 01	+	+
A. balmanni 02	+	+
A. balmanni 03	-	-
Escherichia coli 01	-	+
E. coli 2	-	+
E. coli 3	-	+
E. coli 4	+	+
E. coli 5	-	+
Serratia marcescens	+[p]	-
Xanthomonas perforans 1	+[p]	-
X. perforans 2	+	+
X. perforans 3	+	+
Enterobacter hormaechei	+	+ (Primary host)
Staphylococcus lugdunensis	-	-
Enterococcus faecalis	-	-
Staphylococcus aureus	-	-
Staphylococcus epidermidis	-	-
Lactobacillus Breve	-	-
L. delbrueckii	-	-
L. gasseri	-	-
L. johnsonii	-	-
L. plantarum	-	-
L. sporogenes	-	-

+ Complete lysis – without lysis [p] Partial lysis

BACTERIOPHAGE ISOLATION PURIFICATION AND STORAGE

Samples of water from stream Botafogo in Brazilian Midwest, state of Goiás city of Goiânia, (16° 39' 10.9"S latitude 49° 15' 40.2"W longitude) were collected in dry season in sterilized 1L polypropylene carboys. Phage isolation, enrichment and purification were performed according to (AKREMI *et al*., 2020). Briefly, 50 mL sample from stream Botafogo were treated with 10 % of chloroform and vortexed 2 minutes, then centrifuged 10.000 g at 4° C for 20 minutes. 200 µL of supernatant was inoculated in exponential phase of bacterial

isolates kv1 and Enb1 (10□) in Nutrient Broth (supplemented with 10 mM $CaCl_2$) and incubated in shaker 130 rpm at 30 °C for 48 h for phage enrichment.

Samples were treated and centrifuged as previously. 100 µL of resulting supernatant was diluted in phage buffer (NaCl 100 mM, MgSO4°7H2O 8 mM, Tris-HCl 1 M, pH 7.5 50 mM, H²0 1 L) (SAMBROOK; RUSSELL, 2001). 100 µL of dilutions was mixed in 200 µL of a fresh kv1 and Enb1 culture and incubate at 30 °C for 10 min to allow phages adsorption. After incubate, samples was inoculated in 1 mL of molten agar at concentration 0,5 % and double-layer assay has performed. The plates was incubate overnight at 30° C. A single lysis plaque as picked, this procedure was repeated for six times for purifies viral particles according to ADDY *et al*. (2019). Purified phages were storage in microcentrifuge tubes at 4° C containing 10 % of chloroform (ADDY *et al*., 2019; DAUBIE *et al*., 2022).

MULTIPLICITY OF INFECTION (MOI)

To determine the Multiplicities of infection (MOI), primary host of SvBr38 and NtBr01 has been used to determine best MOI value at different ratios 0.001, 0.01, 0.1 and 1. A fresh culture of kv1 and Enb1 was infected at different MOIs in nutrient broth $CaCl_2$ (NTC) and incubated overnight at 30 °C. The culture was centrifuged at 10.000 g for 20 minutes, the supernatant of each MOI value were treated with chloroform (10 % v/v) (JAGDALE *et al*., 2019). To determine phage titer, 100 µL of resulting supernatant were serially diluted with 900 µL phage buffer, and double-layer assay was performed as before (WEI *et al*., 2017; RAMÍREZ *et al*., 2017). The best MOI resulting in the highest phage titrate after 24 h incubation, was considered as an optimal MOI and was used in the following experiments. The experiments was repeated in triplicate (JAGDALE *et al*., 2019).

ONE-STEP GROWTH ASSAY

One-step growth assay was conducted as described by Wei *et al*. (2017) and Abdelrhim *et al*. (2021) with minor modifications. The experiment was accomplished in triplicate. A single colony of axenic culture of kv1 and Enb1 was inoculated in 5 mL of nutrient broth and incubated in shaker 130 rpm at 30 °C overnight. 100 µL of primary hosts was resuspended in a nutrient broth and standardized for turbidity tube 0,5 of the McFarland scale (1,5 x 10□ CFU/mL). One milliliter of a high titration (10□ PFU/mL) of SvBr38 and NtBr01 was mixed in 1:1 volume of exponential phase culture of kv1 and Enb1 (approximated 10□ CFU/mL) and incubate at 30 °C for 10 min to allow phage adsorption.

The inoculum was centrifuged at 10.000 g for 10 min and the supernatant was discarded and the sample was washed in equal volume of nutrient broth two times to remove any free unadsorbed phage particle. The pellet was resuspended in 1 mL of nutrient broth and 10 µL was added in 10 mL of nutrient broth and incubate at 30 °C. Throughout this incubation period, 100 µL aliquots were sampled every 10 minutes for two h and immediately diluted in phage buffer for titration. The phage titer and thr burst size was determined according to (AKREMI *et al*., 2020). The burst size was calculated as the ratio of the final count of released bacteriophages at the plateau phase to the initial number of infected bacterial cells.

HOST RANGE ANALYSIS AND SPOT TEST OF SVBR38 AND NTBR01

Host range analysis and spot test of both phages, was determined using ten clinical isolates, *Pseudomonas aeruginosa* 1, *P. aeruginosa* 2, *Klebsiella pneumoniae*, *Acinetobacter balmannii* 01, *A. balmanni* 02, *A. balmanni* 03, *Staphylococcus lugdunensis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Multi-resistant environmental isolates of *Escherichia coli* 01, *E. coli* 2, *E. coli* 3, *E. coli* 4, *E. coli* 5

and *Serratia marcescens*. Including phytopathogenic bacteria *Xanthomonas perforans* 01, *X. perforans* 02 and *X. perforans* 03 and six strains of *Lactobacillus* species, *Lactobacillus Breve*, *L. delbrueckii*, *L. gasseri*, *L. johnsonii*, *L. plantarum* and *L. sporogenes* (Table 1). Totaling twenty-five isolates (Excluding the primary hosts). The ability of phages to lyse bacterial cells was determined using spot test as primary technic, followed by double-layer assay as described by Clokie and Kropinski (2009).

Briefly, 200 µL of a overnight culture of kv1 and Enb1 grown at 30 ° C (padronized by 0,5 MacFarland scale, cell density approximated 10□ CFU/mL) was inoculated in 1 mL of soft agar tubes with 0.5 % agar concentration, and poured onto Nutrient agar plate surface. After 10 minutes, 5 µL of a hight phage titration (±10□) was inoculated on plates. Same volume was used of chloroform and 0.85 % (w/v) saline to positive and negative control respectively, and plates was incubate overnight at 30 °C. Lysis zone was observed 24 h after incubate period. Clear zones are recorded as +, negative zones as – and incomplete or partial zones as p. All the positive spot assay are confirmed by double-layer agar assay.

TEMPERATURE, PH AND UV RADIATION STABILITY

To evaluated temperature effect on viral particles viability, were used a suspension of phages in sterilized phage buffer pH adjust to 7.5 and maintained at constant temperature (10, 20, 30, 40, 50, 60, 70, 80 and 90 °C) water batch, (QUIBERONI *et al*., 2003; PARK *et al*., 2018).

For pH experiment, a new suspension of phages were maintained in NTC with different pH values 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. Temperature of samples as kept at 25 °C (LIU *et al*., 2021).

To evaluate the viability of viral particles to UV radiation, aliquots of phages were used in phage buffer as previously described. Viral particles were exposed to UV radiation at a wavelength of 254 nm, 18 µW/cm2. With exposure times of 0, 5, 15, 30, 40, 50 and 60 minutes, at a distance of 20 cm from the light source (PINHEIRO *et al*., 2019).

TRANSMISSION ELECTRON MICROSCOPY

To determine morphology of the viral particle, characterization was performed using transmission electron microscopy (TEM). 10 µL aliquot of high titration of phages 10□ PFU/mL was applied to a grid composed of an ultrahigh carbon support film for electron microscopy (200 mesh) previously coated with Formvar®. A Pilko guide was used to manipulate and maintain the grid in an appropriate position. Excess was removed with the aid of filter paper. 2% (w/v) uranyl acetate was used for 2 minutes to negatively stain the viral particles. After drying the grids with the adsorbed phages, the viral morphology were observed by transmission electron microscopy and measure in open source software ImageJ (LIU *et al*., 2021). Phage names and classification was based according to criteria proposed by (ADRIAENSSENS; BRISTER, 2017).

BACTERIOPHAGE DNA EXTRACTION

For phage DNA extraction, lysates with the highest available titers were employed, following optimization of the Multiplicity of Infection (MOI). Specifically, MOI values of 0.1 and 0.01 were utilized for isolates NtBr01 and SvBr38, respectively. Extraction of phage DNA was carried out as per method given by Jakočiūnė *et al*. (2018) and Jagdale *et al*. (2019) with minor modifications.

Briefly, the extraction was carried out using the silica column purification method. Initially, 100 µl of the sample was homogenized with 250 µl of lysis buffer and incubated for

10 minutes at 24°C. After lysis, 250 µl of ethanol (99%) was added for precipitation and the mixture was transferred to the centrifugation column.

The washing process consisted of two successive steps with specific buffers (A and B), followed by centrifugation at 13,000 rpm to remove ethanol residues. Finally, the nucleic acids were eluted in 45 µl of elution buffer after incubation for 1 minute at room temperature.

For integrity analysis, 5 µl of the sample was mixed with 1 µl of loading dye (Blue Green Loading Dye I). Fractionation was conducted on a 0.5% agarose gel and subsequently analyzed using the open source image editor, Gimp v. 3.0.6.

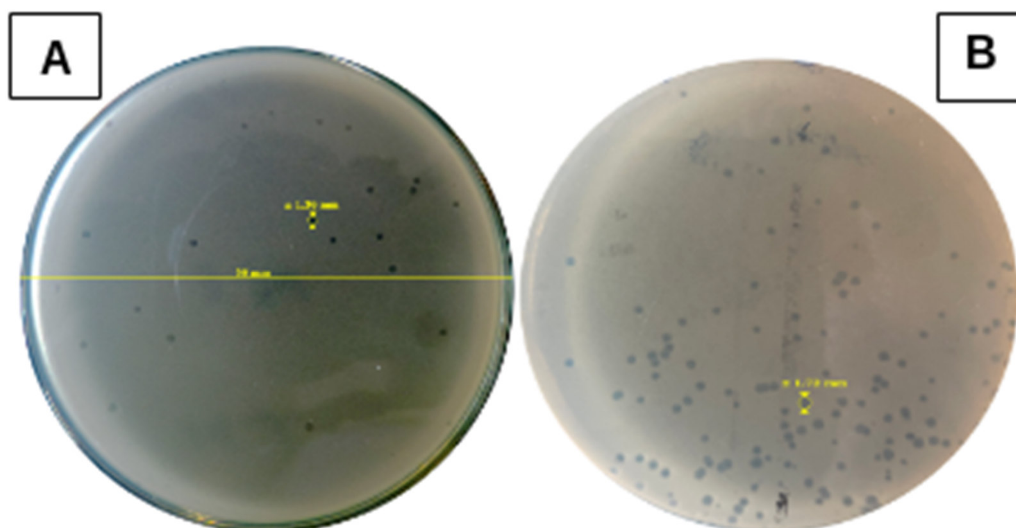
The use of generative artificial intelligence was limited to grammatical review and textual organization, using ChatGPT 5.4 (OpenAI, San Francisco, CA). No scientific content was generated with the assistance of AI. In accordance with Brasil (2026), the authors ensure the integrity, originality, and validation of the results presented.

RESULTS AND DISCUSSION

ISOLATION AND CHARACTERIZATION OF PHAGES FROM WATER SAMPLES IN BRAZILIAN MIDWEST

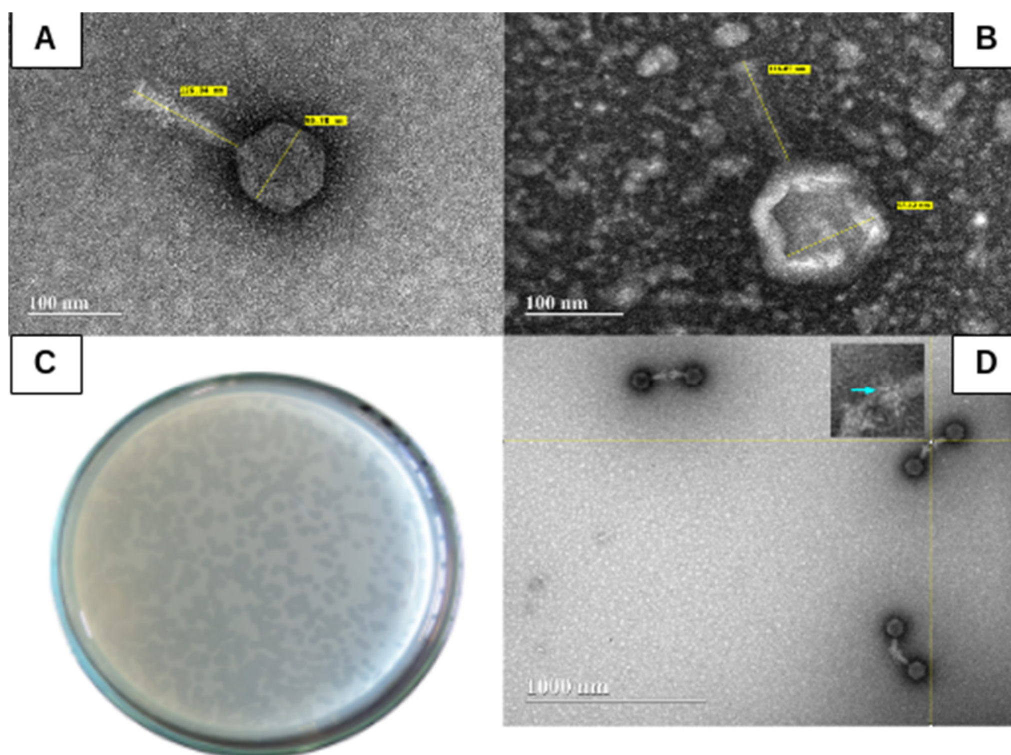
About 5 water samples were collected from Botafogo stream, state of Goiás Brazil, during the dry season. Phages were isolated from each samples, a single lysis plaque obtained from each phage was enriched and purified for further studies, only phages SvBr38 and NtBr01 were successfully purified, showing a range of 1.3 and 1.7 mm diameter of plate size (Figure 1) respectively, on double-layer assay technique with 0.5% of agar using *K. variicola* and *E. hormaechei* as primary hosts. Viral particles showed similar sizes in Electron microscope images, the phage particles had typical morphology of *Caudoviricetes* class (formerly *Myoviridae* family) icosahedral head of approximately 90.78 nm Number of counting (n=10) 126,04 nm (n=10) tail for SvBr38 phage and 98.12 nm (n=10) and 116.87 nm (n=10) tail for phage NtBr01 (Figure 2). Both phages names *Klebsiella phage* vB_KvaM-SvBr38 and *Enterobacter phage* vB_EhoM-NtBr01 were classified according to the phage identifier naming systems (ADRIAENSSENS; BRISTER, 2017) and his taxonomy reallocated according to latest ICTV classification framework (ZHU *et al*., 2022).

FIGURE 1. Formation of Lysis Plaques (zones without bacterial growth). **A** phage SvBr38 on primary host (*K. variicola*) and **B** phage NtBr01 on primary host (*E. Hormaechei*).



Source: Authors (2026)

FIGURE 2. A Morphology of phage SvBr38 **B.** phage NtBr01 **C** phage SvBr38 on host *K. Variicola* after 48 h incubation **D** Cyan arrow showing basal plate and tail fibers of phage SvBr38.



Source: Authors (2026)

Agricultural losses caused by bacterial phytopathogens represent a major threat to global food security. Crops such as tomatoes, potatoes, corn and soybeans are among the most vulnerable Tavares *et al*. (2023). These pathogens are estimated to cause annual losses of 20% to 40% in global production, seriously undermining food security and economic stability (SAVARY *et al*., 2019).

Conventional control strategies, including chemical bactericides and antibiotics, raise increasing concerns related to resistance development and environmental impact. In this context, bacteriophages as promising biological control agents, offering high specificity and the ability to replicate in the bacterial host within the minimal impact on non-target bacterial (BUTTIMER *et al*., 2017).

Two novel phages, designated SvBr38 and NtBr01, were isolated from the Botafogo stream, located in Brazilian Midwest, state of Goiás city of Goiânia, (16° 39' 10.9"S, 49° 15' 40.2"W). Clear plaques, measuring 1.3 to 1.7 mm in diameter, were observed for both phages on their primary host in a double-layer agar assay (Figure 1 A and B). Furthermore, the phages exhibited a broad lytic spectrum, which included other phytopathogens as well as clinical and environmental isolates.

Transmission electron microscopy revealed that the viral particles of both SvBr38 and NtBr01 exhibited typical tailed morphology consistent with members of the class *Caudoviricetes*, as recently redefined by the International Committee on Taxonomy of Viruses (ICTV) (ZHU *et al*., 2022; TURNER *et al*., 2023), featuring an icosahedral head and contractile tail structures (SvBr38: head ≈ 90.78 nm and tail ≈ 126.04 nm; NtBr01: head ≈ 98.12 nm and tail ≈ 116.87 nm; n = 10), (Figure 2 A and B), showing clear lysis plaques in

the double agar layer technique and basal plate (represented by the cyan arrow) in transmission electron microscopy (Figure 2 C and D).

This taxonomic framework supersedes the former morphology-based family designations such as *Myoviridae*, *Podoviridae*, and *Siphoviridae*. The morphological descriptors cited here is only for comparative purposes. Although bacteriophages with activity against *Klebsiella spp*. have been reported from Brazilian stream waters, including two from Minas Gerais state (CAMILO *et al*., 2025).The phages, exhibited a broad lytic spectrum against clinical, environmental, and phyto bacterial isolates from stream Botafogo in Goiás state.

Beyond their activity against agricultural-associated bacteria, phages SvBr38 and NtBr01 exhibited a polyvalence spectrun, for both SvBr38 and NtBr01, spanning multiple ecological niches and taxonomic limits (KLIMENKO *et al*., 2016). These phages exhibited lytic activity against high-priority clinical isolates, such as *Pseudomonas aeruginosa*, *Acinetobacter balmannii* as well as phytopathogens of significant economic impact, including *Xanthomonas spp*. (Table 1). This broad-spectrum performance against gram-negative bacteria suggests an evolved capability to recognize conserved receptors across different gram-negativa bacterial isolates.

The partial lysis observed for phage SvBr38 against *Serratia marcescens* and *Xanthomonas perforans* may reflect incomplete or abortive infection processes, in which phage adsorption occurs without successful replication or virion assembly. Such phenomena may be associated with, partial receptor recognition, or the presence of antiphage defense systems like CRISPR-Cas or abortive infection (LOPATINA *et al*., 2020).

Furthermore, their efficacy extended to multidrug-resistant (MDR) environmental Escherichia coli, a primary agent associated with waterborne diseases in urban ecosystems. A major contact with the multidrug-resistant bacterial host may explain the lysis of E.coli isolates by phage NtBr01 (OSMAN *et al*., 2024).

Despite not infecting gram-positive bacteria, such as *Staphylococcus aureus*, the phage's lack of lytic activity against beneficial species, like *Lactobacillus*, highlights its specificity and possible biosafety (Table 1). This selectivity, which preserves non-target microorganisms, is a vital attribute for biological control agents, suggesting the safe potential of phages SvBr38 and NtBr01 against primary hosts *K. variicola and E. hormaechei* for biocontrol applications.

OPTIMAL MOI VALUE FOR PHAGES SVBR38 AND NTBR01

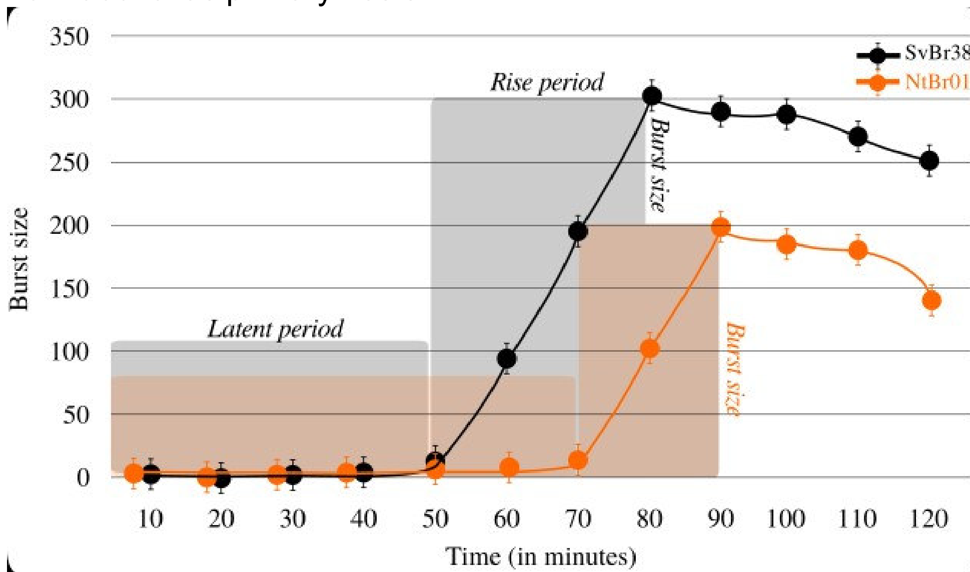
Optimal multiplicity of infection (MOI) of both phages was determined at different ratios (0.001, 0.01, 0.1 and 1 PFU/ml). Between four MOI values analyzed, 0.1 and 0.01 was determined to highest viral titer for phages, 2.1 x 10□ NtBr01 and 1.9 x 10□ for SvBr38. The remaining values generated the lowest phages titer, 1,5 x 10□ and 0,7 x 10□ PFU/ml, after 24 h, respectively (ABDELRHIM *et al*., 2021).

The determination of the optimal Multiplicity of Infection (MOI) is a critical parameter for scaling up phage production, ensuring the maximum viral yield with the minimum initial inoculum. In this study, the highest titers for NtBr01 (2.1×10□ PFU/mL) and SvBr38 (1.9×10□ PFU/mL) were achieved at MOIs of 0.1 and 0.01, respectively. The attractive forces of van der Waals interactions and hydrophobicity overcome the electrostatic repulsion between phage particles, causing them to aggregate. This aggregation, in turn, reduces viral infection efficiency (MATHIEU *et al*., 2018). This phenomenon can explain a low phage titration due to viral agregation (Figure 2D).

EFFICIENCY OF INFECTION OF PHAGES SVBR38 AND NTBR01

One-step growth assays revealed distinct lytic profiles for phages SvBr38 and NtBr01. SvBr38 demonstrated higher replicative efficiency, with a latent period of 50 min and a rise period of 30 min, yielding a burst size of approximately 300 viral particles per infected cell. Conversely, NtBr01 exhibited a prolonged latent period of 70 min followed by a 20 min rise period, resulting in a smaller burst size of 200 particles per cell. Both phages reached their respective plateaus between 80 and 90 min, with a gradual decline in titers observed after 100 min (Figure 3).

FIGURE 3. One-step growth assay for phages SvBr38 and NtBr01 using *K. variicola* and *E. hormaechei* as primary hosts.



Source: Authors (2026)

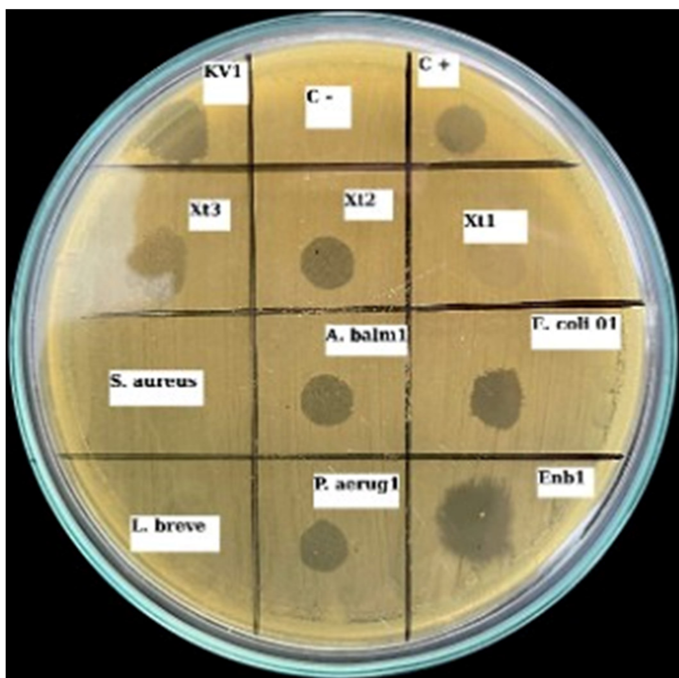
One-step growth curve analysis demonstrated distinct replication dynamics between the two phages, with SvBr38 exhibiting a shorter latent period (~50 min), longer rise period (~30 min), and higher burst size (~300 virions per infected cell) compared to NtBr01, which showed a longer latent period (~70 min), shorter rise period (~20 min), and a burst size of ~200 virions per cell (Figure 3). These differences suggest variation in phage–host interaction efficiencies and intracellular replication strategies, which are consistent with reports of phage biological diversity across related tailed phages. Furthermore our phage display a modest burst size in comparation on other phages, like 10,292 ± 1,097 plaque-forming units (PFUs)/cell of Hafnia paralvei phage Pan *et al*. (2022), nonetheless within expectations for a common burst size used for a biological control (WEI *et al*., 2017).

PRIMATY HOSTS ANALYSIS

Host range analysis revealed that phages SvBr38 and NtBr01 were capable of lysing twelve out of twenty-five of total isolates tested bacterial strains, including clinical, veterinary, and phytopathogenic isolates (Table 1). Notably, no lytic activity was detected against gram-positive bacteria or beneficial *Lactobacillus* species. SvBr38 exhibited a broader host range, uniquely inducing partial lysis in *Xanthomonas perforans* 1 and *Serratia marcescens*. In contrast, NtBr01 showed a higher specificity for all *Escherichia coli* strains tested (Table 1).

Spot test was used a preliminary assay followers a double layer assay as described previously (Figure 4).

FIGURE 4. Spot teste of phage SvBr38. From left to right *Klebsiella variicola*, negative controle, positive control, *Xanthomonas perforans*3, *X. perforans*2, *X. perforans*1, *Staphylococcus aureus*, *Acinetobacter balmannii*, *Escherichia coli*01, *Lactobacillus breve*, *Pseudomonas aeruginosa*1 and *Enterobacter hormaechei*.



Source: Authors (2026)

The host range profiles of phages SvBr38 and NtBr01 reflect the fundamental principle that characterizing host specificity is essential for assessing the efficacy of phage therapy against multiple bacterial strains. This characterization enables targeted efforts toward the treatment of specific bacterial isolates (CHUNG; LIAU; TANG, 2023).

TEMPERATURE PH AND UV STABILITY OF PHAGES SVBR38 AND NTBR01

For thermal assay were used a sterilized phage buffer at 7.5 ph value and 20 min exposure time for each temperature. Phage SvBr38 kept higher titer at 10, 20, 30, 40 and 50 °C with drastic decrease occur in 60 °C (Figure 5). Phage NtBr01 showed more sensitive to thermal effect, with lowest value at 60 °C. No one phage were found in 70, 80 and 90 °C. This data suggest the optimal temperature for phage SvBr38 is 10 to 50 °C and 10 to 40 for NtBr01, and values above 70 °C for both phages are lethal.

The thermal stability of bacteriophages are essential for evaluating bacteriophage stability and persistence on plant surfaces is a decisive factor for their efficacy as biocontrol agents (JONES *et al*., 2012). In this study, the stability of both phages (Figure 5 A) especialy SvBr38 at elevated temperatures is particularly relevant given the climatic conditions of the Brazilian Midwest, where climatic variables can significantly impact food production (FIORINI *et al*., 2024). Similar studies conducted in warm and subtropical regions have demonstrated that phages with greater thermal stability exhibit improved persistence and disease suppression on crops (BALOGH *et al*., 2010; ADDY *et al*., 2019).

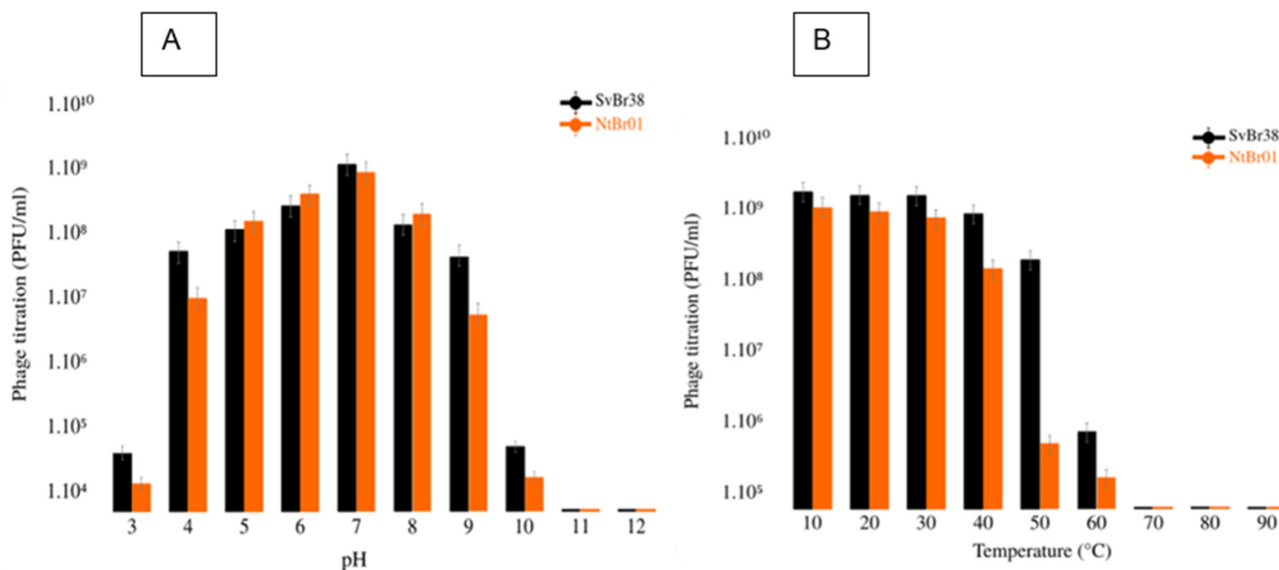
Ph values were analyzed in sterilized NTC with same conditions of exposure. NtBr01 showed stability over pH range 5 and 8 while SvBr38 showed higher stability over pH range 4 and 9. pH range less than 4 and 10 kept viral titer at lowest concentration, for NtBr01 and for SvBr38. No viral particles were detected at pH range 11 and 12 (Card 1). This data showed that SvBr38 is more stable at pH range 4 and 9 than NtBr01.

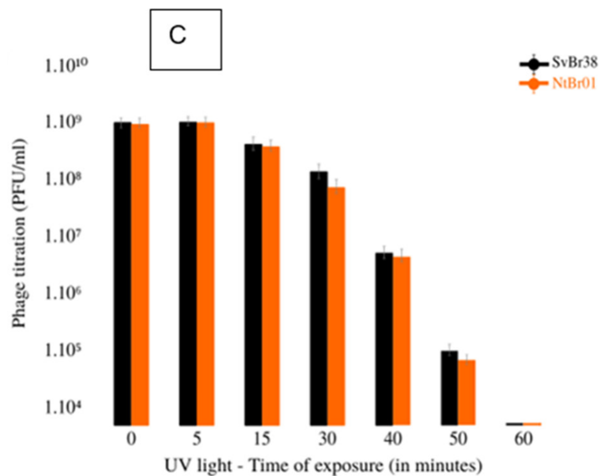
Environmental stability assays demonstrated that both pH and ultraviolet (UV) radiation significantly influence the persistence of phages SvBr38 and NtBr01, factors that are critical for their effectiveness in agricultural applications. NtBr01 remained stable within a pH range of 5–8, whereas SvBr38 exhibited broader tolerance, maintaining higher stability between pH range 4–9. In contrast, extreme acidic or alkaline conditions (pH ≤4 or ≥10) markedly reduced viral titers, with complete inactivation observed at pH 11–12 (Figure 5 B). This data suggesting that the former is better adapted to the soil acidification, or alkaline water, common in certain lands in Brazil (REIS; RODELLA, 2002; FARIA *et al*., 2009).

UV stability assays revealed a similar inactivation profile for both phages, NtBr01 and SvBr38. Exposure to UV radiation (254 nm, 18 μW/cm2) resulted in a progressive decline in viral viability over time (Card 1). Complete inactivation was achieved at the 60 minutes, with no detectable plaque forming units (PFU/mL) for either isolate.

Similarly, both phages showed progressive loss of infectivity under UV radiation exposure (254 nm), reaching complete inactivation after 60 min. UV sensitivity is a well-documented limitation of phage persistence on plant surfaces, largely attributed to nucleic acid damage (ŠEVIĆ *et al*., 2019). Nonetheless both phages, exhibited good stability to UV radiation even after 40 minutes of exposure. Viral titer decrease was observed only after 50 minutes of exposure (Figure 5 C).

FIGURE 5 . Left to right, Thermal test of phages SvBr38 and NtBr01, pH test at range 3 to 12 of phages SvBr38 and NtBr01 and Viability of phages SvBr38 and NtBr01 after ultraviolet (UV) irradiation at 254 nm.



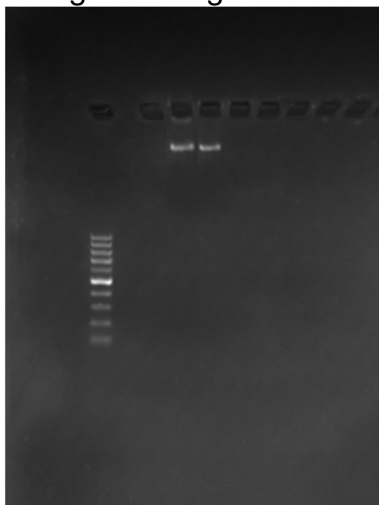


Source: Authors (2026)

MORPHOLOGICAL CHARACTERIZATION AND GENOMIC INTEGRITY OF PHAGES SVBR38 AND NTBR01

Transmission electron microscopy (TEM) analysis revealed the structural morphology of phages SvBr38 and NtBr01. Morphometric measurements performed via ImageJ software ensured the determination of capsid diameters and tail lengths with characteristics compatible with the *Caudoviricetes* class. The genomic extraction yielded high-purity nucleic acids from lysates. Agarose gel electrophoresis (0.5%) was used to assess the integrity and estimated molecular weight of the extracted viral nucleic acids, which appeared as well-defined, high-molecular-weight bands without visible degradation (Figure 5).

FIGURE 6. Assessment of viral DNA integrity by electrophoresis in 0.5% agarose gel showing intact high molecular weight bands.



Source: Authors (2026)

Genomic extraction from phage lysates yielded high-purity viral nucleic acids, as evidenced by agarose gel electrophoresis (0.5%), which revealed well-defined, high–molecular-weight bands with no signs of fragmentation or degradation (JAKOČIŪNĖ; MOODLEY, 2018). The integrity of the extracted genomes indicates efficient purification and

supports their suitability for downstream molecular analyses, including sequencing and genomic characterization in the future.

CONCLUSION

The characterization of phages SvBr38 and NtBr01 establishes them as promising candidates for the biological control of K. variicola and E. hormaechei, pathogens that compromise the quality of major cultivars such as tomato, potato, and soybean. Their stability across a wide range of temperatures and pH levels, combined with significant resilience to UV radiation, suggests high viability and persistence under adverse environmental conditions, particularly in tropical regions like Brazil. Although the primary focus of this work lies in plant disease management, the broad lytic activity of phages SvBr38 and NtBr01 suggests therapeutic potential against emerging and hard-to-treat bacterial isolates.

The successful extraction of high-quality genetic material enables subsequent r whole-genome sequencing, which is essential to confirm the absence of virulence factors and elucidate the mechanisms underlying their extended host range. This study presents an integrated approach within the One Health framework, offering a sustainable alternative for managing pathogens that are increasingly resistant to conventional treatment methods.

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REFERENCES

ABDELRHIM, A. S.; AHMAD, A. A.; OMAR, M. O. A.; HAMMAD, A. M. M.; HUANG, Q. A new Streptomyces scabies-infecting bacteriophage from Egypt with promising biocontrol traits. **Archives of Microbiology**, [*S. l.*], v. 203, n. 7, p. 4233–4242, set. 2021.https://doi.org/10.1007/s00203-021-02415-2.

ADDY, H. S.; AHMAD, A. A.; HUANG, Q. Molecular and Biological Characterization of Ralstonia Phage RsoM1USA, a New Species of P2virus, Isolated in the United States. **Frontiers in Microbiology**, [*S. l.*], v. 10, p. 267, 19 fev. 2019.https://doi.org/10.3389/fmicb.2019.00267.

ADRIAENSSENS, E.; BRISTER, J. R. How to Name and Classify Your Phage: An Informal Guide. **Viruses**, [*S. l.*], v. 9, n. 4, p. 70, 3 abr. 2017. https://doi.org/10.3390/v9040070.

AKREMI, I.; HOLTAPPELS, D.; BRABRA, W.; JLIDI, M.; HADJ IBRAHIM, A.; BEN ALI, M.; FORTUNA, K.; AHMED, M.; MEERBEEK, B. V.; RHOUMA, A.; LAVIGNE, R.; BEN ALI, M.; WAGEMANS, J. First Report of Filamentous Phages Isolated from Tunisian Orchards to Control Erwinia amylovora. **Microorganisms**, [*S. l.*], v. 8, n. 11, p. 1762, 10 nov. 2020.https://doi.org/10.3390/microorganisms8111762.

AKREMI, I.; HOLTAPPELS, D.; BRABRA, W.; JLIDI, M.; HADJ IBRAHIM, A.; BEN ALI, M.; FORTUNA, K.; AHMED, M.; MEERBEEK, B. V.; RHOUMA, A.; LAVIGNE, R.; BEN ALI, M.; WAGEMANS, J. First Report of Filamentous Phages Isolated from Tunisian Orchards to Control Erwinia amylovora. **Microorganisms**, [*S. l.*], v. 8, n. 11, p. 1762, 10 nov. 2020.https://doi.org/10.3390/microorganisms8111762.

BALOGH, B.; JONES, J.; IRIARTE, F.; MOMOL, M. Phage Therapy for Plant Disease Control. **Current Pharmaceutical Biotechnology**, [*S. l.*], v. 11, n. 1, p. 48–57, 1 jan. 2010.https://doi.org/10.2174/138920110790725302.

BRASIL. Conselho Nacional de Desenvolvimento Científico e Tecnológico. Portaria CNPq nº 2.664, de 6 de março de 2026: Institui a Política de Integridade na Atividade Científica do CNPq. Brasília, DF: Ministério da Ciência, Tecnologia e Inovação, 2026. Available at: https://www.in.gov.br/web/dou/-/portaria-cnpq-n-2.664-de-6-de-marco-de-2026-691779232. Accessed on: Apr. 26, 2026.

BRAVO, A.; GILL, S. S.; SOBERÓN, M. Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. **Toxicon**, [*S. l.*], v. 49, n. 4, p. 423–435, mar. 2007.https://doi.org/10.1016/j.toxicon.2006.11.022.

CAMILO, I. E. O.; PEIXOTO, M. D. O.; SANTOS, E. G. C. D.; BRUCKNER, F. P.; CASTELUBER, M. C. D. F. Bacteriophages isolate of stream water in Brazil lyses the superbugs Klebsiella spp. **Research, Society and Development**, [*S. l.*], v. 14, n. 10, p. e151141049849, 25 out. 2025.https://doi.org/10.33448/rsd-v14i10.49849.

CLOKIE, M. R. J.; KROPINSKI, A. M. (org.). **Bacteriophages**. Totowa, NJ: Humana Press, 2009. (Methods in Molecular Biology). DOI:10.1007/978-1-60327-164-6. Available at:: http://link.springer.com/10.1007/978-1-60327-164-6. Accessed on: Jan. 6, 2026.

DANTAS, C. W. D.; MARTINS, D. T.; NOGUEIRA, W. G.; ALEGRIA, O. V. C.; RAMOS, R. T. J. Tools and methodology to in silico phage discovery in freshwater environments. **Frontiers in Microbiology**, [*S. l.*], v. 15, p. 1390726, 31 maio 2024.https://doi.org/10.3389/fmicb.2024.1390726.

DAUBIE, V.; CHALHOUB, H.; BLASDEL, B.; DAHMA, H.; MERABISHVILI, M.; GLONTI, T.; DE VOS, N.; QUINTENS, J.; PIRNAY, J.-P.; HALLIN, M.; VANDENBERG, O. Determination of phage susceptibility as a clinical diagnostic tool: A routine perspective. **Frontiers in Cellular and Infection Microbiology**, [*S. l.*], v. 12, p. 1000721, 21 set. 2022.https://doi.org/10.3389/fcimb.2022.1000721.

DIALLO, M. C. A., SANTOS, R. C., GOMES, E. P., MACHADO, C. A. C., DIAS, C. R. A., SANTOS, E. C. D., ... & LOVATTO, J. Challenges of smart irrigation implementation in water optimization and agricultural sustainability. **Contrib. LAS Cienc. Soc,** v.17, n.13, e13723-e13723, 2024. https://doi.org/10.55905/revconv.17n.13-270.

CHUNG, K. M.; LIAU, X. L.; TANG, S. S. Bacteriophages and Their Host Range in Multidrug-Resistant Bacterial Disease Treatment. **Pharmaceuticals**, [*S.l.*], v. 16, n. 10, p. 1467, 2023. https://doi.org/10.3390/ph16101467.

FAO-FAOSTAT - Food and Agriculture Organization of the United Nations, 2025. Available at: <http://faostat.fao.org/>. Acess: Jun 2025.

FARIA, F. H. D. S.; LIMA, L. A.; RIBEIRO, M. S.; SANTOS, S. R.; RIBEIRO, K. M. AVALIAÇÃO DA SALINIDADE, SODICIDADE E ALCALINIDADE DAS ÁGUAS SUBTERRÂNEAS PARA IRRIGAÇÃO EM JAÍBA E JANAÚBA, MINAS GERAIS.**IRRIGA**,

[*S. l.*], v. 14, n. 3, p. 299–313, 30 set. 2009.https://doi.org/10.15809/irriga.2009v14n3p299-313.

FIORINI, A. C. O.; ANGELKORTE, G.; BAKMAN, T.; BAPTISTA, L. B.; CRUZ, T.; A DIUANA, F.; MORAIS, T. N.; RATHMANN, R.; DA SILVA, F. T. F.; TAGOMORI, I.; ZANON-ZOTIN, M.; LUCENA, A. F. P.; SZKLO, A.; SCHAEFFER, R.; PORTUGAL-PEREIRA, J. How climate change is impacting the Brazilian agricultural sector: evidence from a systematic literature review. **Environmental Research Letters**, [*S. l.*], v. 19, n. 8, p. 083001, 1 ago. 2024.https://doi.org/10.1088/1748-9326/ad5f42.

JAGDALE, S.; AHIWALE, S.; GAJBHIYE, M.; KAPADNIS, B. Green approach to phytopathogen: Characterization of lytic bacteriophages of Pseudomonas sp., an etiology of the bacterial blight of pomegranate.**Microbiological Research**, [*S. l.*], v. 228, p. 126300, nov. 2019.https://doi.org/10.1016/j.micres.2019.126300.

JAKOČIŪNĖ, D.; MOODLEY, A. A Rapid Bacteriophage DNA Extraction Method. **Methods and Protocols**, [*S. l.*], v. 1, n. 3, p. 27, 27 jul. 2018. https://doi.org/10.3390/mps1030027.

JONES, J. B.; VALLAD, G. E.; IRIARTE, F. B.; OBRADOVIĆ, A.; WERNSING, M. H.; JACKSON, L. E.; BALOGH, B.; HONG, J. C.; MOMOL, M. T. Considerations for using bacteriophages for plant disease control.**Bacteriophage**, [*S. l.*], v. 2, n. 4, p. e23857, out. 2012. https://doi.org/10.4161/bact.23857.

KLIMENKO, A. I.; MATUSHKIN, Y. G.; KOLCHANOV, N. A.; LASHIN, S. A. Bacteriophages affect evolution of bacterial communities in spatially distributed habitats: a simulation study. **BMC Microbiology**, [*S. l.*], v. 16, n. S1, p. S10, dez. 2016.https://doi.org/10.1186/s12866-015-0620-4.

LAHLALI, R.; EZRARI, S.; RADOUANE, N.; KENFAOUI, J.; ESMAEEL, Q.; EL HAMSS, H.; BELABESS, Z.; BARKA, E. A. Biological Control of Plant Pathogens: A Global Perspective. **Microorganisms**, [*S. l.*], v. 10, n. 3, p. 596, 9 mar. 2022.https://doi.org/10.3390/microorganisms10030596.

LIU, Y.; LIU, M.; HU, R.; BAI, J.; HE, X.; JIN, Y. Isolation of the Novel Phage PHB09 and Its Potential Use against the Plant Pathogen Pseudomonas syringae pv. actinidiae. **Viruses**, [*S. l.*], v. 13, n. 11, p. 2275, 14 nov. 2021.https://doi.org/10.3390/v13112275.

LLOYD, D. H. Alternatives to conventional antimicrobial drugs: a review of future prospects. **Veterinary Dermatology**, [*S. l.*], v. 23, n. 4, p. 299, ago. 2012.https://doi.org/10.1111/j.1365-3164.2012.01042.x.

LOPATINA, A.; TAL, N.; SOREK, R. Abortive Infection: Bacterial Suicide as an Antiviral Immune Strategy. **Annual Review of Virology**, [*S. l.*], v. 7, n. 1, p. 371–384, 29 set. 2020.https://doi.org/10.1146/annurev-virology-011620-040628.

MATHIEU, J.; YU, P.; ZUO, P.; DA SILVA, M. L. B.; ALVAREZ, P. J. J. Going Viral: Emerging Opportunities for Phage-Based Bacterial Control in Water Treatment and Reuse. **Accounts of Chemical Research**, [*S. l.*], v. 52, n. 4, p. 849–857, 16 abr. 2019.https://doi.org/10.1021/acs.accounts.8b00576.

MEFTAUL, I.; VENKATESWARLU, K.; DHARMARAJAN, R.; ANNAMALAI, P.; MEGHARAJ, M. Pesticides in the urban environment: A potential threat that knocks at the door, Science of the Total Environment, v.711, p.1-55, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/31810707/>. Accessed: JUN. 6, 2025. doi: 10.1016/j.scitotenv.2019.134612.

SEKYERE, J.; RETA, M. A. Global evolutionary epidemiology and resistome dynamics of *Citrobacter species ,Enterobacter hormaechei ,Klebsiella variicola ,and Proteeae clones*.**Environmental Microbiology**, [*S. l.*], v. 23, n. 12, p. 7412–7431, dez. 2021.https://doi.org/10.1111/1462-2920.15387.

OSMAN, M.; DAABOUL, D.; TAJANI, A. G.; EL OMARI, K.; BISHA, B.; HASSAN, J.; CAZER, C. L.; FIORELLA, K. J.; KARAH, N.; ABBARA, A.; HAMZE, M.; CUMMINGS, K. J.; NAAS, T.; KASSEM, I. I. Multidrug-resistant pathogens contaminate river water used in irrigation in disenfranchised communities. **Journal of Global Antimicrobial Resistance**, [*S. l.*], v. 36, p. 175–180, mar. 2024.https://doi.org/10.1016/j.jgar.2023.12.016.

PACÍFICO, C.; HILBERT, M.; SOFKA, D.; DINHOPL, N.; PAP, I.-J.; ASPÖCK, C.; CARRIÇO, J. A.; HILBERT, F. Natural Occurrence of Escherichia coli-Infecting Bacteriophages in Clinical Samples. **Frontiers in Microbiology**, [*S. l.*], v. 10, p. 2484, 31 out. 2019.https://doi.org/10.3389/fmicb.2019.02484.

PAN, L.; LI, D.; SUN, Z.; LIN, W.; HONG, B.; QIN, W.; XU, L.; LIU, W.; ZHOU, Q.; WANG, F.; CAI, R.; QIAN, M.; TONG, Y. First Characterization of a Hafnia Phage Reveals Extraordinarily Large Burst Size and Unusual Plaque Polymorphism. **Frontiers in Microbiology**, [*S. l.*], v. 12, p. 754331, 8 fev. 2022.https://doi.org/10.3389/fmicb.2021.754331.

PARK, J.; LEE, G. M.; KIM, D.; PARK, D. H.; OH, C.-S. Characterization of the Lytic Bacteriophage phiEaP-8 Effective against Both Erwinia amylovora and Erwinia pyrifoliae Causing Severe Diseases in Apple and Pear. **The Plant Pathology Journal**, [*S. l.*], v. 34, n. 5, p. 445–450, 1 out. 2018.https://doi.org/10.5423/PPJ.NT.06.2018.0100.

PINHEIRO, L. A. M.; PEREIRA, C.; FRAZÃO, C.; BALCÃO, V. M.; ALMEIDA, A. Efficiency of Phage φ6 for Biocontrol of Pseudomonas syringae pv. syringae: An in Vitro Preliminary Study. **Microorganisms**, [*S. l.*], v. 7, n. 9, p. 286, 23 ago. 2019.https://doi.org/10.3390/microorganisms7090286.

QUIBERONI, A.; GUGLIELMOTTI, D. M.; REINHEIMER, J. A. Inactivation of Lactobacillus delbrueckii bacteriophages by heat and biocides.**International Journal of Food Microbiology**, [*S. l.*], v. 84, n. 1, p. 51–62, jul. 2003.https://doi.org/10.1016/S0168-1605(02)00394-X.

RAKHUBA, D. V.; KOLOMIETS, E. I.; DEY, E. S.; NOVIK, G. I. Bacteriophage Receptors, Mechanisms of Phage Adsorption and Penetration into Host Cell. **Polish Journal of Microbiology**, [*S. l.*], v. 59, n. 3, p. 145–155, 2010.https://doi.org/10.33073/pjm-2010-023.

RAMÍREZ, M.; NEUMAN, B. W.; RAMÍREZ, C. A. Bacteriophages as promising agents for the biological control of Moko disease (Ralstonia solanacearum) of banana. **Biological Control**, [*S. l.*], v. 149, p. 104238, out. 2020.https://doi.org/10.1016/j.biocontrol.2020.104238.

REIS, T. C.; RODELLA, A. A. Cinética de degradação da matéria orgânica e variação do pH do solo sob diferentes temperaturas. **Revista Brasileira de Ciência do Solo**, [*S. l.*], v. 26, n. 3, p. 619–626, set. 2002.https://doi.org/10.1590/S0100-06832002000300006.

RIVERA, B., ORELLANA-SERRADELL, O., SERVILI, E., SANTOS, R., BRAUCHI, S., & CERDA, O. The odyssey of the TR (i) P journey to the cellular membrane. **Frontiers in Cell and Developmental Biology,** v.12, p.1414935, 2024. https://doi.org/10.3389/fcell.2024.1414935

RODRÍGUEZ-MEDINA, N.; BARRIOS-CAMACHO, H.; DURAN-BEDOLLA, J.; GARZA-RAMOS, U.*Klebsiella variicola* : an emerging pathogen in humans. **Emerging Microbes & Infections**, [*S. l.*], v. 8, n. 1, p. 973–988, jan. 2019.https://doi.org/10.1080/22221751.2019.1634981.

SAMBROOK J, RUSSELL DW. Molecular Cloning A LABORATORY MANUAL. New York, 2001.
SAVARY, S.; WILLOCQUET, L.; PETHYBRIDGE, S. J.; ESKER, P.; MCROBERTS, N.; NELSON, A. The global burden of pathogens and pests on major food crops. **Nature Ecology & Evolution**, [*S. l.*], v. 3, n. 3, p. 430–439, 4 fev. 2019.https://doi.org/10.1038/s41559-018-0793-y.

SANTANA, C. S., SANTOS, R. C., DE CARVALHO, T. I., JORDAN, R. A., SANCHES, A. C., FILHO, R. R. G., ... & DE ALBUQUERQUE MOURA, G. B. Artificial neural network for assessment of impacts of rural anthropization in a tropical climate region. **Revista Brasileira de Geografia Física,** v.17, n.2, p.1071-1085. 2024. https://doi.org/10.26848/rbgf.v17.2.p1071-1085

ŠEVIĆ, M.; GAŠIĆ, K.; IGNJATOV, M.; MIJATOVIĆ, M.; PROKIĆ, A.; OBRADOVIĆ, A. Integration of biological and conventional treatments in control of pepper bacterial spot. **Crop Protection**, [*S. l.*], v. 119, p. 46–51, maio 2019.https://doi.org/10.1016/j.cropro.2019.01.006.

TACCA, L. M. A.; LIMA, R. N.; PASCOAL, P. V.; OLIVEIRA, M. A.; AZEVEDO, C. A .X.; BAMBIL, D.; MIZOTTE, P.; ROSINHA, G. M. S.; BITTENCOURT, D. M. C.; SIGNOR, D.; MOURA, M. S. B.; TRINDADE, J. P. P.; VOLK, L. B. S.; FERNANDES, F. A.; LOPES, R.; ARAÚJO, L.; FREIRE, M.; RECH, E. Bridging Gaps in Soil Ecology: Metagenomic Insights into Microbial Diversity and Functionality Across Brazil's Biomes.BioRxiv, p. 1-26, may. 2025. https://doi.org/10.1101/2025.05.16.654316.

TAVARES, R. S.; MACHADO, L. B.; CARNEIRO, L. C.; VIEIRA, M. M.; VIEIRA, J. D. G. BACTERIOPHAGES AS BIOLOGICAL CONTROL AGENTS AND NEW TRENDS IN MODERN AGRICULTURE. ENCICLOPÉDIA BIOSFERA, v. 20, n.44, p. 53, 2023. https://DOI: 10.18677/EnciBio_2023B4.

TURNER, D.; et al. Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. **Archives of Virology**, [*S. l.*], v. 168, n. 2, p. 74, fev. 2023.https://doi.org/10.1007/s00705-022-05694-2.

WEI, C.; LIU, J.; MAINA, A. N.; MWAURA, F. B.; YU, J.; YAN, C.; ZHANG, R.; WEI, H. Developing a bacteriophage cocktail for biocontrol of potato bacterial wilt. **Virologica**

Sinica, [*S. l.*], v. 32, n. 6, p. 476–484, dez. 2017.https://doi.org/10.1007/s12250-017-3987-6.

ZHU, Y.; SHANG, J.; PENG, C.; SUN, Y. Phage family classification under Caudoviricetes: A review of current tools using the latest ICTV classification framework. **Frontiers in Microbiology**, [*S. l.*], v. 13, p. 1032186, 16 dez. 2022.https://doi.org/10.3389/fmicb.2022.1032186.