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Evaluation of Bacteriophages for the Biocontrol of Tomato wilt Disease Caused by *Ralstonia solanacearum*

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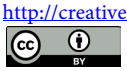


Abstract

Aim: This study was carried out in order to evaluate the potential of bacteriophages in controlling tomato bacterial wilt disease caused by *Ralstonia solanacearum*. Study design: A purposive sampling technique was used to collect samples from bacterial wilt hot spot tomato growing areas in Kenya. Place and duration of study: The research work was done at Jomo Kenyatta University of Agriculture and Technology, between June 2020 and July 2021. Methodology: Thirty diseased plants and corresponding soil were collected from five counties, Nyeri, Kajiado, Nyandarua, Kiambu and Taita Taveta. Bacteria were isolated and characterized, and then used as hosts to propagate the phages. Tests done were gram stain, oxidation test, potassium hydroxide solubility test, H2S production test catalase test, NaCl test and sugar fermentation test. Molecular analysis and phenotyping were also done in order to identify the bacteria. The bacteriophages were then isolated through a double overlay method using *R. solanacearum* as the host. They were characterized and assayed in a greenhouse setting to determine their effectiveness in controlling bacterial wilt. Results: Six host bacteria were isolated and all belonged to biovar II. Four phages were observed based on morphology. Upon characterization the phages were stable at 30˚C, PH range between 6 - 7 and tolerance of more than an hour under UV light. In the greenhouse experiment, treatment of plants with bacteriophage prevented wilting after subsequent inoculation with the pathogen. A bacteriophage mix of SN1 and WT1 were used for efficacy tests due to their efficiency in plating and infection. Phage SN1 and WT1 exhibited high lytic activity and relatively high thermotolerance and acid tolerance, thereby showing great potential in the biocontrol of bacterial wilt infection across a variety of conditions. Conclusion: The results obtained in this research show that bacteriophages offer potential for the biocontrol of bacterial wilt.

Keywords

Ralstonia solanacearum, Bacteriophages, Bacterial Wilt, Bio-Control, Tomatoes

1. Introduction

1.1. Background of the Study

Tomato (*Solanum lycopersicum*), is the second most important vegetable crop in the world after potato [1]. It belongs to the solanaceae family which includes other known species such as round potato, pepper and eggplant [2]. The crop originated in the South Andes, then later, from Europe to Asia, the Middle East, and Africa [3]. Bacterial wilt is an important plant disease in many crops, including tomatoes. It damages more than 200 species in 50 botanical families occurring widely in the world, and persists in the environment [4]. The causal organism for the disease is a bacterial pathogen*, Ralstonia solanacearum* [5].

Tomato production is globally estimated at 161,793,834 tonnes per year with production of 33.6 tonnes per hectare implying the potential of the crop to develop into a high value crop [6]. It is proclaimed that global tomato production has increased by about 300% in the last four decades [7].

In Africa, Kenya is ranked 6th among tomato producers with an approximate production of 70 - 80 tonnes /hectare annually [8]. In Kenya, tomato farming is an important economic activity for smallholder farmers [9]. It is among the most promising crops in horticultural expansion. It accounts for 14% of total vegetable production and 6.72% of the total horticultural crops [10]. Tomatoes are either grown in open fields or under greenhouse technology. The major tomato growing counties are Kirinyaga, Kajiado, Nyandarua, Taita Taveta and Kiambu [9]. The crop is widely grown because of its importance and value. It is one of the most highly consumed vegetable crops in Kenya. Very large quantities of tomatoes are processed and preserved in a variety of forms, the most predominant form being tomato paste used to make products such as pastes and soups. Tomatoes also decrease the risk of conditions such as cancer, osteoporosis and cardiovascular diseases. Tomatoes are not only food but also act as medicine, a flavoring ingredient, detoxificant and human system cleanser [11].

Tomato production however is affected by both living and non-living factors leading to losses of approximately 25% - 42% worldwide [2]. Among the living factors, bacterial wilt has been termed as the most devastating factor especially for countries in the tropics and subtropics whereby Kenya is included. The bacteria is well adapted to the soil and thrives in the rhizobium. It has a diverse range of virulence factors and enters the roots from artificial or Natural openings and populates vascular tissues leading to internal and external symptoms hence causing wilting of the plant [5].

The standard management measures of bacterial disease in place involve use of

chemicals and other cultural practices like crop rotation. However the development of resistance, the wide host range of the bacterium, and its ability to survive for long periods in the environment restrict the effectiveness of cultural and chemical measures [12]. For instance antibiotics streptomycin, ampicillin, tetracycline and penicillin show hardly any effect on the bacteria, in fact, and streptomycin application increased the occurrence of bacterial wilt in Egypt.

Bacteriophages offer an alternative to the standard control measures for managing bacterial wilt [13]. Bacteriophages are viruses that infect bacteria with no direct negative effects on animals or plants [14]. Colonization of a bacterium by a virulent bacteriophage leads to rapid viral replication, followed by lysis of the bacterium and the release of massive progeny bacteriophages. These bacteriophages can then move on to infect nearby bacteria. Therefore the number of bacteriophages will multiply when the target pathogen is met and the remedy will essentially be increased in response to the bacterial infection [14].

Studies indicate that the potential bacteriophages have been isolated from different environmental sources (such as soil, river water, and sewage phyllo spheres) and successfully used in controlling different bacteria in plants [15]. For instance cabbage rot disease that is attributed to *Xanthomonas campestris pv. Campetris* [16], bacterial spot disease of pepper caused by *Xanthomonas campestris pv. vesicatoria* as well as blackleg disease of potato caused by *Pectobacterium carotovorum subsp. atrospeticum* [17].

Advantages of Biocontrol over chemical control [18].

Low toxicity: Biocontrol is safe for people, workers, and consumers.

Short or no pre-harvest intervals: Biocontrol can be used without waiting long periods before harvesting.

Environmentally friendly: Biocontrol is made from natural products that break down quickly.

Doesn't disrupt ecosystems: Biocontrol doesn't harm biodiversity or ecosystems.

Energy self-sufficient: Biocontrol can be self-sufficient in terms of energy.

Slows down pest resistance: Biocontrol can use multiple modes of action, which slows down resistance to pests.

Cost-effective: Biocontrol can be cost-effective, especially over the long term.

Incorporates well into integrated pest management: Biocontrol can be easily integrated into integrated pest management (IPM) programs.

However, pests can develop resistance to natural enemies, as they can with pesticides.

1.2. Problem Statement

The control of bacterial diseases has become a challenge globally. This is due to various characteristics of the causative agent *Ralstonia solanacearum*. Such include, its genetic variation, its ability to overcome plant genetic resistance, the emergence of resistant bacterial strains, the ability to achieve high populations in a relatively short span of time and lack of effective bactericides for use [19]. While

economic losses realized from *R. solanacearum* remain high, control by conventional measures such as spraying using chemicals and mixtures of herbs and ash have proved fruitless. For instance, chemical control is ineffective. Antibiotics streptomycin, ampicillin, tetracycline and penicillin show hardly any effect; in fact, streptomycin application increased the occurrence of bacterial wilt in Egypt [17].

The ability of the pathogen to persist in soil for long periods restricts the effectiveness of cultural and chemical control measures [17].

Moreover, biocontrol of this *Ralstonia solanacearum* using bacteriophages has not been evaluated in Kenya.

1.3. Justification

Tomatoes are important for food and a source of income for small-scale farmers [11]. However, the crop is massively affected by bacterial wilt that is attributed to *Ralstonia solanacearum* [4].

Moreover, there are no known methods of eradicating these bacteria, except chemical and cultural methods which have been proven ineffective.

In addition, the cultural methods are not feasible for small-scale farmers hence may lead to losses and low production, which makes it inadequate for consumers in terms of food security. There is therefore need to find an affordable and sustainable method to control tomato bacterial wilt.

Phages offer an alternative for this because phages are very specific in infection and have no direct effect on other microbes that maybe beneficial to the plant or the plant itself [14]. Bacteriophages also have mechanisms to avert the host's resistance and are very efficacious.

They also self-replicate and easily magnify the response towards the pathogen within a short span of time. Globally, bacteriophages have been successfully exploited as biocontrol agents against plant pathogenic bacteria.

In Kenya however bacteriophage application for biocontrol of plant diseases is non-existent. This study therefore aimed at exploiting the potential of bacteriophages to control bacterial wilt in tomatoes.

2. Methodology

2.1. Sample Collection

A total of thirty diseased tomato plants showing typical wilting symptoms were picked from farmers' fields for assessment, using purposive sampling. The sampled areas were Njambini-Nyandarua County (NN), Birika-Kajiado County (BK), Sagana-Nyeri County (SN), Wundanyi-Taita, and Taveta County (WT).

To confirm that the wilt was due to *R. solanacearum*, the stem was cut eight Centimeters in length from the root base of the wilted plant and the stem portion was placed in a clear glass beaker filled with clear water. The presence of oozing milky exudates from the cut stem section was proof that the pathogen was *R. solanacearum* [14]. Labelling of each sample was done with the sample's location,

date and sample identification number.

For isolation of bacteriophage, a hundred grams of soil samples were collected from 10 to 30 cm deep using a soil sampling tube (3 cm in diameter) and put into sterile plastic bags. Samples were transported to SAJOREC Laboratory-JKUAT in a cooler box, and stored at 4˚C until further work.

2.2. Isolation and Identification of *R. solanacearum* Bacteria

To isolate *R. solanacearum* bacteria, plant stems were thoroughly washed in tap water, and surface sterilized by dipping into 70% ethanol and flaming on a Bunsen burner. They were then chopped into 1 cm pieces into sterilized water in a sterile capped bottle. The stem pieces were maintained in the water for 30 min to diffuse the bacteria into the water. After 30 min, 2 loopfuls of the water suspension were streaked on triphenyl tetrazolium chloride (TTC) medium [14] containing 10 g peptone; 10 g dextrose; 1 g casamino acid (Difco), 18g agar, 1liter distilled water, and filter sterilized 1% aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride, to give a final concentration of 0.005%, and then incubated at 30˚C for 48 h. After 48 h incubation, purification of *R. solanacearum*-looking colonies was made by streaking technique. Colonies were selected on the basis of their fluidity, color, and morphology and stored in sterile distilled water in test tubes at room temperature [20] to reduce mutation. The strains were routinely cultured on tetrazolium chloride (TTC) medium to maintain their viability.

2.3. Biochemical Characterization of *R. solanacearum.*

2.3.1. Gram Staining

Pure cultures (48 hrs.) were used for gram staining reaction according to [21].

2.3.2. Oxidase Test, Catalase Test, KOH Solubility, NaCl Tolerance and Growth at 37 and 41˚C

The above tests were performed according to [22].

2.3.3. Oxidase Test

Oxidase activity was detected by the method of Kovacs. Freshly grown (24 to 48 h) cultures from nutrient agar with 1% glucose were patched onto a filter paper moistened with a fresh oxidase reagent (1% w/v aqueous solution of tetramethyl-para-18 phenylene diamine dihydrochloride) using a wooden stick. A purple reaction in 30 s was recorded as oxidase positive.

2.3.4. Catalase Test

One ml of a 3% solution of hydrogen peroxide was added to a Petri dish and a loop-ful of fresh culture grown on CPG agar medium was added into the solution. The release of bubbles from the culture was recorded as catalase positive.

2.3.5. KOH Solubility Test

48 h cultures were used. Three drops of 3% KOH were put onto a glass slide and the colony of test strain was stirred into the solution with clean loop for 5 to 10 s. When the solution was viscous enough to stick to the loop causing a thin strand

of slime, then the test was recorded as positive.

2.3.6. NaCl Tolerance Test

NaCl broth was used which contained: peptone 5 g, yeast extract 3 g, glucose 5 g, distilled water 1 L, and either 0.5, 1.0, 1.5 or 2.0 g of NaCl, pH 7. The broth was autoclaved at 121˚C for 15 min and dispensed into sterile 100 ml flasks.

Test strains were inoculated into the flasks and incubated on a rotary shaker at 30˚C with 100 rpm up to 14 days. Growth was assessed every 2 days for each test tube.

2.3.7. Growth at 37 and 41˚C

To determine the growth at 37 or 41˚C, strains were cultured in flasks for 3 days in CPG broth on a rotary shaker at different temperatures and growth was read by spectrophotometer.

2.4. Carbohydrate Oxidation/Biovar Test

Biovars of *R. solanacearum* were determined on the basis of carbon utilization in disaccharides and hexose alcohols. The disaccharides were cellobiose, lactose, and maltose while the hexose alcohols were dulcitol, mannitol and sorbitol. The basal medium described by [23] was used. The medium constituents were, $NH_4H_2PO_4$. 1.0 g, KCl 0.2 g, $MgSO_4{\cdot}7H_2O$ 0.2 g, bromothymol blue (1%w/v) 0.3 ml, agar 1.5 g, distilled water 1litre. The pH was adjusted to 7.1 With 40% (w/v) NaOH solution before adding the agar cellobiose, lactose, and maltose.

Solutions were filter-sterilized, while Mannitol and sorbitol were autoclaved for 20 min as 10% (w/v) solutions [23]. Dulcitol was added directly to the basal medium, which was then autoclaved for 20 min. Five ml of each sugar and sugar alcohol solution were added to 45 ml of molten cooled Hayward's basal medium and 10 ml volumes of the resulting amended medium were dispensed into test tubes [23]. The medium without sugar or sugar alcohol carbon source served as control. A suspension of each strain grown on CPG for 48 h at 30˚C was prepared by inoculating 300 µl of sterile water with a wire loopful of cells. The test tubes of Hayward's medium were inoculated with 30µl of the prepared suspensions and incubated at 30˚C and checked for acid production (yellow color) at various intervals for up to 5 weeks [23]. Liquid culture media inoculation was used as negative control.

R. solanacearum strains were classified into biovars according to their ability to produce acid from sorbitol, mannitol, dulcitol, lactose, maltose and cellobiose.

2.5. DNA Extraction

One ml of the culture was used for the extraction of genomic DNA. Bacterial DNA was extracted with a commercially available kit (Quick-DNA Fungal/Bacterial Miniprep Kit by Zymo-Research, Irvine, California, USA) according to the manufacturer's instructions.

The quality and quantity of the DNA samples were checked by measuring

their A260/A280 ratios using a NanoDrop One Microvolume UV-Vis Spectrophotometer (Instrument operating Software version 1.2.0, Thermo Fisher Scientific, Inc.)

2.6. Detection of *R. solanacearum* Using PCR

To determine and confirm isolates as *R. solanacerum*, PCR-based identification was done using FliC pair primers which amplifies the flagellin gene sequence. The primer sequences for Ral-*FliC*-R and Ral-*FliC*-F were (5'-GAACGCCAACGGTGCGAACT-3') and (5'-GGCGGCCTTCAGGGAGGTC-3') respectively, with an expected PCR product of 400bp) [24].

PCR amplification was performed using NEB One Taq Quick-Load 2X Master Mix (New England Biolabs, Inc). For PCR amplification a DNA thermal cycler (PeQlab Cyclone 25) was used. Briefly, PCR conditions were: pre-denaturation at 94˚C for 5 minutes, 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 60˚C for 2 minutes, and Initial extension at 72˚C for 1 minute. Final extension was done at 72˚C for 10 minutes and cooling to 4˚C. Amplified products (25 µl) were resolved by gel electrophoresis in 1.5% agarose gel in 1 × TAE (10 mM Tris-HCl and 1mM EDTA) buffer, pH 8.0, at 120 voltage. The gel was stained with Gel Red Nucleic acid stain (Millipore Sigma) and visualized and photographed under 300 nm ultraviolet light.

2.7. Isolation of Bacteriophages Using Isolated *R. solanacearum* as Host

The isolated bacteria were used as the host to propagate the target bacteriophages. Bacteriophages were isolated from soil samples with a modification of the method described by Bhunchoth [25], briefly, 5 g soil sample was homogenized with 5 ml of SM buffer (Sodium Chloride-Magnesium Sulfate (SM) buffer (50 mMTris-HCl (pH 7.5), 100 mM NaCl, 10 mM $MgSO_4$)). After the addition of 100 µl of an overnight culture of *R. solanacearum*, initial enrichment was performed for 24h at 30˚C in 250ml conical flasks. After centrifugation, the supernatant was filtered through 0.45 µm pore-size filters (Sartorius, Germany). Ten-fold serial dilutions of these filtrates were used in spotting assay against *R. solanacearum* to confirm the presence of bacteriophage by the formation of plaques. Bacteriophage was purified using five-time repeated overlay assays by picking plaques and eluting in SM buffer *R. solanacearum* was used to propagate the bacteriophages. Phage stock was stored in SM buffer at 4˚C conditions until further experiments [26].

2.8. Determination of Physiological Characteristics of the Bacteriophage (pH, Temperature, UV).

Each of the physicochemical experiments was repeated three independent times.

For temperature stability, bacteriophage aliquots (10^6 PFU/ml) in SM buffer were incubated at 20˚C, 30˚C, 40˚C, 50˚C and 60˚C for 1 hour. After incubation, viable phage titers were enumerated using plaque assay.

For pH stability, phage aliquots were added to SM buffer (final concentration 10^5 PFU/ml) adjusted with HCl or NaOH to pH 3-12, and incubated at 4˚C for 16 h. After incubation, viability was determined using plaque assay [27].

For UV stability, to test the ultraviolet stability of the phages, 1 mL of the phage suspension (10^8 PFU/mL) was incubated for 15, 30, 45, 60, and 75 minutes under an ultraviolet lamp (100 W). Phage titers were then determined by plaque assay.

2.9. Evaluation Efficacy of the Phages as Biocontrol Agent for Bacterial Wilt

To test the efficacy of the bacteriophages in relation to disease, medium composed of yard manure:soil:sand in a ratio of 1:3:2 v/v was used. The medium was sterilized at 121˚C for 20 minutes. Tomato seeds were obtained from the *Simlaw* Company, Kenya. Tomato seedlings were transplanted to the planting bags at three leaf-stage (4 - 5 weeks after sowing). Two weeks after transplanting, tomato plants were inoculated with *R. solanacearum* (Set A, B and Set C) according to the method described by [28]. The stem was punctured at the axis of the third fully expanded leaves with a needle dipped in the inoculum. The OD value of the bacterial suspension was adjusted spectrophotometrically to reach OD of 0.26 at 600nm (approximately 1×10^8 CFU/ml). A high-titer phage suspension (1×10^8 PFU/ml) prepared in SM buffer was then applied by spraying.

Set A: (Positive control) neither inoculated with *R. solanacearum* nor treated with phage mixture but sterile water.

Set B: (Experimental) The phage mixture was applied after the inoculation with *R. solanacearum* bacteria.

Set C: (Negative control) Inoculated only with *R. solanacearum*.

The plantlets were grown in an enclosed greenhouse and disease progress was monitored for 14 days (recorded every 2 days). The same set of treatment structures was repeated for each *R. solanacearum* strain and phage samples. The plants were maintained in the greenhouse at temperatures of 24 to 28˚C and 75 to 90% relative humidity and seedlings were watered with sterile water when necessary. Disease severity was assessed and recorded (1 = no symptom, 2 = two or three leaves wilted, 3 = four or more leaves wilted, 4 = plant died).

3. Results & Discussion

3.1. Sampling of *R. solanacearum* Bacteria

Bacterial wilt caused by *Ralstonia solanacearum* is a predominant disease in warm humid tropical and temperate regions of the world. In the present study, Samples were collected from some of the major tomato growing areas, also considered as bacterial wilt hot spot areas; Njambini-Nyandarua County, Birika-Kajiado County, Sagana-Nyeri County, Wundanyi-Taita, Taveta County.

Higher disease incidence was observed in Nyandarua. This may be due to humid climatic conditions favoring easy transmission of disease in this area. *Ralstonia solanacearum* was isolated using the selective media of Triphenyl Tetrazolium

Chloride agar (TTC).

The sampled plants were then subjected to a confirmatory ooze test for streaming of milky slime to be able to distinguish it from other vascular wilts which turned out positive (**Figure 1**).

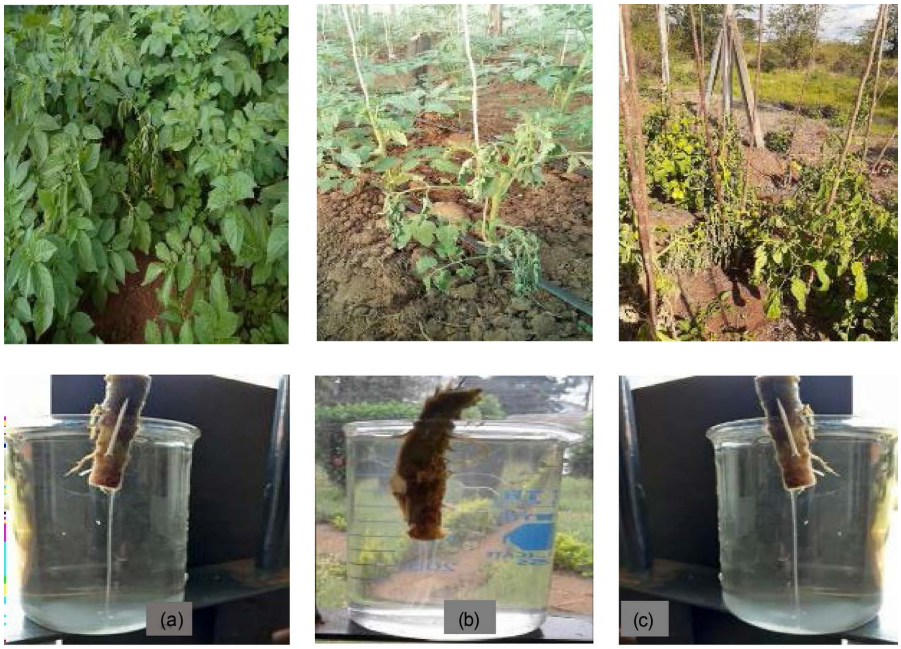


Figure 1. Wilted plants showing typical bacterial wilt symptoms. The three (a, b, c) showed wilting symptoms differently depending on the variety of tomato. All the three yielded positive results on ooze test, as indicated in **Figure 1**.

3.2. Morphological Characteristics

Out of the 30 samples, a total of six (6) seemingly *R. solanacearum* bacteria were isolated. Morphological variability in terms of growth was observed. Two types of colonies were observed on agar plates, fluidal or mucoid and afluidal. The bacterial isolates that yielded fluidal and irregular colonies with pink/light red center and whitish periphery after 48 h of incubation at 30˚C on Triphenyl Tetrazolium Chloride agar (TTC) were four (4) While the afluidal ones, smaller in size with red centres were two (**Figure 2**). On Casamino Peptone Glucose (CPG) agar medium the strains produced irregular, smooth, creamy-white colonies (3.4) according to [21]. The mucoid substance in the fluidal colonies is produced by the accumulation of an exopolysaccharide (EPS) which causes these mucoid colonies to exhibit a typical irregularity of their surfaces with characteristic whorls in the centre.

Under certain conditions, *R. solanacearum* colonies change in morphology from fluidal to afluidal and are linked to a great reduction in disease inducing capacity of these cells. A phenomenon is known as phenotypic conversion (PC). PC-type variants were easily observed by prolonged culture on agar plates [29] and when the strains were grown in a non-aerated liquid medium with glucose and an organic source of nitrogen [29] (**Figure 3**).

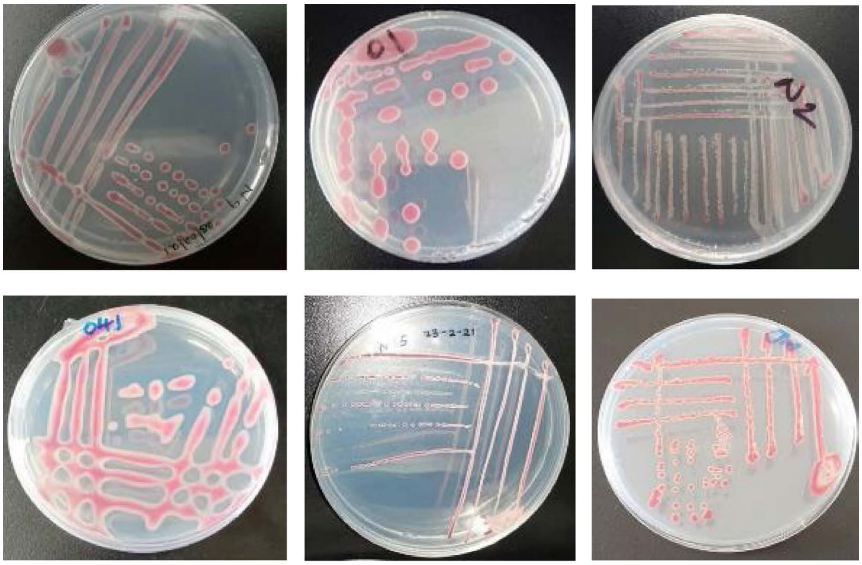


Figure 2. Cultural characteristics of the *R. solanacearum* strains on triphenyl tetrazolium chloride (TTC) agar medium incubated at 30˚C for 48 hours.

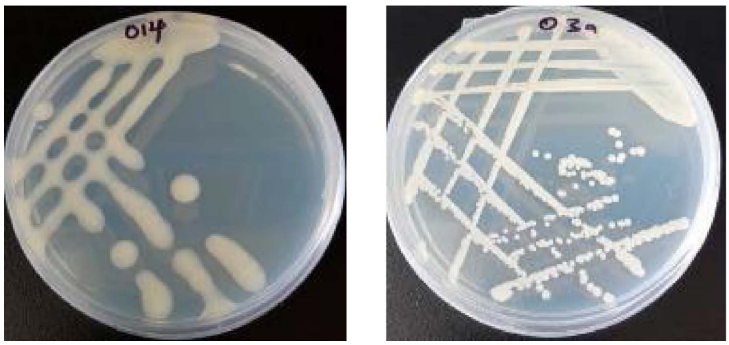


Figure 3. Cultural characteristics of the *R. solanacearum* strains on casamino peptone glucose (CPG) agar medium incubated at 30˚C for 48 hours. On CPG, the colonies appear creamy white.

3.3. Biochemical Characterization of *R. solanacearum* Bacteria

KOH solubility test: All the strains produced a thin strand of slime when drops of KOH were added on a glass slide and the colonies of the bacteria were lifted up with loop after stirring into the solution indication of gram negative.

Oxidase and catalase: All strains were oxidase positive; a purple color appearing within 30 s after addition of culture to the oxidase reagent.

Catalase: They were all catalase positive; release gas upon addition of hydrogen peroxide.

H_2S production: All the strains produced black discoloration on lead acetate impregnated paper strips hanged on medium of strains indicating production of H_2S.

NaCl tolerance: In all the strains, heavy growth (turbidity) appeared in 0.5 and 1.0% NaCl medium with weak growth in 1.5% NaCl. As is the characteristics of *R. solanacearum*, none grew at 2% NaCl.

Carbohydrate oxidation test: For all the tests 24 - 48 hours old cultures were used. Carbohydrate oxidation test based on the ability of strains of *R. solanacearum* to differentially produce acid from several carbohydrate sources like disaccharides sugars (Cellobiose, lactose, maltose) and hexose alcohols (Dulcitol, Mannitol, Sorbitol), revealed that the *R. solanacearum* isolates oxidized disaccharides, but not alcohols. After 15 days of incubation, the medium in tubes showed a green colour for the alcohols and yellow color for the sugars indicating that the strains belong to biovar II [30] (**Figure 4**). Phylogenetic analysis clustered the bacterial isolates into a homogenous group of Genus *Ralstonia* with over 99% similarity level. Biochemical tests results are summarized in **Table 1**.

Figure 4. Carbohydrate tests: yellow colour for the sugars and green colour for the alcohols. The bacteria was able to oxidize the sugars which led to the formation of acidic byproducts which turned the phenol red indicator in the media to yellow. The bacteria however was unable to oxidize the alcohols hence no color change.

Table 1. Summary of the *R. solanacearum* biochemical tests.

		TK1	NN1	NN2	BK1	SN1	WT1
BIOCHEMICAL TESTS							
Gram staina		−ve	−ve	−ve	−ve	−ve	−ve
KOHa		+ve	+ve	+ve	+ve	+ve	+ve
Oxidasea		+ve	+ve	+ve	+ve	+ve	+ve
Catalasea		+ve	+ve	+ve	+ve	+ve	+ve
H2S[a]		+ve	+ve	+ve	+ve	+ve	+ve
NaCl tolerance							
	0.5%	+++	+++	+++	+++	+++	+++
	1%	++	++	++	++	++	++
	1.5%	+	+	+	+	+	+
	2%	−	−	−	−	−	−
Temperatureb							
	37˚C	+	+	+	+	+	+
	41˚C	−	−	−	−	−	−

Continued

Carbohydrates test							
	Cellobios E	+	+	+	+	+	+
	Maltose	+	+	+	+	+	+
	Lactose	+	+	+	+	+	+
	Sorbitol	–	–	–	–	–	–
	Mannitol	–	–	–	–	–	–
	Dulcitol	–	–	–	–	–	–
BIOVAR		**II**	**II**	**II**	**II**	**II**	**II**

a "+ve" positive reaction; "–ve" negative reaction; b "+++" very turbid; "++" turbid; "+" less turbid; "–" no observable change; d "+" (Yellow color produced due to change in pH); "–" means negative reaction (Green color remained as pH did not change).

3.4. Molecular Analysis and Phylotyiping

The quality and quantity of the DNA samples as checked by measuring their A260/A280 ratios using a Nanodrop One Microvolume UV-Vis Spectrophotometer showed that the purity of the DNA was good it was above 1.8 [31].

The ratio of absorbance at 260 and 230 was used as a secondary measure for the DNA purity which was slightly below the range, showing slight impurities (**Table 2**).

Table 2. Summary of the Spectrophotometer readings showing DNA quality.

Sample	Nucleic acid (ng/µl)	A260 (Abs)	A280 (Abs)	260/280	260/230
TK1	92.8	1.856	0.890	2.09	0.69
NN1	75.8	1.515	0.777	1.95	0.17
NN2	90.8	1.816	0.931	1.95	0.15
BK1	218.8	4.375	2.781	1.57	0.53
SN1	103.7	2.073	1.099	1.89	0.24
WT1	293.8	5.876	2.882	2.04	0.75

Each of the 6 isolates generated a 400-bp gene fragment product with the RalfliC primer. The PCR products obtained from the 6 strains belonged to *R. solanacearum* strains (biovar 2) according to [24] (**Figure 5**).

3.5. Bacteriophage Isolation and Physiological Characterization

Bacteriophages were isolated using *R. Solanacearum* as host. The samples assay for the presence of lytic bacteriophages against *R. Solanacearum* produced lytic plaques on assay plates. Single plaques were isolated from each assay plate for further purification, amplification and characterization. Different bacteriophages were observed based on morphology *i.e.* NN1 (a) SN1 (b) NN2(c), TK1 (d), all of which displayed effective lytic activity on *R. Solanacearum* (**Figure 6**), according to [32].

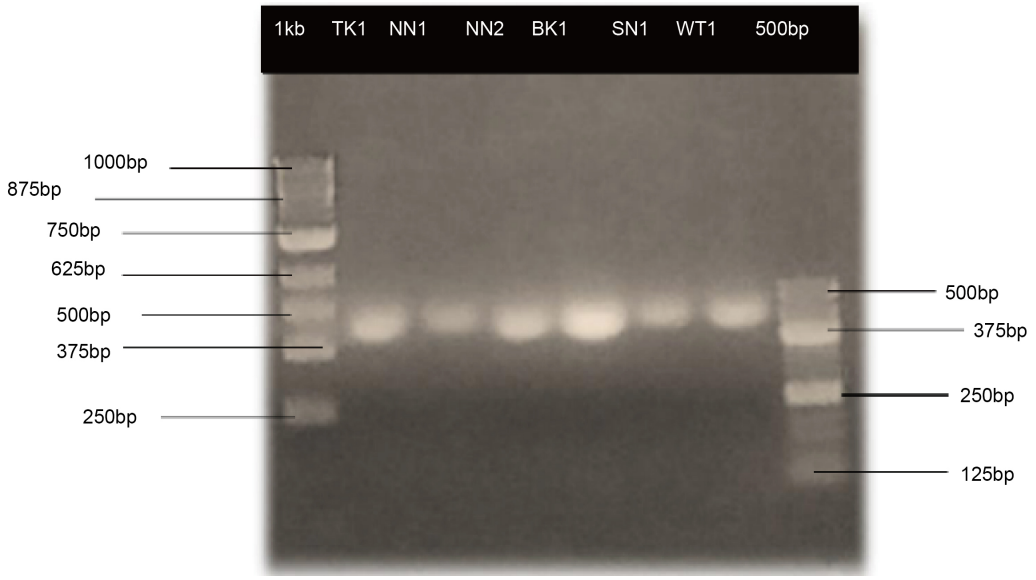


Figure 5. Agarose gel bands showing representative patterns of bacterial isolates generated by PCR amplification with Ral-*fliC* primers. Lane 1; 1 kb DNA Ladder, 1 (TK1) 2 (NN1) 3 (NN2) 4 (BK1) 5 (SN1), with an amplicon size of ~400 bp according to [24].

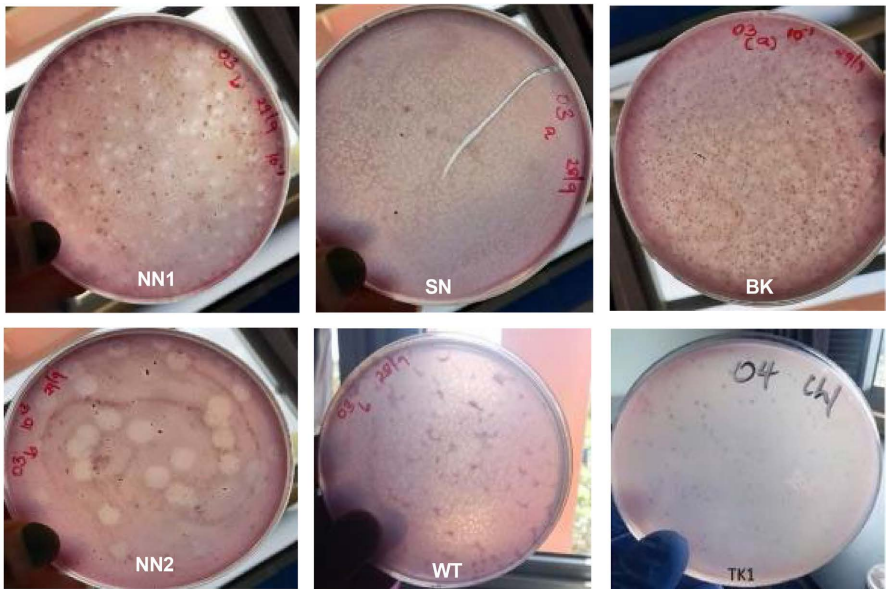


Figure 6. Bacteriophage lytic activity on *R. solanacearum* bacteria on double layer agar plates. Four bacteriophages were observed that with that on NN1 appearing similar to that of BK1, and SN1 similar to WT1. However NN2 differed from TK1.

Upon characterization the bacteriophages were stable at 30˚C - 37˚C temp, pH of range 6 - 7 and tolerated UV for over 1 hour (**Figure 1**, **Figure 2**).

Temperatures define the outcome of phage-bacteria interactions. At higher temperatures for our case 37˚C, the bacteriophage predominantly goes through a lytic cycle, but at lower temperatures, 20˚C and below (**Figure 1**), the phages remain temperate hence low phage concentrations (see ANOVA Analysis, **Tables 3-8**).

Table 3. Stability of bacteriophages at various temperatures.

	Df Sum	Sq Mean	Sq F	value	Pr(>F)	
Temp	4	230.18	57.54	70.23 < 2e−16***		
Residuals	55	45.06	0.82			
Signif. codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '	1

Phage titre Log (PFU/mL).

Table 4. Tukey multiple pairwise comparisons, 95% family-wise confidence level.

Temp	diff	lwr	upr	padj
20˚C - 10˚C	1.4083333	0.3661161	2.4505506	0.0031174
30˚C - 10˚C	3.9583333	2.9161161	5.0005506	0.0000000
40˚C - 10˚C	0.2583333	−0.7838839	1.3005506	0.9558377
50˚C - 10˚C	−2.0166667	−3.0588839	−0.9744494	0.0000115
30˚C - 20˚C	2.5500000	1.5077828	3.5922172	0.0000001
40˚C - 20˚C	−1.1500000	−2.1922172	−0.1077828	0.0236126
50˚C - 20˚C	−3.4250000	−4.4672172	−2.3827828	0.0000000
40˚C - 30˚C	−3.7000000	−4.7422172	−2.6577828	0.0000000
50˚C - 30˚C	−5.9750000	−7.0172172	−4.9327828	0.0000000
50˚C - 40˚C	−2.2750000	−3.3172172	−1.2327828	0.0000009

Fit: aov (formula = temp $ Value ~ temp $ Temp, data = temp).

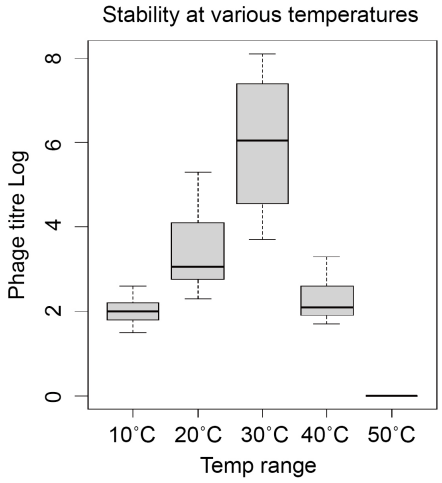


Table 5. Tolerance of bacteriophages under UV lamp.

	Df Sum	Sq Mean	Sq F	value	Pr(>F)	
pH	4	94.89	23.722	144.2 < 2e−16***		
Residuals	55	9.05	0.165			
Signif. codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '	1

Table 6. Tukey multiple pairwise comparisons, 95% family-wise confidence level.

$pH	diff	lwr	upr	padj
pH10 - pH2	−0.0500000	−0.5170107	0.4170107	0.9981348
pH4 - pH2	0.9083333	0.4413226	1.3753441	0.0000104
pH6 - pH2	3.4083333	2.9413226	3.8753441	0.0000000
pH8 - pH2	0.9333333	0.4663226	1.4003441	0.0000060
pH4 - pH10	0.9583333	0.4913226	1.4253441	0.0000035
pH6 - pH10	3.4583333	2.9913226	3.9253441	0.0000000
pH8 - pH10	0.9833333	0.5163226	1.4503441	0.0000020
pH6 - pH4	2.5000000	2.0329893	2.9670107	0.0000000
pH8 - pH4	0.0250000	−0.4420107	0.4920107	0.9998794
pH8 - pH6	−2.4750000	−2.9420107	−2.0079893	0.0000000

Fit: aov (formula = Value ~ pH, data = ph).

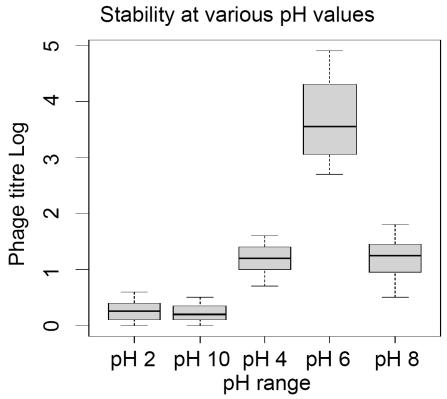


Table 7. Tolerance of bacteriophages under UV Lamp.

	Df Sum	Sq Mean	Sq F	Value	Pr(>F)	
Min	5	411.5	82.30	396.6 < 2e−16***		
Residuals	66	13.7	0.21			
Signif. codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '	1

Phage titre Log (PFU/mL).

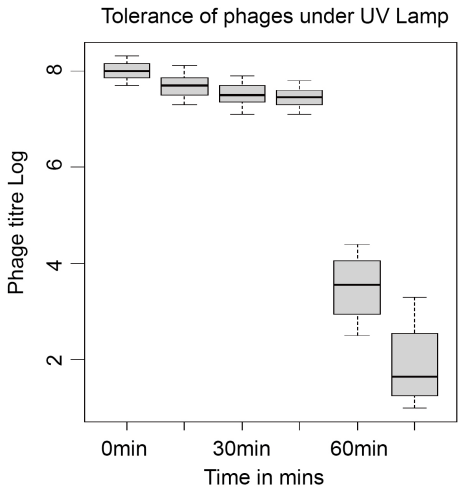
Table 8. Tukey multiple pairwise comparisons, 95% family-wise confidence level.

$Min	diff	lwr	upr	padj
15 min - 0 min	−0.325	−0.8708277	0.220827664	0.5060871
30 min - 0 min	−0.475	−1.0208277	0.070827664	0.1236106
45 min - 0 min	−0.550	−1.0958277	−0.004172336	0.0472096
60 min - 0 min	−4.500	−5.0458277	−3.954172336	0.0000000
75 min - 0 min	−6.100	−6.6458277	−5.554172336	0.0000000

Continued

30 min - 15 min	−0.150	−0.6958277	0.395827664	0.9653375
45 min - 15 min	−0.225	−0.7708277	0.320827664	0.8305180
60 min - 15 min	−4.175	−4.7208277	−3.629172336	0.0000000
75 min - 15 min	−5.775	−6.3208277	−5.229172336	0.0000000
45 min - 30 min	−0.075	−0.6208277	0.470827664	0.9985657
60 min - 30 min	−4.025	−4.5708277	−3.479172336	0.0000000
75 min - 30 min	−5.625	−6.1708277	−5.079172336	0.0000000
60 min - 45 min	−3.950	−4.4958277	−3.404172336	0.0000000
75 min - 45 min	−5.550	−6.0958277	−5.004172336	0.0000000
75 min - 60 min	−1.600	−2.1458277	−1.054172336	0.0000000

Fit: aov (formula = Value ~ Min, data = min).



4. Results Interpretations

4.1. Stability of Bacteriophages at Various Temperatures

Interpretation of ANOVA and Tukey Multiple Comparisons:

The ANOVA results indicate a highly significant effect of temperature on phage titre (Log (PFU/mL)), with an F value of 70.23 and a p-value less than 2e−16. This suggests that temperature plays a crucial role in determining the stability of bacteriophages, and we reject the null hypothesis of no difference in means across the temperature groups. The Tukey HSD test provides detailed pairwise comparisons between the different temperature conditions:

20˚C vs. 10˚C:

There is a significant increase in phage titre at 20˚C compared to 10˚C, with a mean difference of 1.4083 (95% CI: 0.3661 to 2.4506) and a p-value of 0.0031. We reject the null hypothesis of no difference in means.

30˚C vs. 10˚C:

Phage titre is significantly higher at 30˚C compared to 10˚C, with a mean

difference of 3.9583 (95% CI: 2.9161 to 5.0006) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

40˚C vs. 10˚C:

There is no significant difference in phage titre between 40˚C and 10˚C, with a mean difference of 0.2583 (95% CI: −0.7839 to 1.3006) and a p-value of 0.9558. We fail to reject the null hypothesis of no difference in means.

50˚C vs. 10˚C:

Phage titre is significantly lower at 50˚C compared to 10˚C, with a mean difference of −2.0167 (95% CI: −3.0589 to −0.9744) and a p-value of 0.0000115. We reject the null hypothesis of no difference in means.

30˚C vs. 20˚C:

There is a significant increase in phage titre at 30˚C compared to 20˚C, with a mean difference of 2.5500 (95% CI: 1.5078 to 3.5922) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

40˚C vs. 20˚C:

Phage titre is significantly lower at 40˚C compared to 20˚C, with a mean difference of −1.1500 (95% CI: −2.1922 to −0.1078) and a p-value of 0.0236. We reject the null hypothesis of no difference in means.

50˚C vs. 20˚C:

There is a significant decrease in phage titre at 50˚C compared to 20˚C, with a mean difference of −3.4250 (95% CI: −4.4672 to −2.3828) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

40˚C vs. 30˚C:

Phage titre is significantly lower at 40˚C compared to 30˚C, with a mean difference of −3.7000 (95% CI: −4.7422 to −2.6578) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

50˚C vs. 30˚C:

There is a significant decrease in phage titre at 50˚C compared to 30˚C, with a mean difference of −5.9750 (95% CI: −7.0172 to −4.9328) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

50˚C vs. 40˚C:

Phage titre is significantly lower at 50˚C compared to 40˚C, with a mean difference of −2.2750 (95% CI: −3.3172 to −1.2328) and a p-value of 0.0000009. We reject the null hypothesis of no difference in means.

4.2. Stability of Bacteriophages at Various pH Values

Interpretation of ANOVA and Tukey Multiple Comparisons

The ANOVA results indicate a highly significant effect of pH on phage titre (Log (PFU/mL)), with an F value of 144.2 and a p-value less than 2e−16. This suggests that pH significantly affects the stability of bacteriophages, and we reject the null hypothesis of no difference in means across the pH levels. The Tukey HSD test provides pairwise comparisons between the different pH conditions:

pH 4 vs. pH 2:

There is a significant increase in phage titre at pH 4 compared to pH 2, with a mean difference of 0.9083 (95% CI: 0.4413 to 1.3753) and a p-value of 0.0000104. We reject the null hypothesis of no difference in means.

pH 6 vs. pH 2:

Phage titre is significantly higher at pH 6 compared to pH 2, with a mean difference of 3.4083 (95% CI: 2.9413 to 3.8753) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

pH 8 vs. pH 2:

Phage titre is significantly higher at pH 8 compared to pH 2, with a mean difference of 0.9333 (95% CI: 0.4663 to 1.4003) and a p-value of 0.0000060. We reject the null hypothesis of no difference in means.

pH 4 vs. pH 10:

There is a significant increase in phage titre at pH 4 compared to pH 10, with a mean difference of 0.9583 (95% CI: 0.4913 to 1.4253) and a p-value of 0.0000035. We reject the null hypothesis of no difference in means.

pH 6 vs. pH 10:

Phage titre is significantly higher at pH 6 compared to pH 10, with a mean difference of 3.4583 (95% CI: 2.9913 to 3.9253) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

pH 8 vs. pH 10:

Phage titre is significantly higher at pH 8 compared to pH 10, with a mean difference of 0.9833 (95% CI: 0.5163 to 1.4503) and a p-value of 0.0000020. We reject the null hypothesis of no difference in means.

pH 6 vs. pH 4:

There is a significant increase in phage titre at pH 6 compared to pH 4, with a mean difference of 2.5000 (95% CI: 2.0330 to 2.9670) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

pH 8 vs. pH 4:

Phage titre at pH 8 is not significantly different from pH 4, with a mean difference of 0.0250 (95% CI: −0.4420 to 0.4920) and a p-value of 0.9998794. We fail to reject the null hypothesis of no difference in means.

pH 8 vs. pH 6:

Phage titre is significantly lower at pH 8 compared to pH 6, with a mean difference of −2.4750 (95% CI: −2.9420 to −2.0080) and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

4.3. Interpretation of ANOVA and Tukey Multiple Comparisons

ANOVA

The ANOVA results indicate a highly significant effect of exposure time to UV Lamp (Min) on phage titre (Log (PFU/mL)), with an F value of 396.6 and a p-value less than 2e−16. This suggests that exposure time significantly impacts the tolerance of bacteriophages to UV radiation, leading us to reject the null hypothesis of no difference in means across exposure times.

Tukey HSD Test: The Tukey HSD test provides pairwise comparisons between different exposure times:

15 min vs. 0 min:

There is no significant difference in phage titre between 15 minutes and 0 minutes of exposure, with a mean difference of −0.325 and a p-value of 0.5061. We fail to reject the null hypothesis of no difference in means.

30 min vs. 0 min:

There is no significant difference in phage titre between 30 minutes and 0 minutes of exposure, with a mean difference of −0.475 and a p-value of 0.1236. We fail to reject the null hypothesis of no difference in means.

45 min vs. 0 min:

There is a significant decrease in phage titre after 45 minutes compared to 0 minutes of exposure, with a mean difference of −0.550 and a p-value of 0.0472. We reject the null hypothesis of no difference in means.

60 min vs. 0 min:

There is a significant decrease in phage titre after 60 minutes compared to 0 minutes of exposure, with a mean difference of −4.500 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

75 min vs. 0 min:

There is a significant decrease in phage titre after 75 minutes compared to 0 minutes of exposure, with a mean difference of −6.100 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

30 min vs. 15 min:

There is no significant difference in phage titre between 30 minutes and 15 minutes of exposure, with a mean difference of −0.150 and a p-value of 0.9653. We fail to reject the null hypothesis of no difference in means.

45 min vs. 15 min:

There is no significant difference in phage titre between 45 minutes and 15 minutes of exposure, with a mean difference of −0.225 and a p-value of 0.8305. We fail to reject the null hypothesis of no difference in means.

60 min vs. 15 min:

There is a significant decrease in phage titre after 60 minutes compared to 15 minutes of exposure, with a mean difference of −4.175 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

75 min vs. 15 min:

There is a significant decrease in phage titre after 75 minutes compared to 15 minutes of exposure, with a mean difference of −5.775 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

45 min vs. 30 min:

There is no significant difference in phage titre between 45 minutes and 30 minutes of exposure, with a mean difference of −0.075 and a p-value of 0.9986. We fail to reject the null hypothesis of no difference in means.

60 min vs. 30 min:

There is a significant decrease in phage titre after 60 minutes compared to 30 minutes of exposure, with a mean difference of −4.025 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

75 min vs. 30 min:

There is a significant decrease in phage titre after 75 minutes compared to 30 minutes of exposure, with a mean difference of −5.625 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

60 min vs. 45 min:

There is a significant decrease in phage titre after 60 minutes compared to 45 minutes of exposure, with a mean difference of −3.950 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

75 min vs. 45 min:

There is a significant decrease in phage titre after 75 minutes compared to 45 minutes of exposure, with a mean difference of −5.550 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

75 min vs. 60 min:

There is a significant decrease in phage titre after 75 minutes compared to 60 minutes of exposure, with a mean difference of −1.600 and a p-value less than 0.0001. We reject the null hypothesis of no difference in means.

4.4. Factors That Affect the Stability of Phages

4.4.1. Temperature

Temperature plays a vital role in a phage's survival and stability. The lysogenic phase cycle is dominated by attachment, penetration, multiplication, and the duration of the latent phase temperature. Only a few phages genetic materials can infiltrate the host cell when the temperature is below the optimum temperature, and only a few phages participate in phage multiplication. [33] discovered that phages that are kept at a high temperature could extend the latent stage. Furthermore, the speed, viability, and storage of phages are all influenced by temperature. Phages may thrive in hot springs, which are uncommon habitats with temperatures that range from 40 to 90˚C.

More than 75% of the phages persist even after incubation on the ice at around 0˚C. Phages are also vulnerable to higher temperatures. 68% - 70% of phage particles disintegrate when boiled at 105˚C [34] discovered that a thermal treatment inactivates phages in dewatered sludge and raw sewage. This study suggests that phages are more resistant to thermal treatment than bacteria. The most significant parameter in regards to determining phage activity is the storage temperature. Tailed phages are the most resistant to storage and have the most extended longevity. Some phages, are viable after 10 - 20 years at 4˚C [33]. Phages generally resist freezing and thawing, so repeated short-term treatments can antagonistically affect them. [18] discovered that 4˚C (k 40 days) in wastewater is the best temperature for phage storage. The temperature must be kept below −80˚C in order to retain the phage activity for a longer period. The phage viability is nil after

84 days in an SM buffer at 42˚C, whereas no phage activity was found after 120 days at 37˚C. According to [34] phages should not be stored below −20˚C, because ice crystals form at this temperature, which can kill phages.

4.4.2. pH of the Environment

Another critical factor that regulates phage activity is the environment's acidity. Scientists investigated the presence of phages in wine, particularly those associated with the lactic acid bacterium *Leuconostocoenos*. According to [35] phages can grow in an acidic environment, such as in sauerkraut. After 60 and 100 days in a sauerkraut fermentation tank, 24 phages were identified (pH 3.5). [17] investigated T7 phage stability in several pH (3 - 11) buffers, which included citrate, phosphate, phosphate-borate, borate buffers, and citrate-phosphate, for 1 - 2 weeks at 0.5 - 2˚C. The optimal pH for phage physical stability for long storage is between 6 and 8. The T7 phage is most active at pH 7, and it has the best stability in a phosphate buffer, which only loses 20% of its activity. It was unstable at pH 4 and lost most of its infectivity after 96 h in citrate or citrate-phosphate buffers. Also, it entirely lost its activity after 1 h at pH 3. T7 phages demonstrate at least 30% activity at pH 9 in alkaline conditions, and their infectivity lasts for 15 days. The T7 activity was almost eliminated after 24 h in a borate buffer with a pH > 10. Their activity was limited by a pH < 3.5 and a total concentration of SO_2 of 50 mg/L. The phage is generally stable in the pH range of 5 - 9, with an optimal pH of 5 - 6. Their instant coagulation occurs at pH 2, whereas the phages precipitated at pH 3 and 4. However, it was alterable at the greater value, and the phages could be redisposed by shaking them. The researchers found that irreversible coagulation and precipitation might be the limiting factor of the phage activity. A little loss of infectivity nearby at pH 7 was also observed. The PM2 phage was sensitive at a low pH, completely losing activity at pH 5.0. Particles of the T1 phage vanished at pH 3.0, and the M13 phage survived even at pH 2. These interpretations show that the alteration in environmental pH may shelter the phage activity at a low temperature. [36] studied bacterial viruses in source-separated urine, concluding that these viruses could be found there (pH 9). Inactivation was roughly twice as high at 20˚C as at 5˚C in PBS (pH 7.4), which was utilized as a control. The decrease in the phage titer at 20˚C could have been caused by the conversion of urea to ammonia, a component that inactivates viruses. These findings were verified by [37]. They discovered that urine dilution and a lower incubation temperature increased the stability of phage 28B, MS2, and phiX174. Their findings on T4 phage stability in urine after 4 weeks of incubation at 6˚C and at room temperature in a urine sample indicated no significant fluctuations in the phage titer, which indicates strong viral stability. The MS2 survival is better in diluted or fresh urine than in preserved urine, according to [15]. The temperature and pH had a more significant impact on the phage inactivation at 30˚C than at 15˚C. The concentration of hydrogen ions alters the phage aggregation when the pH is less than or equal to the phage isoelectric point (pI = 3.9). For example, the MS2 phages showed significant potential to aggregate.

4.4.3. Salinity and Ions

Phages have been reported to be inactivated by osmotic stress. According to [34] psychrophilic *Pseudomonas* phages showed less perseverance in highly concentrated NaCl and sugar solutions. The vitality of the phages reduces by 99% when diluted in 4 mol/L NaCl [33]. However, the viability of the phage only reduces by 26% [21]. The effectiveness of both phages was reduced by up to 30% in 0.1% citrate on a soft agar medium. Several phages were recovered from different salinities of seawater. [24] grouped the phages into three families. 11 belong to the *Myoviridae*, 7 to the *Siphoviridae*, and 4 to the Podoviridae. This site found no DNA structural similarity across phages from different families. In addition, [21] investigated the stability of five marine phages in various inorganic salt media. All the phages were shown to be more inactivated in a medium enriched with 0.5% NaCl than in the other media [15].

4.5. Bioassay on Tomato Seedlings Testing the Efficacy of the Bacteriophages

In the plant challenge experiments it was observed that upon infection with the host bacteria, all strains of *R. solanacearum* with mucoid colonies were virulent while EPS-deficient mutants were avirulent to the plant and so no symptoms were observed. Upon treatment of plants with bacteriophage prevented wilting after subsequent inoculation with the pathogen. Bacteriophage mix of SN1 and WT1 were used for efficacy test due to their efficiency in plating and infection. Phage SN1 and WT1 exhibited high lytic activity and relatively high thermos tolerance and acid tolerance, thereby showing great potential in the biocontrol of bacterial wilt infection across a variety of conditions [28] (**Figure 7**).

Figure 7. Plant bioassay on tomato seedlings testing the efficacy of the bacteriophages. (a) Negative control neither inoculated with *R. solanacearum* nor treated with the phage. (b) Experimental, the phage mixture applied to the soil once after the inoculation with a *R. solanacearum* bacteria. (c) Positive control: Inoculated only with *R. solanacearum* [22].

Table 9. Bacterial wilt disease progression on experimental tomato plantlets.

	Days						
	2	**4**	**6**	**8**	**10**	**12**	**14**
Set A	1	1	1	1	1	1	1
Set B	1	1	1	1	1	1	1
Set C	1	1	2	3	3	3	4

Key: 1 = no symptom, 2 = two or three leaves wilted, 3 = four or more leaves wilted, 4 = plant died.

From Table 9, it was observed that the plants remained healthy after spraying with 1×10^8 PFU/ml phage titre as a biocontrol agent unlike set B where the plant died after being infected with *R. solanacearum* for lack of treatment.

5. Conclusions

The results indicate that phage stability is highest at 30˚C, with significantly higher titres compared to 10˚C, 20˚C, 40˚C, and 50˚C. In contrast, the stability is lowest at 50˚C, showing significantly lower titres compared to all other temperatures. The non-significant difference between 40˚C and 10˚C suggests that phage stability is similar at these two temperatures.

These findings highlight the importance of maintaining optimal temperature conditions to ensure the stability of bacteriophages.

The results indicate that pH significantly influences the stability of bacteriophages. pH levels of 6 and 8 show the highest phage titres, significantly higher than pH levels of 2, 4, and 10. pH 4 also exhibits significantly higher titres compared to pH 2 and 10. pH 8 is the only pH level that does not significantly differ from pH 4, indicating a similar phage stability at these two pH conditions. These findings underscore the importance of pH regulation for maintaining the stability and effectiveness of bacteriophages in various applications.

The results indicate that exposure time to UV Lamp significantly affects the tolerance of bacteriophages, with longer exposure times leading to a significant reduction in phage titre. Specifically, exposure times of 60 minutes and 75 minutes show substantial decreases in phage titre compared to shorter exposure times (0, 15, 30, and 45 minutes). These findings underscore the importance of UV exposure duration in controlling bacteriophage populations in various applications.

The results also show that bacteriophages offer the potential for the biocontrol of bacterial wilt and can replace the use of chemicals if they are exploited more.

Further research regarding genetic and molecular aspects will facilitate understanding of phage and bacteria interaction. This study has identified promising candidates for phage biocontrol as well as established a foundation for further studies on the interaction of hosts and their bacteriophages.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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