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Isolation and Characterization of Two Phages Infecting *Streptomyces scabies*

AlKhazindar M*, Sayed ETA, Khalil MS, and Zahran D

Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt.

ABSTRACT

Potato scab is an important wide spread disease caused by *Streptomyces scabies*. Actinophages affect the composition and diversity of bacterial population, thereby, can be used as biological control. Two specific phages against *S. scabies* were isolated from different potato fields in different locations in Giza, Egypt. Phages were partially characterized using electron microscopy and genome structure. The first phage is suggested to belong to family podoviridae and was named vB_StscP-G1 however, the second phage belongs to family siphoviridae and was named vB_StscS-G2. Physical properties including dilution end point, longevity *invitro*, thermal inactivation point and host range were studied for the two isolated phages. Both phages were thermostable and were not affected by storage up to 180 days at room temperature**.** Furthermore, they were infective in acidic and alkaline conditions but, were totally inhibited at high alkaline conditions. Antiviral activity was also studied using different plant extracts and chemicals. Some plant extract showed a significant inhibitory effect on the isolated phages. However, sodium chloride and copper sulphate showed significant increase in the activity of the phages. Our results provide a useful data for designing a control strategy against potato scab disease.

Keywords: *potato scab, actinophages, electron microscopy,* podoviridae*, siphoviridae, titre, antiviral activity*

Corresponding author

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INTRODUCTION

Potato (*Solanum tuberosum*L.) is one of the most important vegetable crops in Egypt and the fourth most important food crop after corn, rice and wheat. Potato crop is susceptible to a number of bacterial pathogens among which is *Streptomyces scabies*, causing potato scab disease. This disease is characterized by corky tuber lesions, which may be superficial or pitted, causing economic loss with respect to plant yield and quality of tubers [1].

Phages are thought to be ecologically important in controlling bacterial number and activity. They represent a potentially important biotic factor affecting bacterial soil populations thereby, affecting agricultural productivity [2]. Phage populations exist in the soil at an estimated level of 1.5×10^8 phages/g of soil[3].*Streptomyces* phages are viruses that specifically infect *Streptomyces sp.*.All actinophages posses six morphological types and are classified within three families: *Myoviridea, Siphoviridea* and *Podoviridea*[4].

Streptomyces phages have been isolated from different environments. Phages can be isolated from different environment such sea water, soil, sewage water/sludge. They occur abundantly in soil, particularly in compost and soil rich in organic matter [5]. They have been found to be effective against almost all the pathogenic bacteria [6]. However, few studies have focused on using them to control plant-pathogenic bacteria [7,8]. Therefore, the objective of this study was to isolate and characterize *Streptomyces* phages infecting *S. scabies* from potato soils and to determine their activity spectra.

MATERIALS AND METHODS

Streptomyces species and culture conditions

S. scabies was purchased from Leibniz Institute DSMZ-German collection of micro-organisms and cell cultures GmbH, Germany. Twelve *Streptomyces* species used in the host range test were isolated from different soils in Giza governorate farms, Egypt. The identification of *S.* species were done using the key of Küster [5] and Whitman *et al.*[9].

S. coelicolor, S. flaveolus, S. griseus, *S. violaceoruber* were purchased from Microbiological Resource Center (Cairo MIRCEN Center). All *Streptomyces* species used in this study were maintained in starch nitrate media [10], stored in 20% (v/v) glycerol at -20^{o}C and refreshed every 8 weeks.

Isolation of *Streptomyces* phages from soil

Phages specific to *S. scabies* were isolated from soils of potato field located in different regions: farm of Faculty of Science, farm of Faculty of Agriculture, Cairo University and different locations in Giza, Egypt. In order to isolate phages, 25 g from the fresh soil samples were mixed with 50ml of nutrient broth medium and enriched by 5ml of 24 hours old *S. scabies* (10^{-7}cfu/ml). The mixture was incubated at 200 rpm on the shaker for 24 hours followed by centrifugation at 4000rpm for 20 minutes. The presence of phages was determined by the double layer technique according to Dowding [11]. The presence of plaques was detected after incubation of the plates at 30°C for 24 hours.

Purification of single plaque

The purification of single plaque was done according to Pringsulaka *et al.* [4]. A single plaque was picked and immersed into 1ml of nutrient broth. Subsequently, 5 ml of the host was added and incubated using a shaking incubator for 24 hours at 30°C. The mixture was filtered through a sterile 0.45µm pore size cellulose acetate filter and tested using the double layer technique as described before. The purification step was repeated 5 times to test the ability of the phage to reinfect the host.

Preparation of high concentration of phage lysate

High concentration of phage lysate was prepared according to Othman *et al*. [12]. Ten ml of nutrient broth was inoculated with 1 ml of 24 hours old culture of *S. scabies* host. Ten plaques were picked from the purified phages plates, added to the mixture and shaked for 24 hours at 30ºC. Following the shaking

incubation, the mixture was centrifuged at 4000 rpm for 20 minutes. Phage lysate was filtered and stored at 4°C.

Transmission electron microscopy

One drop of purified phage preparation (10^{-7}pfu/ml) was placed on coated carbon copper grid for 2 minutes and the excess was drawn off with filter paper. The sample was negatively stained with 2%w/v phosphotungestic acid (PTA), pH 6.8. The grids were air dried and examined with a JOEL Jem_1400 transmission electron microscope (Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Egypt). The magnifications used were 72,000x and 100,000x.

Phage genome extraction

Phage nucleic acid was extracted from viral lysate as described by Yoshida *et al*. [13]. Twenty µl of 10% SDS and 1 µl of 20 mg/ml protinase K were added to 500 µl of phage lysate and incubated in 65°C water bath for 1 hour. After incubation, an equal volume of phenol was added and centrifuged at 6000 rpm for 5 minutes. A1:1 phenol: chloroform extraction was performed followed by overnight incubation with two volumes of 100% ice cold ethanol at -20°C. Following centrifugation, 70% ethanol was added to the pellet and centrifuged. The precipitated nucleic acid was air dried and resuspended in TE buffer.

Determination of nucleic acid type

Nucleic acid type was determined using RNase and DNase digestion tests. 5µl of the extracted nucleic acid was added to 1µl of DNase 1 or RNase (Eppendorf). The mixtures were incubated at 37°C for 2 hours. The digested nucleic acids were electrophoresed through 0.8 % agarose gel in 1X TAE buffer stained with 0.5µg/ml ethidium bromide. The gel was visualized by UV photography.

Phage nomenclature

The isolated phages were named according to Kropinski *et al.* [14]. The name is preceded by the prefix vB (bacterial viruses) followed by two components: the isolation host (first two letters of the host genus and species) and virus family. The second part provides the specific laboratory designation.

Determination of phage titre

The phage lysate was serially diluted with phosphate-buffered saline (PBS). Each dilution was subjected to plaque assay using the double layer method as described before. Plaques were counted in the plates containing 50-300 plaques and expressed as plaque forming unit per milliliter (pfu/ml**)**.

Host range

The host range of the isolated phages was examined on 16 different *Streptomyces sp*. together with *S. scabies*. Four were purchased from Microbiological Resource Center (Cairo MIRCEN Center) *S. coelicolor, S. flaveolus, S. griseous and S. violaceoruber*. Twelve were isolated from soil samples in different localities and identified using biochemical and molecular studies [5,9]. Equal volume 500 µl of each phage lysate and 500 µl of 24 hours old culture of each *Streptomyces* species (previously incubated at 150 rpm in shaking incubator) were mixed and subjected to double layer technique. The presence of plaques was examined after 24 hours [15].

Thermal sensitivity test

The determination of the effect of temperature on the isolated phages was done as described by Phumkhachorn and Rattanachaikunsopon [16].A 100 µl of each phage solution (at the final concentration 10^{-7} pfu/ml) was subjected to preheated water bath and adjusted at desirable temperatures ranging from 50 to 100°C for 10 minutes. Subsequently, the tubes were cooled and the surviving phages were assayed by the double layer method.

pH sensitivity test

The influence of pH range on the infectivity of the isolated phages was determined as described by Pringsulaka *et al.* [4].Nutrient broth (500µl) with various pH values (1 to14) was used for phage cultivation. The culture with phage (concentration 10^{-7} pfu/ml) was incubated for 1 hour at room temperature. The number of plaques was examined by double layer method.

Longevity *in vitro*

The effect of aging on the isolated phages was determined at room temperature (25-30°C) and in the fridge (4°C).Tubes containing 500 µl phage lysate was preserved at room temperature and others were placed at 4°C. Phage assay using double layer method was done after 2 days incubation, followed by 10 days interval from 10 to 180 days. The same test was done but the tubes were incubated at 4°C.

Effect of different plant extracts on the isolated phages and their host

The effect of some plant extracts as listed in table (1) was tested for their inhibitory effect on *S. scabies* and the isolated phages. The infectivity of the phage was tested by double layer technique. The extraction of plants was done according to Thilza *et al*. [17]. Twenty grams of each plant was air dried, ground into fine powder and mixed with 200 ml distilled water. The mixture was subjected to rotatory shaker for 3 days at room temperature. This was followed by heating for 10 minutes and filtration using sterile Whatman No.1 filter paper.

To test the effect of the different plant extracts on *S. scabies*, wells were prepared on nutrient agar media plates using sterile cork borer. The extract of each plant was placed in a well. The plates were incubated at 30°C for 3 days and monitored for any inhibition zone. Plant extract that did not show any inhibitory effect on *S. scabies* was used to test its antiviral activity. Plant extract (500 µl) was mixed with an equal volume of the phage solution (at the final concentration 10^{-7} pfu/ml). The mixture was incubated for 10 minutes at room temperature. The number of plaques was determined by the double layer method. Control test was prepared by mixing bacterial suspension with phage without plant extract. The same test was performed on the second phage solution.

Table (1): List of plant species used to test their effect on *Streptomyces scabies* and isolated phages

Latin name	Common name	Plant organ used
Camellia sinensis L.	Tea	Leaves
Capsicum frutescens L.	Chili pepper	Fruit
Cinnamomum cassia (Nees&T.Nees)	Cinnamon	Bark
Cuminum cyminum L.	Cumin	Seeds
Curcuma longa L.	Tumeric	Rhizome
Ellicium verum Hook L.	Star anise	Fruit
Matricaria chamomilla L.	Chamomile	Flower
Mentha sativa	Peppermint	Leaves
Nigella sativa L.	Black cumin	Seeds
Origanum majorana L.	Sweet majoranum	Leaves
Psidium guajava L.	Guava leaves	Leaves
Tilia Americana L.	Tilia	Leaves
Zingiber officinal Roscoe	Ginger	Rhizome

Effect of different chemicals on isolated phages and their host

Monovalent, divalent and trivalent chemicals were chosen to be tested on *S. scabies* and the isolated phages KCl, NaCl, $CaCl_2.2H_2o$, $CoCl_2$, $HgCl_2$, $MgCl_2.6H_2o$, $ZnCl_2$, $AlCl_3$, $AgNO_3$ and $CuSO_4$. Stock solutions (100 mM) of each salt were prepared. Each salt was diluted into 0.1, 0.2, 0.3, 0.4 and 0.5 mM [18].The effect of each of the chemicals chosen was tested on *S. scabies* using the nutrient agar media plate method as described before. The plates were visualized for any inhibition zone. To test the effect of chemicals on the isolated phages, each dilution of each salt (500 µl) was mixed with an equal volume of the phage solution (at the final concentration 10^{-7} pfu/ml). The mixture was incubated for 10 minutes at room temperature. The number of plaques was determined by the double layer method. Control test was prepared by mixing bacterial

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suspension with phage without the tested chemicals. The same test was performed on the second phage solution.

Statistical analysis

All data generated were the mean of three replicates of each test. The data was statistically analyzed using one way ANOVA test, tabulated and plotted graphically.

RESULTS

Isolation and purification of *Streptomyces* phages

Potato soil samples were tested for the presence of phages using *S. scabies* as a host. Using differences in plaque morphology and size, two clearly different phages were selected for single plaque purification. The phage that showed clear plaques with average size 0.1cm was designated phage1 (P1), while the phage that showed larger plaques (0.8cm) was designated phage2 (P2) as shown in **Fig. (1).**

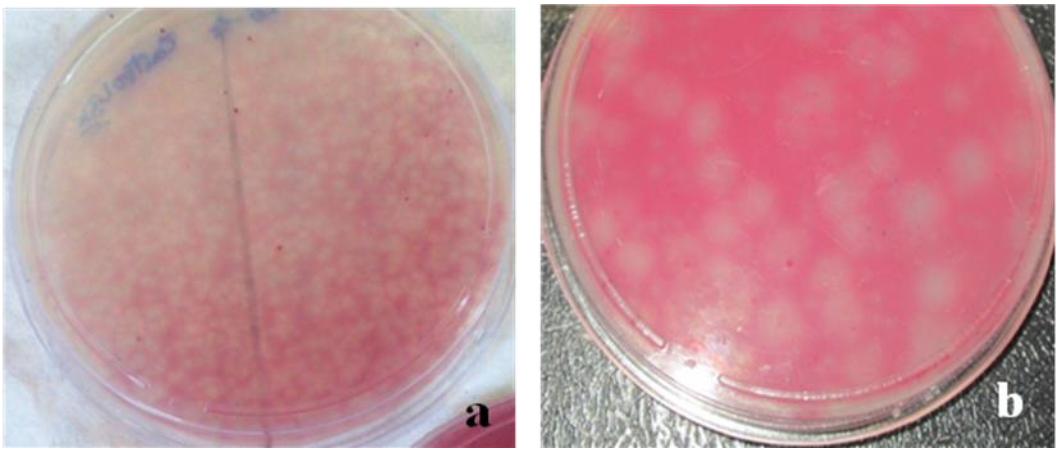

Fig. (1): Plaques produced on *S. scabies* from soil using double layer technique showing different types of phages: (a) phage 1with plaques size ≈ 0.1cm and (b) phage 2 with plaques size ≈ 0.8 cm in diameter.

Phage morphology

Morphological characterization of the two isolated phages (P1 and P2) using transmission electron microscope showed that P1 had a hexagonal head (59.9 nm in diameter) and a short tail (10.4 nm long) as shown in Fig. (2.a). Phage 2 had a hexagonal head (67.2 nm in diameter) and a long tail (114 nm long) as shown in Fig. (2.b).

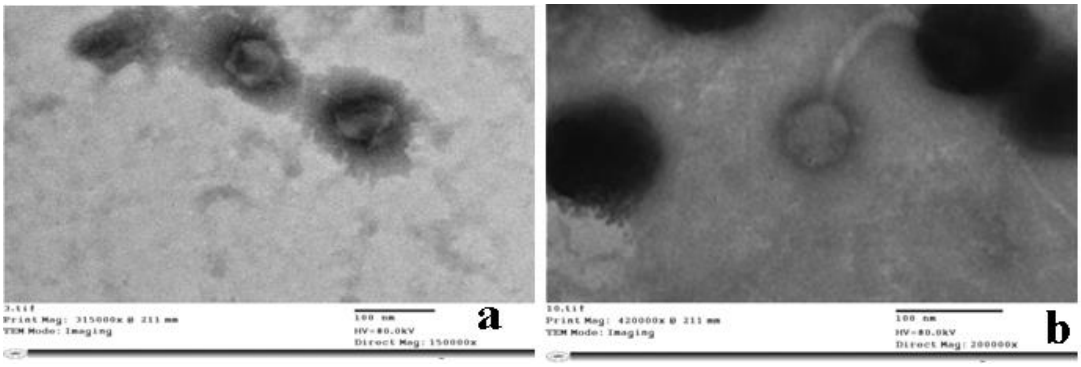

Fig. (2): Transmission electron micrograph of phages negatively stained with phosphotungestic acid; (a) phage1 and (b) phage 2. The bar represents 100 nm.

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Phage genome

Nucleic acid of the two isolated phages P1 and P2 were isolated and subjected to enzymatic digestion analysis using RNase and DNase. Both phages were not affected with the RNase, however, they were digested using the DNase (Fig.3).

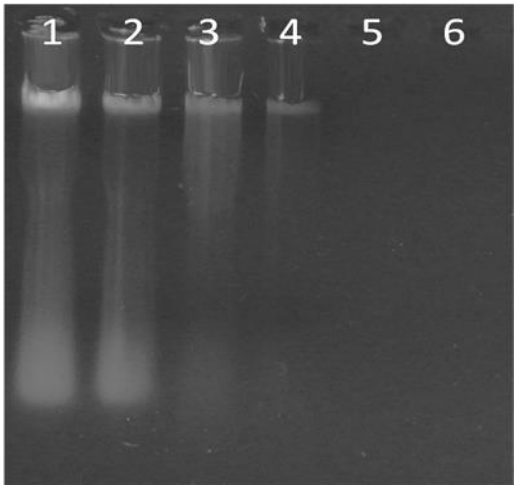

Fig. (3): Gel electrophoresis of the total nucleic acid of the two isolated phages lane 1: phage 1 and lane 2: phage 2. RNase digestion of genomic DNA of Phage 1 (lane 3) and Phage 2 (lane 4). Lanes 5 & 6 represent DNase digestion of the phages 1 & 2 respectively, showing total digestion.

Phage nomenclature

The two isolated phages were named based on the nucleic acid type and the morphology using electron microscope. Both viruses posses DNA genome and tailed, therefore, they belong to order Caudovirales. Furthermore, P1 that has a short tail and P2 that has a long tail belong to family podoviridae (P) and siphoviridae (S) respectively. Therefore, the phages were named bacterial virus (vB); the first two letters of the host genus and species *Streptomyces scabies* (Stsc); followed by the virus family. The second part was designated based on the location of isolation which was Giza governorate (G). Thus, phage 1 was named: vB_StscP-G1 and phage 2 was named vB_StscS-G2.

Determination of phage titre

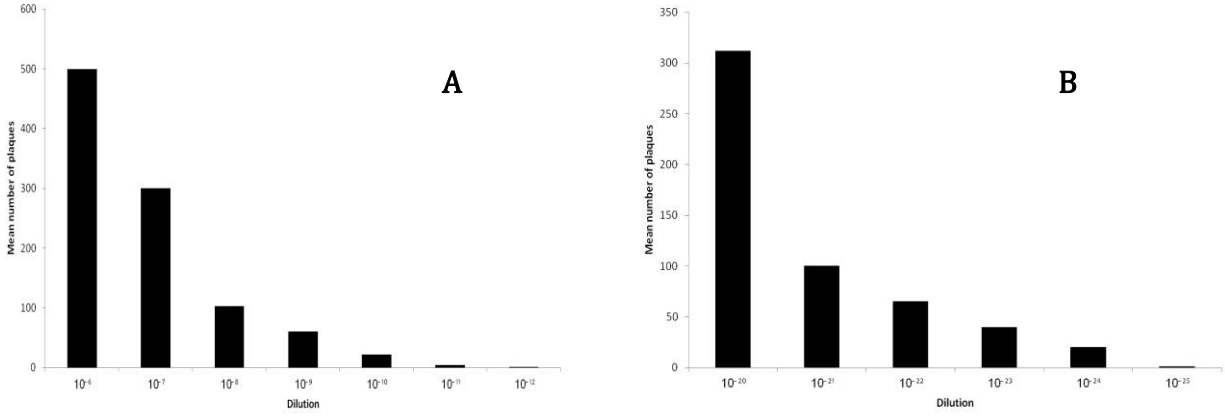

Fig. (4): The dilution end point of both phages: (A) vB_StscP-G1 at 10^{-12} and (B) vB_StscS-G2 at 10^{-25}.

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Nutrient broth with various dilutions of phage lysate was used to determine the phage titre. Low dilution for both phages (1 & 2) showed overcrowded plaques. The dilution end point for phage 1 was 10^{-12}, while that for phage 2 was 10^{-25}(Fig. 4).

The dilution that showed the best separated countable plaques were used in the next experiments (103 plaques at the dilution 10^{-8}) for vB_StscP-G1 and (322 plaques at the dilution 10^{-20}) for vB_StscS-G2.The recorded values are the mean of three replicates.

Host range

The host range of the isolated phages was examined on 16 different *Streptomyces* sp. together with *S. scabies*. The *Streptomyces* species isolated from soil were biochemically and molecularly identified as *S. aureofaciens, S. enissocaesilis, S. rochei, S. flavoviridis, S. labeadae, S. lateritius, S. lienomycini, S. plicatus, S. tendae, S. tritolerans, S. variabillis* and *S. vinaceusdrappus.* Other than the primary host (*S. scabies*), vB_StscM-G1 was susceptible to *S. aureofaciens, S. coelicolor, S. enissocaesilis, S. flavoviridis, S. griseus, S. tendae, S. tritolerans*and *S. vinaceusdrappus.* Phage vB_StscM-G2 was susceptible to *S. coelicolor, S. flavoviridis, S. griseus, S. lateritius, S. tendae*and *S. vinaceusdrappus.* However, *Streptomyces* species (*S. flaveolus, S. labeadae, S. lienomycini, S. plicatus, S. rochei, S. variabillis* and *S. violaceoruber*) were not infected by any of the phages under study as shown in table (2).

Table (2): Host range of the two isolated phages against different *Streptomyces* species

***Streptomyces* species**	Isolated phages	
	vB_StscM-G1	vB_StscM-G2
S. aureofaciens	+	-
S. coelicolor	+	+
S. enissocaesilis	+	-
S. flaveolus	-	-
S. flavoviridis	+	+
S. griseus	+	+
S. labeadae	-	-
S. lateritius	-	+
S. lienomycini	-	-
S. plicatus	-	-
S. rochei	-	-
S. scabies	+	+
S. tendae	+	+
S. tritolerans	+	-
S. variabillis	-	-
S. vinaceusdrappus	+	+
S. violaceoruber	-	-

Symbol: (+) infected, (-) not infected

Heat stability

The infectivity at different temperatures was high for both phages. The thermal inactivation point (TIP) for vB_StscP-G1 was 90°C and was 85°C for vB_StscS-G2 . No plaques were found at 100°C as shown in Fig. (5). The recorded values are the mean of three replicates.

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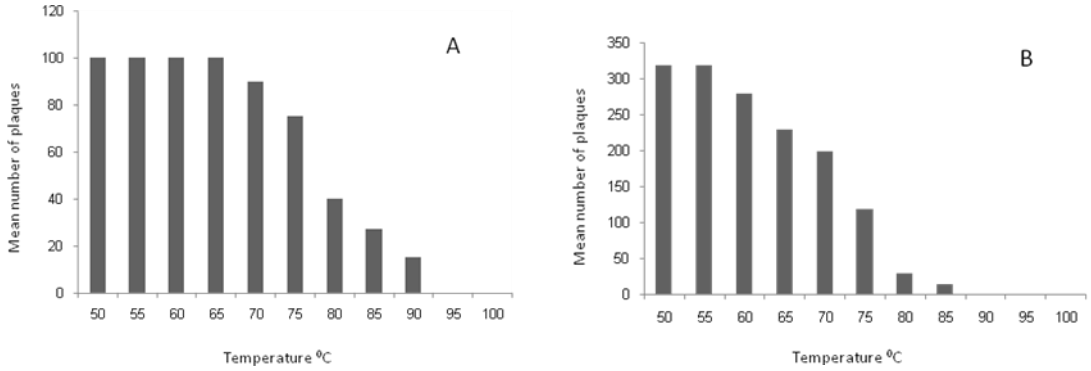

Fig 5: Thermal inactivation point of both phages . (A) vB_StscP-G1 at 90°C and (B) vB_StscS-G2 at 85°C.

pH sensitivity test

The optimum infectivity of vB_StscP-G1 was obtained at pH 7. The phage infectivity was totally inhibited at pH 12 (Fig. 6.A). However, vB_StscS-G2 maintained its infectivity over the pH range 7-9. It showed significant infectivity drop at highly acidic and alkaline conditions (pH1 & 11). Infectivity was lost at pH 13 (Fig. 6.B).The recorded values are the mean of three replicates.

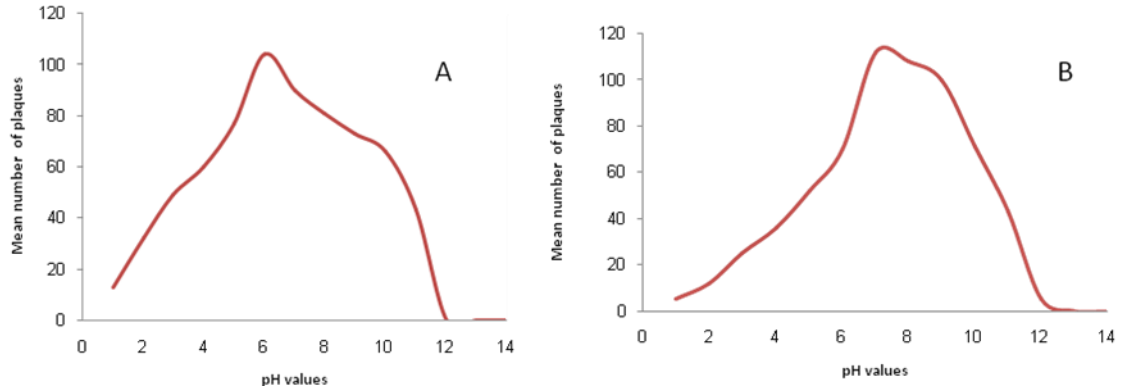

Fig. (6): Stability of vB_StscP-G1 (A) and vB_StscS-G2 (B) treated with different pH values.

Longevity *in vitro*

The results showed that both phages were not affected by storage neither at room temperature (25-30°C) nor at 0°C**.** The infectivity was not affected during the whole storage time (10-180 days).

Effect of different plant extracts on *Streptomyces scabies* and the isolated phages

Among the 13 different plant extracts used to test their effect on *S. scabies,* both turmeric and chili pepper showed bacteriocidal effect. An inhibition zone (3 & 2.3 cm) occurred when using turmeric and chili pepper, respectively**.** Both plants were eliminated in the test on phages. Plant extracts of tea, cinnamon, star anise, guava and ginger showed significant total loss of infectivity of vB_StscP-G1. However, cumin, chamomile peppermint, black cumin, majoranum and tilia did not show significant effect (Fig. 7.A). In case of vB_StscS-G2, plant extracts of cumin, guava and ginger showed significant loss of phage activity. However, tea, cinnamon, star anise, chamomile peppermint, black cumin, majoranum and tilia did not show significant inhibitory activity (Fig. 7.B). The recorded values are the mean of three replicates.

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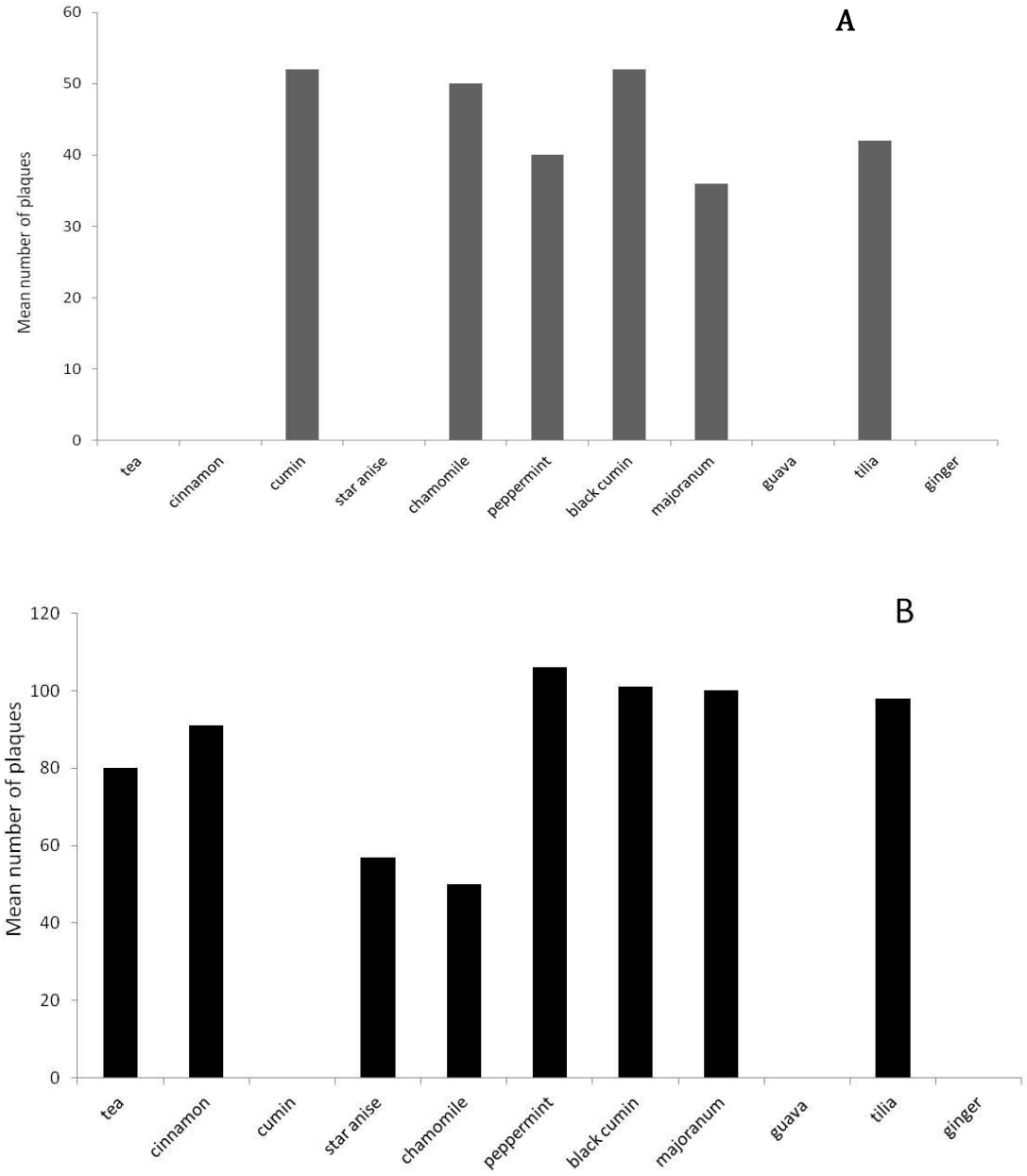

Fig 7: Effect of different plant extracts on the infectivity of (A) vB_StscP-G1 and (B) vB_StscS-G2

Effect of chemicals on *Streptomyces scabies* and the isolated phages

Among the ten chemicals tested, cobalt chloride ($CoCl_2$), mercuric chloride ($HgCl_2$) and silver nitrate ($AgNO_3$) caused inhibition zones ranging from 3-5 cm in diameter on *S. scabies*. The three chemicals were excluded in the further tests on the phages under test. By increasing the chemical concentrations (from 0.1 to 0.5 mM), sodium chloride (NaCl) and copper sulphate ($CuSO_4$) showed significant increase in the activity of both phages compared to the control. However, zinc chloride ($ZnCl_2$) and aluminum chloride ($AlCl_3$) showed significant loss in the infectivity of both phages. Calcium chloride ($CaCl_2.2H_2O$), magnesium chloride ($MgCl_2$) and potassium chloride (KCl) increased the activity of vB_StscP-G1 however, such increase was insignificant. Furthermore, the same chemicals did not show any activity increase for vB_StscS-G2 when compared to the recorded controls (Fig. 8).

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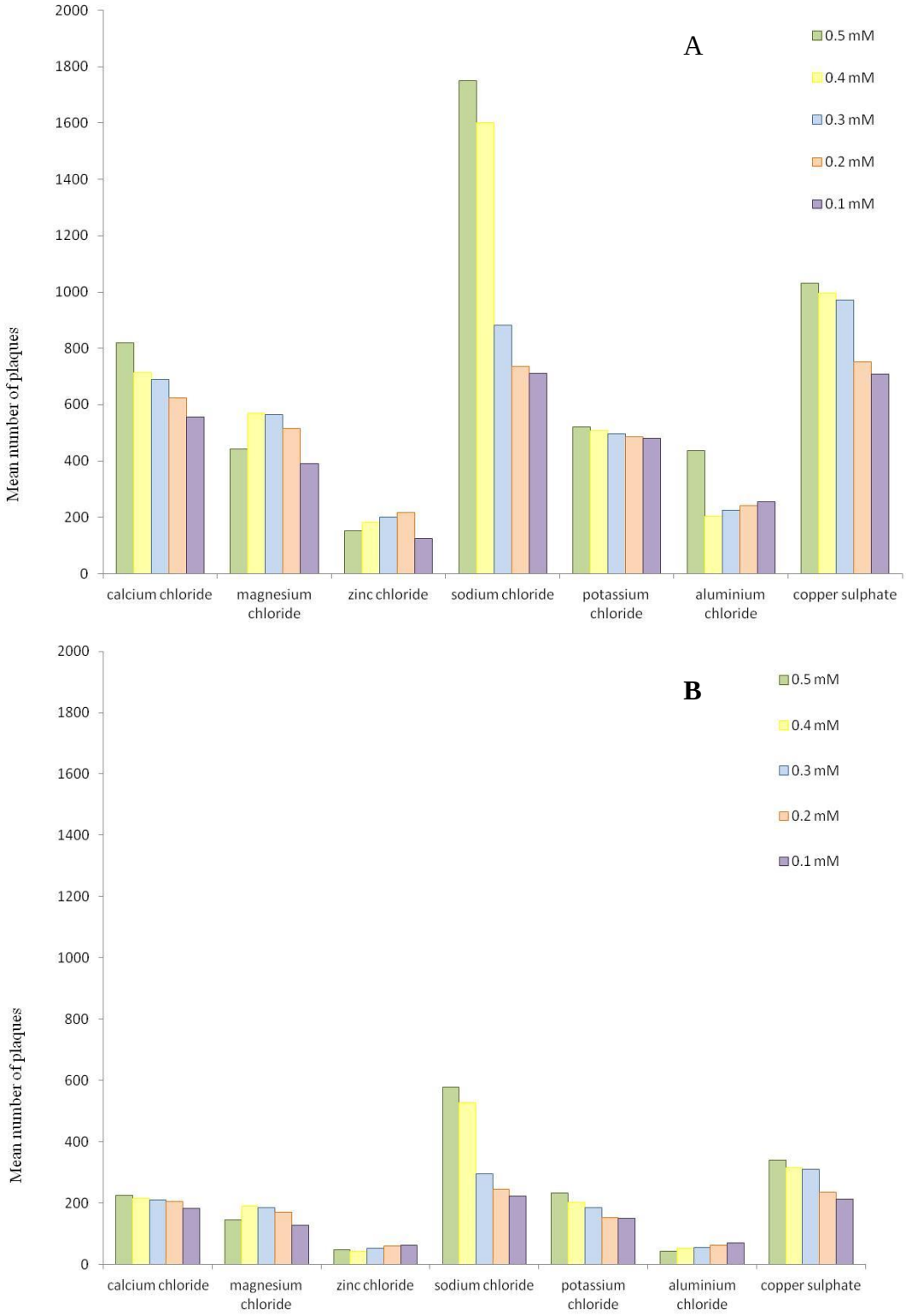

Fig 8: Effect of different chemicals on the infectivity of (A): vB_StscP-G1 and (B): vB_StscS-G2.

DISCUSSION

Potato scab is an important wide spread disease caused by *Streptomyces scabies*. Methods of control including the chemical control are not efficient. Phage therapy is one of the promising approaches to control bacterial infections [16]. Phages are generally isolated from environments that are habitats for their host [19].

In our study, two phages were isolated from potato soil. They showed different plaque sizes that were subjected to purification. The isolation was successfully done using the double layer technique using the enrichment method with $CaCl_2$. Such enrichment increased the adsorption of both phages. Our results were in agreement with Lillehaug [20] who stated that high rate of phage diffusion was observed using the double layer agar which improves the plaque visibility and counts. Also, Dowding [11],Dagert and Ehrlich [21] and Chung *et al.*[22]used $CaCl_2$ as a supplement in the media to increase the *Streptomyces* phage activity. On the contrary, EL-Tarabily [15] and Ackermann *et al.* [23] isolated the *Streptomyces* phages without the enrichment technique.

In our study, the purified phages were named phage 1 (P1) and phage 2 (P2) as a preliminary nomenclature. The morphology of P1 consisted of a hexagonal head and a short tail. However P2 had a hexagonal head and long tail. The genome of both phages was DNA because it was digested by DNase but not by RNase. Our findings of the phage shape and genome places them in order Caudovirales that contains three families of tailed viruses [24]. Possession of P1 with a hexagonal head and short tail would place it in family: Podoviridae, while the possession of a long tail would place P2 in family Siphoviridae. Therefore, according to method of nomenclature reported by Kropinski *et al.* [14], P1 was named vB_StscP-G1 and P2 was named vB_StscS-G2. In agreement to our results, previous studies showed that isolated phages from soil samples using *S. scabies* comprised of icosahedral head and a long tail [7,25].However, Goyer[2] showed the morphology of two isolated actinophages specific to *S. scabies* as tailless icosahedral heads and the other was tailed.

In our study, the dilution end point for both phages was determined. The titre of vB_StscP-G1 was 10^{-8} while that of vB_StscS-G 2 was 10^{-20}. Furthermore, the isolated phages were thermo stable at high temperatures (up to 90°C for vB_StscP-G1 and 85°C for vB_StscS-G2). This is in accordance with Weinbauer [26] who reported that phages have been found to retain their infectivity over wide range temperature conditions. Our results showed that the activity of phages were not affected by storage up to 180 days neither at room temperature (25-30°C) nor at 0°C. Furthermore, both phages were infective in acidic and alkaline conditions but, were totally inhibited at high alkaline conditions (pH 12). The optimum infectivity of vB_StscM-G1 was obtained at pH 7 and vB_StscM-G2 maintained its infectivity over the pH range 7-9. This is in agreement with Sridhar *et al.* [6] and Krasowska *et al*.[27] who stated that the optimum pH value for phages are neutral and the phage infectivity is inversely proportional to the increase of acidity (below pH4) and alkalinity (above pH11). This is attributed to the denaturation of phages proteins as reported by Sykes *et al.* [28]. On the contrary, Goyer [2] isolated *S. scabies* phage (Stsc3) however, it was inactivated in neutral pH.

In our study, among soil-inhabiting *Streptomyces* sp. tested, most species were susceptible to the phages infection. Phages used in phage therapy are preferable to have broad host range because they can inhibit several strains of the pathogen [19]. Also, Krasowska *et al*. [27] recorded that both wide and narrow host range may be useful. While narrow host range will enable distinction of closely related strains in strain typing and wide host range will be beneficial for biocontrol.

In our study, some plant extract showed a significant inhibitory effect on the isolated phages. Tea, cinnamon, star anise and guava showed significant total loss of infectivity of vB_StscP-G1. However, star anise did not show significant inhibitory activity for vB_StscS-G2. Contrary to our results, Schiffenbauer *et al.* [29] showed that star anise is 99.9% effective against T1,T3,T4,T5 and T7 phages. They also showed that tea inactivates bacteriophage T1. Also, Cock and Kalt [30] showed the effective inhibition of against MS2 bacteriophage that attack *Staphylococcus aureus*. In our study, cumin and concentrated guava and ginger showed significant inhibitory effect on vB_StscS-G2. This is may be attributed to the presence of polyphenols together with other chemicals that are effective antiviral agents.

In this study, sodium chloride and copper sulphate showed significant increase in the activity of both phages. Calcium chloride, magnesium chloride and potassium chloride increased the activity of vB_StscP-G1 but did not show any activity increase for vB_StscS-G2. Such increase in phage activity is probably attributed to the increase of adsorption and penetration rates. Our results are in accordance with Watanabe and Takesue [31],Balan and Padill [32], Binetti *et al.*[33], Quiberoni *et al.* [34] who showed that calcium and magnesium ions increase the process of phages infectivity. Absence of these ions prevents adsorption or lysis cycle. Also, Anne *et al.* [35] showed that the plaque formation of phages depend on calcium and magnesium ions

concentrations in medium. Concentration of calcium ions for plaque formation varied from phage to another, but magnesium ions were not required for plaque formation when the medium containing calcium ions. However, Carpa *et al.* [36] showed that calcium ions were not necessary neither for adsorption rate nor lytic cycle of PL-1 and J-1 phages. However, lysis of phages was faster in presence of cations. Also, Nakano *et al.*[37] showed that concentration of calcium ions made the outline of plaques difficult to discern because agar became turbid. On the contrary, this did not happen in our study since plaques were clear even with high salt concentrations. In our study, zinc chloride and aluminum chloride showed significant loss in the infectivity of both phages. This is in accordance with Robert and Charles [38] who showed that aluminum caused viral inactivation. This is due to the dissociation of viral capsid proteins.

In conclusion, we isolated and partially characterized two specific phages to *Streptomyces scabies*. Our results will provide a useful data for designing a biocontrol strategy against potato scab disease in the future work.

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