Application of Bacilli Phages for Controlling *Bacillus Subtilis* Associated Date Palm Plantlets Malformation in Tissue Culture

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Abstract

Date palm considered one of the most important crops in Arab region. *Bacillus subtilis* causes malformation on date palm in tissue culture stage. Three bacteriophages were isolated specific to *Bacillus subtilis* thus named BS1, BS2, and BS3. Results reveled that morphology of isolated phages belonged to family *Myoviridae*, *Siphoviridae*, and *Myoviridae* for BS1, BS2 and BS3 respectively. Bradford showed the total protein of phages were 0.23, 0.14 and 0.28 mg/ml for BS1, BS2 and BS3 respectively. SDS-PAGE showed molecular weight of protein was ~ 97, 70, 37, and 35 KDa in BS1. In BS2 was ~120, 97, 70, 35 and 25 KDa. BS3 was ~97, 70, 35 and 27 KDa. DNA. RAPD-PCR showed. BS1 amplified to 3 fragments with 1000, 600 and 500 bp, phage BS2 amplified to 2 fragments with 1000 and 600 bp, BS3 phage amplified to 2 fragments with 1000 and 500 bp . Five formulas were used to protect phages from UV irradiation. The best used formulas for that used with all phages were Beet-root juice and Casein formulas.

Keywords: Date palm, *Bacillus subtilis*, bacteriophage, biocontrol, pH, UV, SDS

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Introduction

The bacterial disease like sudden decline (*Erwinia chrysanthemi*) Abdalla (2001), wilt and malformation in plantlets resulting from tissue culture (*Bacillus* sp.) (Hadeel *et al*. 2007), and pink rot inflorescence (*Serratia marcescens*) (Riaz *et al*. 2009) Bacteriophages (phages) are ubiquitous obligate viral parasites of bacteria that reproduce in concert with their hosts in diverse natural environments (Koskella and Meaden, 2013). Bacteriophage abundance correlates with bacterial population densities in a given niche and the global phage population has been estimated at over 1031 phages, reflecting over 1025 infections per second (Lima *et al*., 2007). This intimate interaction between phages and their hosts results in rapid co-evolution that has been observed under both natural environmental and laboratory conditions (Westra *et al*., 2012). Bacteriophages consist of a nucleic acid genome enclosed within a protein or lipoprotein coat and like all viruses are absolute parasites, inert particles outside their hosts, deprived of their own metabolism. Inside their hosts, phages are able to replicate using the host cell as a factory to produce new phages particles identical to its ascendant (Rohwer, 2003). Bacteriophages are distributed in different locations populated by bacterial hosts, such as soil bacteriophages have been proposed as potential biological control agents against plant pathogenic bacteria. Serratia

and Bacillus bacteriophages are viruses that replicate and infect within a main host bacterium (Matsushita *et al*., 2009). Phage therapy or viral phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Phage therapy has many potential applications in human medicine as well as dentistry, veterinary science, and agriculture (McGrath and Sinderen, 2007). Phage therapy presents many potential advantages over the use of antibiotics which are intrinsic to the nature of phages. Phages are highly specific and very effective in lysing the target pathogen, preventing dysbiosis, that is, without disturbing the normal flora and thus reducing the likelihood of super-infection and other complications of normal-flora reduction that can often result following treatment with chemical antibacterials. This high specificity means that diagnosis of the bacteria involved in the infection is required before therapy is employed. The specificity of phages also enables their use in the control of pathogenic bacteria (Matsuzaki *et al*., 2000). The study aims to using the bacteriophages as specific bio-agent for control *Bacillus subtilis* contaminated cussing Date Palm plantlets malformation in tissue culture. Isolation and identification of some phages specific for *Bacillus* sp. and application of them as bio-control agents to eliminate or/and reducing *Bacillus subtilis* the total count of the pathogenic bacteria.

Materials and methods

This study was carried out in the Greenhouses, Virology labs, Agric. Microbiology Dept., Fac. of Agric., Ain Shams Univ., and Greenhouses Date Palm Pathology and Protection Dept., Central Lab. for Research of date palm, Agric. Res. Center Egypt.

1. Source of bacteria

Identified isolated *Bacillus subtilis* that infect plantlet in date palm (*Phoenix dactylifera* L.) in tissue culture stage was kindly obtained from central laboratory of date palm (Department of date-palm diseases and protection).

2. Pathogenicity test of *Bacillus subtilis*

Decimal serial dilution of the pathogenic bacteria were prepared from 10^{-1}-10^{-6} and crude of suspension ($1x10^{12}$ cfu\ml with OD_{600}=0.538) were used to inject date palm that planted in pots (aged 60 days).Pathogenicity test for *Bacillus subtilis* isolate was done according to Riaz *et al.* (2009). Ten plantlet (tissue culture) in jars were inoculated with 10 ml of *Bacillus subtilis* isolate (aged 24 hrs, $1X10^{6}$ CFU\ml conc.) and incubated in growth chamber.

3. Antibiotic sensitivity

Antibiotic sensitivity of *Bacillus subtilis* isolate was assayed according to Sethuraman *et al.* (2011). The bacteria growth was streaked on NA media containing ampicillin antibiotic desk with concentration of 10, 50, 100, 200, 400, 1000 mg/L. The plates were incubated at 37°C/24hr for *B.subtilis* and 25˚C/24hr for the isolated bacteria.

4. Source of bacteriophage

Bacteriophages specific to *B.subtilis* were isolated from free soil from Agricultural research center (ARC). Giza, Egypt. Detection of bacteriophages that isolated from soil and plant was assayed qualitatively using the spot test according to Borrego *et al*. (1987).

5. Characterization of the isolated bacteriophages

5.1. Morphological character

Bacteriophages morphology were examined according to Lewaa *et al.,* (2023) as follow: Solution of 2% of phosphotungestic acid (PTA) was prepared with pH 7. Negative staining method was done by Equal bacteriophage and PTA were mixed (10:20µl V/V) and placed on grid coated with carbon film for about 20 sec. then allowed to dry in the air. The grids were examined by transmission electron microscope (TEM, Jeol JEM-J1400 in Fac. of Agric. Res. Park (FARP), Cairo Univ.

5.2. UV spectra properties

UV absorbance of the purified phages was determined according to Setlow and Boyce (1960). The purified phages were red on UV spectrophotometer (Orion Aquamate 8000 UV/VIS) from 200-300 nm with interval wavelength 10 nm and the spectrophotometric data (ratio of A_{260}, A_{280}, A_{260}/A_{280}, A_{280}/A_{260}) were record.

6. Thermal inactivation point (TIP)

TIP was carried out according to Basdew and Laing (2014) by exposure phages (with initial concentration at $1x10^7$, $2x10^7$ and $1x10^7$ pfu/ml for BS1, BS2 and BS3 phages respectively to temperature 30 to 100˚C/ 10 min intervals 10˚C in large scale of temperature and then for 52, 54, 56, 58 and 60˚C. Each treated phage was assayed using spot test assay.

7. Propagation and purification of phages

The isolated Phages were propagated in the main host and then phage suspensions were centrifuged at 3000 rpm/30 min. About 10% chloroform was added to the supernatant to remove the small contaminated bacteria and then centrifuged at 16000 rpm/2hrs/4°C. The supernatant was discarded and the pellet was re-suspended in 1ml CM buffer (6ml/L 1M Tris buffer; 2.5g/L MgSO4.7H2O; 0.735g/L CaCl2; 0.05g/L gelatin; pH 7.5) and centrifuged at 3000 rpm for 30min. and then the supernatant was centrifuged at 16000rpm/2hrs /4°C. Finally, the pellet was re-suspended in CM buffer. This method was carried out with some modification according to (Bachrach and Friedmann, 1971).

8. Stability of pH

Stability of the phages to pH degrees was assayed according to Taj *et al.*(2014) by exposure the phages (with initial concentration $1x10^7$, $2x10^7$ and $1x10^7$ pfu/ml for BS1, BS2 and BS3 phages respectively) at overnight to 5, 6, 7, 8 and 9 degrees of pH. The infectivity of phages was assayed by plaqe assay.

9. Effect of freezing and thawing times on activity of *B.subtilis* lytic phages

Freezing and thawing on bacteriophages was tested according to Clark *et al*. (1962). The isolated phages (with initial concentration, 1x107, 2x107 and 1x107 pfu/ml for BS1, BS2 and BS3 phages respectively) were exposure to freezing (-20˚C) and then thawed at room temperature. The previous steps were repeated for 4 times. The phages infectivity were tested was by plaque assay.

10. Molecular characterization of isolated phages

10.1. DNA Pattern

The total nucleic acids were extracted from purified phages using extraction method according to Maniatis e*t al.* (1982) with minor modification (Campos e*t al.*, 2003) was uses. Fifteen ml of high titer phage suspension were incubated at 37 °C for 30 min. with DNase and RNase at final concentration of 1µg/ml each, to get rid of contaminating bacterial DNA and RNA.

10.2. Random amplified polymorphic DNA–PCR (RAPD-PCR)

RAPD-PCR was used to detection the polymorphism among phage's genome using random primer that carried out according to (Williams *et al*., 1990) as following: The reaction was prepared using 25µl per tube, containing 2µl DNA of each isolate, 0.15 µl of Taq DNA polymerase enzyme (1 unit), 5µl 10X buffer, 2µl MgCl2 (25 mM), 2µl dNTPs (2.5 mM of each), 2µl OP A-12 primer (TCGGCGATAG) as general random primer (that obtained from operon company) and 12µl H2O.. RAPD-PCR was amplified using the following program (Table 1) according to Shamloul *et al.* (1999).

Table (1): Program of PCR

Steps	Temp. and time	Number of cycles
Denaturation	94°c for 4 min.	1 cycle
Denaturation	94ºc for 30 sec.	
Annealing	36ºc for 30 sec.	40 cycles
Extension	72ºc for 45 sec.	
Extension	72ºc for 7 min.	1 cycle

11. Protein pattern

Isolated phages protein were determined using the colorimetric method as used by Bradford (1976).

12. SDS-Polyacrylamide Gel

Polyacrylamide gel consists of stacking (upper) gel and separating (lower) gel. The stacking gel was used to get better band resolution. Gel was prepared according to Laemmli (1970).

13. UV radiation

Stability of the isolated phages was assayed according to Born *et al.* (2015). 1ml of isolated phages with initial concentration $6x10^{12}$pfu/ml) was exposure to UV lamp (254 nm) for 5, 10, 15, 20, 25 and 30 min at hight of 60 cm from the UV lamp. The infectivity of the exposure particles was determined qualitatively by the spot test and quantitatively by the double layer agar technique.

14. Longevity *In vitro* (LIV)

Longevity *In vitro* for isolated phages determined according to Yoshida *et al.* (2006). Each isolated phage was saved at room temperature (25°C) and the infectivity of phages was test each 7 days till 90 days using plaque technique.

15. Application of the Phages as a therapeutic agent

15.1. Preparation of formulas

Carrot juice and beet-root juice were diluted in SM buffer to final concentration 20% (v/v). Casein was dissolved in 1M NaOH with final concentration 20 mg/ml. Sodium alginate was prepared by dissolving 2gm in 100ml d.H_2O and put in water bath at 65°C/4hrs and then the solution was pasteurized on 100°C/30min. mixture of skim milk (0.5g) and sucrose (0.5g).

15.2. Protections of phages

All prepared formulas were added with each isolated phage with rate of 1:1. One gram of each treated phage was exposure to UV lamp (254nm) for 20, 40 and 60 min at hight of 60 cm from the UV lamp. The infectivity of the exposure particles was determined qualitatively by spot test and quantitatively by the double agar layer technique. Data were statistically analyzed according to the standard procedure in completely random design using ANOVA two ways using SPSS program version 19 as mentioned by Snedecor and Cochran (1980).

15.3. Phage as therapeutic agent

The isolated phages were examined as preventative and preservative for date palm plants from bacterial infection according to Hernández (2017) (with MOI =0.01) *In vitro* and *In vivo.*

15.4. Preparation of phages inoculum

The isolated phages of the isolated pathogenic bacteria were prepared with concentration $1x10^8$ pfu/ml for each phage. Three isolated phages of isolated pathogenic bacteria were mixed with ratio 1:1:1. The concentration of each phage cocktail for isolated pathogenic bacteria was estimated using spot test.

16. Prevention

Five replicate of sterilized date plants seedling for each treated were wounded using blade method according to Lidia *et al.* (2008) and soaked the wounded plants the phages of the isolated pathogenic bacteria and 5 replicate wounded plants were soaked in phage

cocktail specific for the isolated pathogenic bacteria. The replicated seedling plants were soaked for 0, 30 and 60min in the phage suspensions, then the treated plants were washed with sterile dH_2O and soaked in the isolated pathogenic bacterial suspensions.

17. Preservation

Sterilized date plants seedling (aged 60 days) were wounded (five replicates for each treatment) using blade method according to Lidia *et al.* (2008) and soaked the wounded plants for 0, 30 and 60 min in the isolated pathogenic bacterial suspension Then, the treated plants were washed with sterile dH_2O and soaked in individual propagated phage suspensions for their main host. Phage cocktail suspension specific for the isolated pathogenic bacteria suspension were treated with mixture the isolated pathogenic bacterial suspension.

The treated plants in prevention and preservation experiments were washed with sterile dH_2O and planted in the tissue culture media and incubated at 25°C in growth room with 16hrs illumination as *In Vitro* and in nursery as *In Vivo* experiment. Data were statistically analyzed according to the standard procedure in completely random design as mentioned by Snedecor and Cochran (1980) using SPSS program version 19 using ANOVA two ways.

18. Detection bacteria and the phages

B.subtilis and their phages were treated plants (*In vitro*) .The bacteria and phages were detected after 21days from the treatment. The detection was carried out by grinding the treated plants well in sterilized d.H2O and streaking on NA media for *B.subtilis* and incubated for 25°c/24hr. The isolated phages were detected in the treated plants qualitatively by spot test technique.

Results

Morphology of phages (size and shape)

Morphology of the isolated phages specific for *B.subtilis* were examined using transmission electron microscope (TEM). As shown in Fig (1) and Table (2), BS1 phage has head with 110x85 nm in diameter and long contractile tail with 191nm in length that belongs to *Myoviridae.* Phage BS2 has a head with 85x79 nm in dimensions with long non-contractile tail with length 245 nm which belongs to *Siphoviridae.* In case of phage BS3, the phage has isometric head of about 110 nm in diameter and long contractile tail of 149 nm in length that belongs to *Myoviridae*.

Table (2): Morphology and taxonomy of the isolated phages.

Phage	Head dimensions (nm)	Tail length (nm)	Family
BS1	110x85	191	*Myoviridae*
BS2	85x79	245	*Siphoviridae*
BS3	110	149	*Myoviridae*

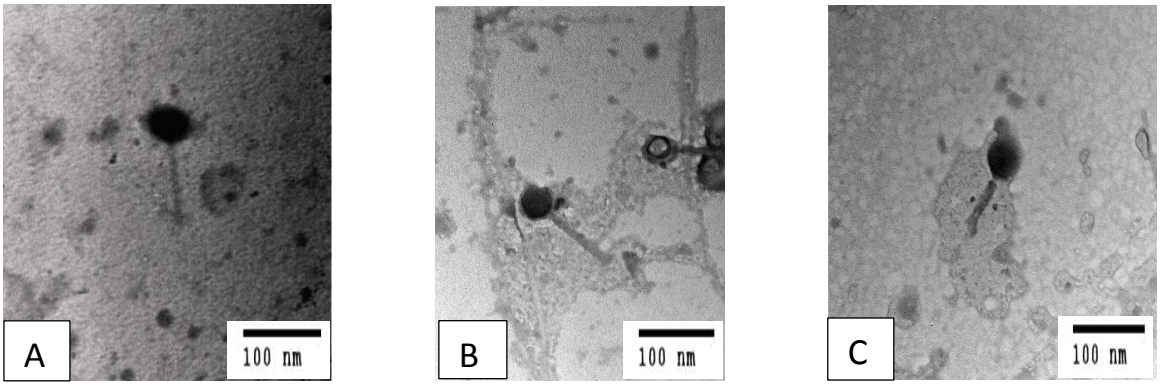

Fig. (1): Electron micrographs of negatively stained particles of phages BS1 (A), BS2 (B) and BS3 (C) specific to *B.subtilis*.

Characteristic spectrum of *B.subtilis* phages

Absorption spectrum properties of *B.subtilis* phages. The absorbance of *B.subtilis* phages was red off and successive reading were taken at for distance 5 nm intervals of the whole range of wavelength (220-300 nm). The obtained data are presented in Fig (2) and table (3). Data showed that *B.subtilis* virulent phages BS1, BS2 and BS3 have maximum absorbance at 260, 260 and 255 nm. Minimum absorbance at 250, 230 and 235. The ratio of A260 nm at A280 nm were 1.1, 1.3 and 1.2 and the ratio of absorbance at A280 to at A260 were 0.82, 0.77 and 0.82 for BS1, BS2 and BS3 phages respectively.

Table (3): UV absorbance for the *Bacillus subtilis* phages

Purified Virus	**A maximum in nm**	**A minimum in nm**	**A_{260}/A_{280}**	**A_{280}/A_{260}**
BS1	260	250	1.1	0.82
BS2	260	230	1.3	0.77
BS3	255	235	1.2	0.82

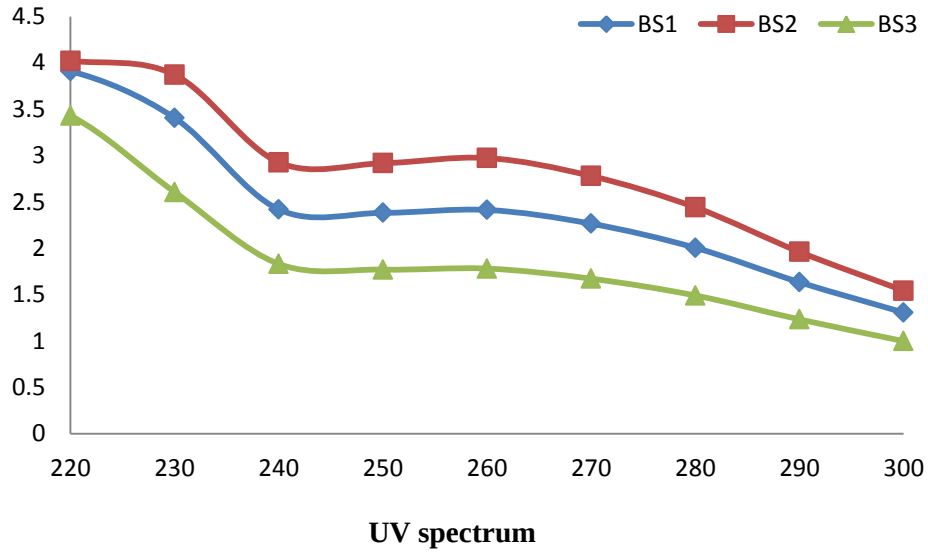

Fig. (2): U.V spectrum of purified suspension of *Bacillus* phages

Heat stability

As shown in table (4), thermal inactivation point (TIP) of *B.subtilis* phages was determined at different temperature degrees from 30˚C to 100˚C with intervals 2˚C and cooled immediately and assayed using spot test. Results showed that TIP were 58±1, 46±1 and 54±1 for phages BS1, BS2 and BS3 respectively.

Table (4): Phage Stability for TIP range

Phage	TIP
BS1	58±1
BS2	46±1
BS3	54±1

Phage stability for pH values

Stability of *B.subtilis* phages to different pH values (5, 6, 7, 8 and 9) was tested. Results in table (5) and illustrated by fig (3) showed that phage BS1 inhibited completely in pH 5 and partially affected by pH 6, pH7, pH 8 and pH9. Its activity reduced at rate of 5 log with $1x10^2$ pfu/ml in pH 6, 1.4 log with $4x10^5$ pfu/ml in pH 7 and 4.5 log with $3.1x10^2$ pfu/ml in pH 8 comparing with the control ($1x10^7$ pfu/ml). BS2 lost the activity in pH 5 completely and partially with $3x10^3$ pfu/ml with reduction rate 3.8 log in pH 6, $3.7x10^5$ pfu/ml with reduction rate 1.7 log in pH 7, with $6x10^3$ pfu/ml with reduction rate 3.5 log in pH 8 and with $2.1x10^2$ pfu/ml with reduction rate 4.9 log in pH 9 comparing with the control ($2x10^7$ pfu/ml). phage BS3 inhibited partially in pH 5, pH 6, pH 7 and pH 8 and lost the activity completely in pH 9 which its activity reduced with rate of 3.7 log with $2x10^3$ pfu/ml in pH 5, 2.5 log with $3.3x10^4$ pfu/ml in pH6, 1.6 log with $2.3x10^5$ pfu/ml in pH 7 and 3 log with $1x10^4$ pfu/ml in pH 8 comparing with control ($1x10^7$ pfu/ml). The results showed also the

phages differed in their stability when treated with different pH values and it's sensitive to acidity more than alkalinity.

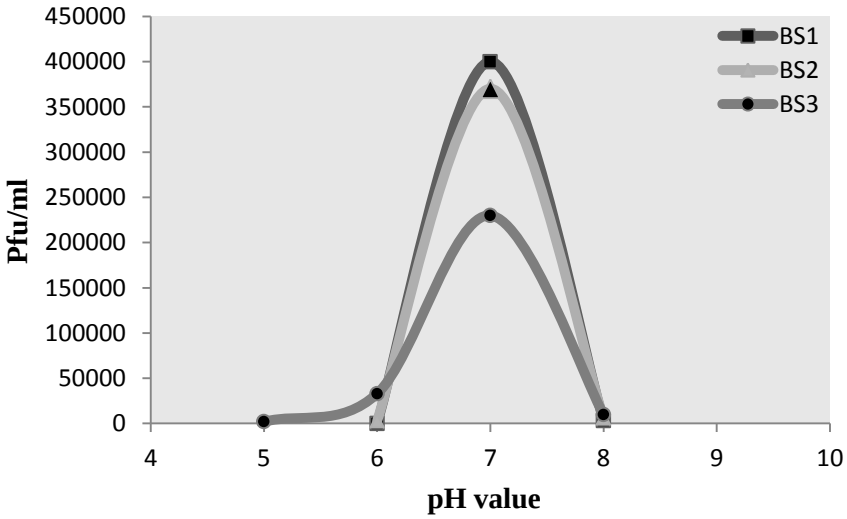

Fig. (3): Effect of pH values on *B.subtilis* lytic phages stability.

Effect of freezing and thawing times on activity of lytic phages

Bacillus subtilis phages were exposed to freezing followed by thawing 4 times and their activity were assayed quantitatively by plaque assay technique and data were illustrated by fig (4). The obtained data showed that phage BS1 titer reduced after every cycle of freezing and thawing. Titer of phage BS2 reached zero pfu/ml after 2 cycles. Titer of phage BS3 reached zero pfu/ml after 1cycle. BS1 phage titer reduced from $2x10^6$ pfu/ml (with 0.69 log reduction) to $2x10^5$ pfu/ml (with 1.7 log reduction) after 2 cycles; from $1x10^7$ pfu/ml to $1x10^5$ pfu/ml (with 2 log reduction) after 3 cycles and from $1x10^7$ pfu/ml to $3x10^3$ pfu/ml (with 3.5 log reduction) after 4 cycles. BS2 Phage decreased from $4.6x10^4$ pfu/ml (with 2.6 log reduction) to $2.6x10^3$ pfu/ml (with 3.8 log reduction) after 2 cycles. In case of BS3, the titer decreased from $4.1x10^3$ pfu/ml (with 3.3 log reduction) to zero in 2, 3 and 4 cycles. The obtained results showed that phage SB1 is the most stable one to freezing and thawing.

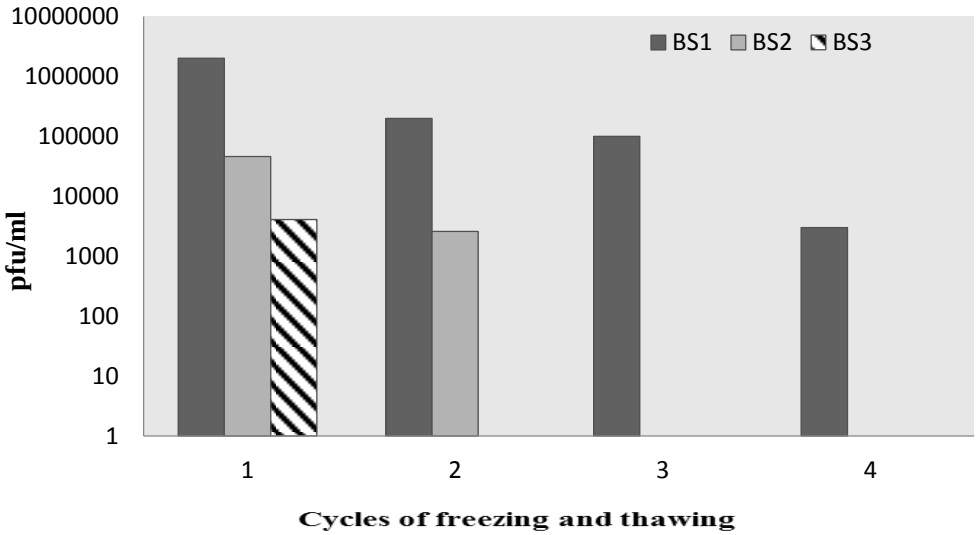

Fig (4): Effect of freezing and thawing cycles on *Bacillus* phages

RAPD-PCR analysis

RAPD-PCR was used in this study to find out the variability of genome DNA of phage isolates. DNA polymorphisms amplified by arbitrary primer was successive to a genetic marker among phages. The total number polymorphism 3 fragments (Fig 5) of amplified *Bacillus* phages' DNA. The results showed that phage BS1 amplified to 3 fragments with 1000, 600 and 500 bp, phage BS2 amplified to 2 fragments with 1000 and 600 bp, BS3 phage amplified to 2 fragments with 1000 and 500 bp, Monomorphic bands among *Bacillus* phages was 1 out of 3 monomorphic bands with percentage 33.33 %. Polymorphic bands (specific bands for each phage) were 2 out of 3 with percentage 66.67 %. As showed in Table, 23 and illustrated in Fig. (26). Polymorphic showed that genome variability among the 3 isolates.

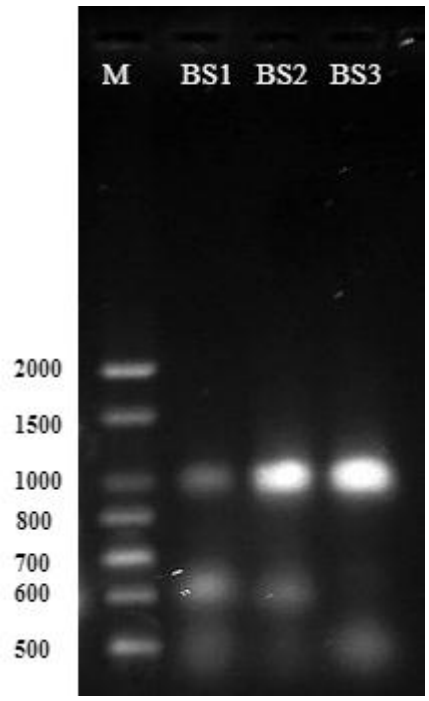

Fig. (5): RAPD-PCR for *Bacillus* phages` DNA

Protein quantity in *B.subtilis* lytic phage particles

The amount of protein in viral particle was determined by Bradford method. The viral protein of *B.subtilis* lytic phages BS1, BS2 and BS3 was red at 595 nm using the spectrophotometer and the amount of protein was calculated from the standard curve (fig 6) that prepared by bovine serum albumin. The amount of protein in phages BS1, BS2 and BS3 was found to be 0.23, 0.14 and 0.28 µg/ml, respectively.

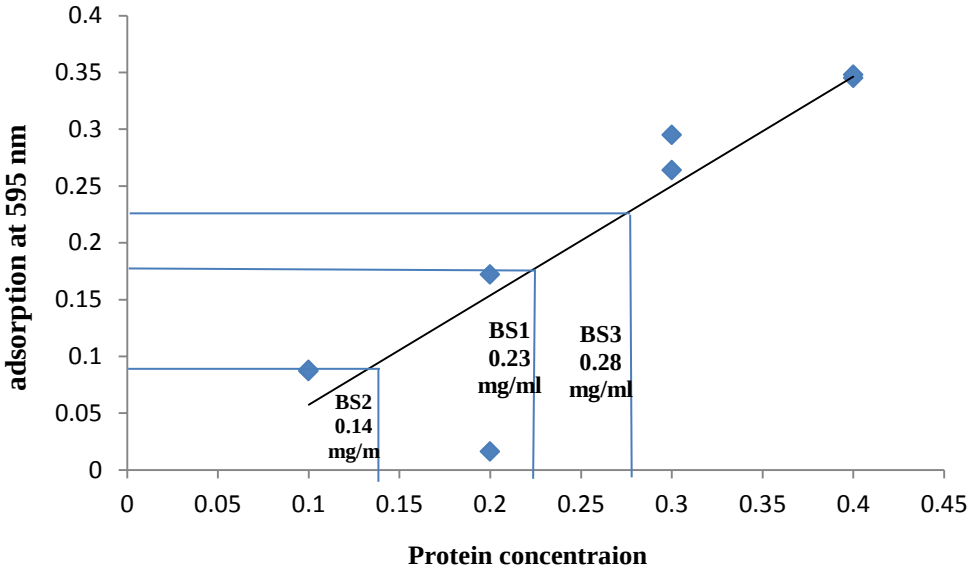

Fig. (6): Quantitation of *Bacillus* phages protein using Bradford method

Protein pattern of *Bacillus subtilis* lytic phages

The structural protein of *Bacillus subtilis* lytic phages was determined using 12% SDS-polyacrylamide gel electrophoresis. The obtained results in fig (7) showed that the number of the structural proteins and their molecular weight of the phages BS1, BS2 and BS3. As shown in fig (31) phage BS1 has 4 structural proteins of molecular weight about 97, 70, 37 and 35 KDa. BS2 phage has 5 major structural proteins of molecular weights about 120, 97, 70, 35 and 25. BS3 phage has 5 major major structural proteins of molecular weights about 97, 70, 55, 35 and 27 KDa. Data in table (28) showed that BS1 phage has unique band of molecular weight 37 KDa and 2 monomorphic band of molecular weights 70 and 35 KDa and 1 polymorphic band of molecular weight 97 KDa. The phage BS2 has 2 unique bands of molecular weights 120 and 25 KDa, 1 polymorphic band of molecular weight 97 KDa and 2 monomorphic bands of molecular weight 70 and 35 KDa. BS3 phage has 2 unique bands of molecular weights 55 and 27 KDa and 1 polymorphic band of molecular weight 97 KDa and 2 monomorphic bands of molecular weights 70 and 35 KDa. On the basis of the obtained results, it can conclude that *Bacillus subtilis* lytic phages are different in their content of the structural proteins qualitatively and quantitatively.

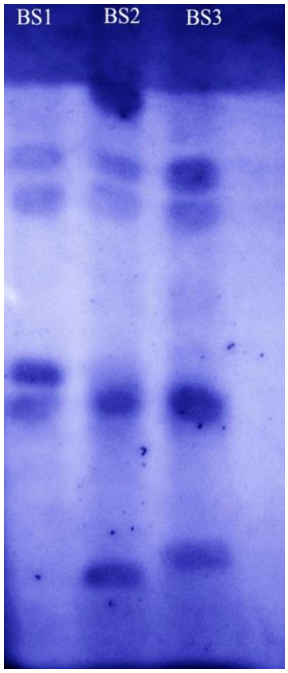

Fig. (7): SDS-PAGE 12% of purified serratia and bacillus phages

Stability of *B.subtilis* lytic phages to the UV-radiation

Stability of *B.subtilis* lytic phages to UV-radiation was assayed after exposure of the viral suspensions to UV at distance of 60 cm for 5, 10, 15, 20 and 25 min. The results illustrated by fig (8) showed that, titers of the phages decreased when the phages are exposed to UV and the decrease proportionate with the time of exposure. The phage BS1, decreased with

rate of 1.6 log with $2.1x10^6$ pfu/ml, 2.9 log with $1.3x10^4$ pfu/ml, 4 log with $1x10^3$ pfu/ml and 4.9 with $1.1x10^2$ pfu/ml after 5, 10, 15 and 20 min from the exposure respectively. In BS2 phage, the phages' titer decreased with rate of 1.3 log with $1x10^6$ pfu/ml, 3.1 log with $1.3x10^4$ pfu/ml and 5.2 log with $1.1x10^2$ pfu/ml, after 5, 10, and 15 min from the exposure respectively. In case of BS3 phage, the phages' titer decreased with rate of 0.69 log with $2x10^6$ pfu/ml, 1.6 log with $2.5x10^5$ pfu/ml, 4 log with $1x10^3$ pfu/ml and 4.8 log with $4.2x10^2$ pfu/ml after 5, 10, 15 and 20 min from the exposure respectively.

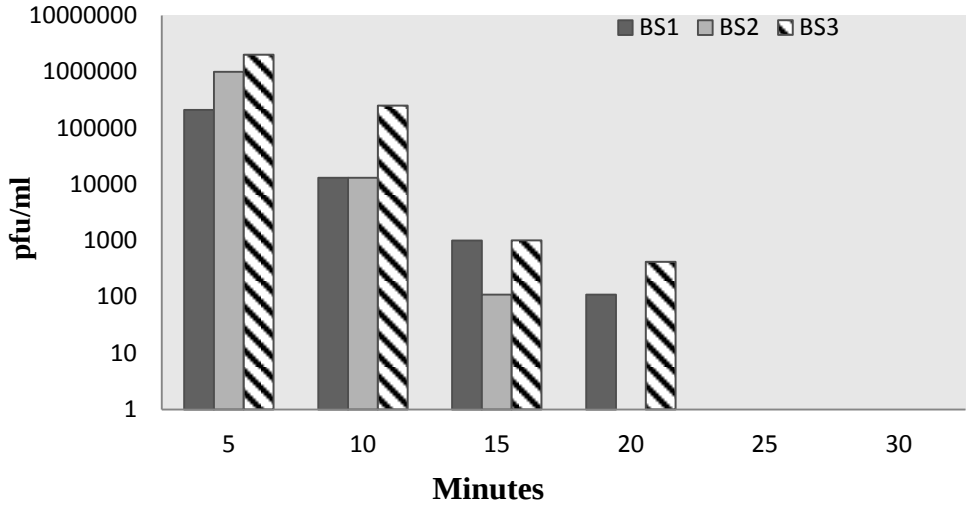

Fig (8): Stability of *Bacillus* phages for UV irradiation

Aging of *B.subtilis* lytic phages

Lysate of each phage of *B.subtilis* (BS1, BS2 and BS3) was placed in 12 eppendorf tubes (1ml/tube) and left at room temperature for 90 days. The activity of each phage was examined using the spot test technique every week to determine their aging. The obtained results along the 90 days indicated that the phages still active and could be lyses their bacterial host. Data in Fig (9) illustrated that the concentration of BS1 phage was $2.3x10^5$ pfu/ml with reduction ratio 1.6 log, $1x10^4$ with reduction ratio 3 log and $1.1x10^2$ with reduction ratio 4.9 log treated with skim milk+ sugar and exposed to UV for 20, 40 and 60min, respectively. Moreover, $3.6x10^6$ with reduction ratio 0.44 log, $2.1x10^5$ with reduction ratio 1.6 log and $3x10^3$ with reduction ratio 3.5 log treated with beet-root juice and exposed to UV for 20, 40 and 60min, respectively. In addition, $7.4x10^5$ with reduction ratio 1.1 log, $4.2x10^4$ with reduction ratio 2.3 log and $2.6x10^3$ with reduction ratio 3.6 log treated with carrot juice and exposed to UV for 20, 40 and 60min, respectively. Furthermore, $2.8x10^4$ with reduction ratio 2.5 log and under-range (32) with reduction ratio 5.5 log treated with sodium alginate and exposed to UV for 20 and 40min, respectively. Whereas, $6.6x10^5$ with reduction ratio 1.2 log, $7x10^4$ with reduction ratio 2.1 log and $2.1x10^3$ with reduction ratio 3.6 log treated with casein and exposed to UV for 20, 40 and 60min, respectively. The statistical analysis of the obtained data for treatments showed that the carrot juice, sodium alginate and casein formulas are no significant difference and each other formulas are significant difference between them. The statistical analysis of the time

showed that there is no significant difference between 40 and 60 min. and significant difference with 20 min.

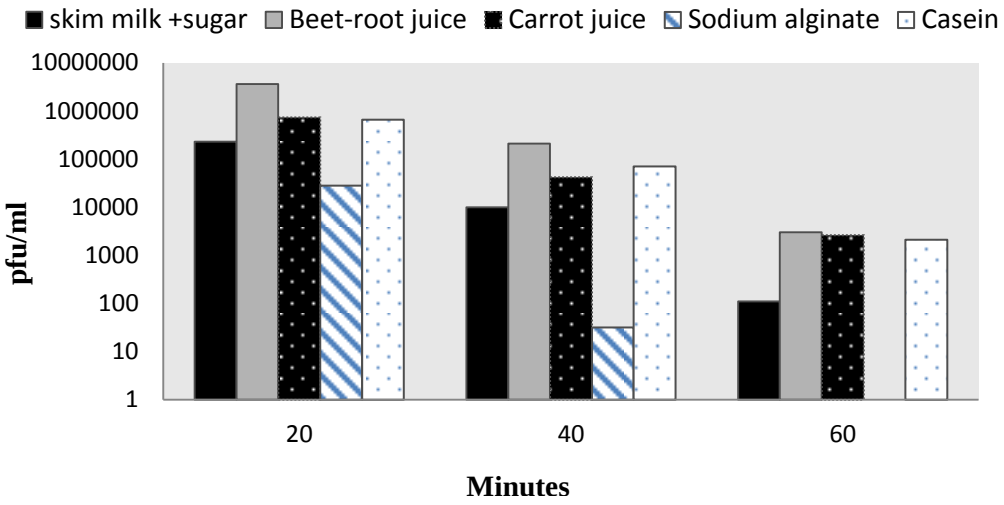

Fig (9): UV stability with treated BS1 phage with several formulas

In BS2 phage, the concentration of treated phage was $3.4x10^4$ pfu/ml with reduction ratio 2.7 log and under-range (42) with reduction ratio 5.6 log treated with skim milk+ sugar and exposed to UV for 20, 40 and 60min, respectively. Whereas, $4.3x10^5$ with reduction ratio 1.6 log, $1x10^4$ with reduction ratio 3.3 log and $1.4x10^3$ with reduction ratio 4.1 log treated with beet-root juice and exposed to UV for 20, 40 and 60min, respectively. In addition, $2x10^5$ with reduction ratio 2 log, $2.8x10^4$ with reduction ratio 2.8 log and $6x10^2$ with reduction ratio 4.5 log treated with carrot juice and exposed to UV for 20, 40 and 60min, respectively. Furthermore, $6.8.x10^3$ with reduction ratio 3.4 log and under-range (65) with reduction ratio 5.4 log treated with sodium alginate and exposed to UV for 20 and 40min, respectively. Moreover, $1x10^6$ with reduction ratio 1.3 log, $7x10^4$ with reduction ratio 2.4 log and $1.2x10^3$ with reduction ratio 4.2 log treated with casein and exposed to UV for 20, 40 and 60min, respectively as shown in Fig (10).The statistical analysis of the obtained data for treatments showed that all formulas are no significant difference between them. The statistical analysis of the time showed that there is no significant difference between 40 and 60 min. and significant difference with 20 min.

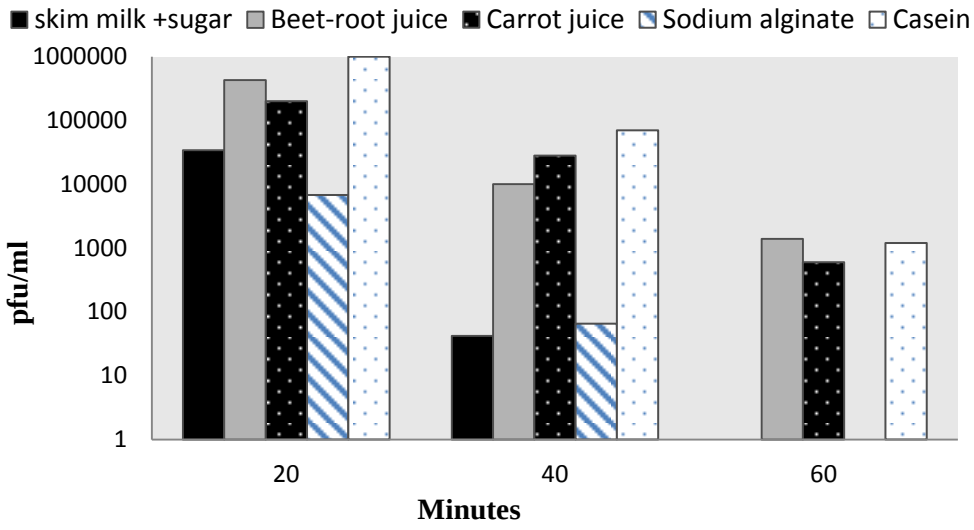

Fig (10): UV stability with treated BS2 phage with several formulas

Concentration of BS3 phage was $4.2x10^4$ pfu/ml with reduction ratio 2.3 log and under-range (26) with reduction ratio 5.6 log treated with skim milk+ sugar and exposed to UV for 20 and 40 min, respectively. Moreover, $1x10^5$ with reduction ratio 2 log, $2.7x10^3$ with reduction ratio 3.5 log and $2.6x10^2$ with reduction ratio 4.6 log treated with beet-root juice and exposed to UV for 20, 40 and 60min. respectively. In addition, $3.4x10^5$ with reduction ratio 1.4 log and $1.5x10^3$ with reduction ratio 3.8 log treated with carrot juice and exposed to UV for 20 and 40 min, respectively. Whereas, $2x10^4$ with reduction ratio 2.7 log and under-range (58) with reduction ratio 5.2 log treated with sodium alginate and exposed to UV for 20 and 40 min, respectively. Furthermore, $1.2x10^5$ with reduction ratio 1.9 log, $6.1x10^4$ with reduction ratio 2.2 log and $1x10^3$ with reduction ratio 4 log treated with casein and exposed to UV for 20, 40 and 60 min, respectively as shown in fig (11). The statistical analysis of the obtained data for treatments showed that all formulas are no significant difference between them. The statistical analysis of the time showed that there is no significant difference between 40 and 60 min. and significant difference with 20 min.

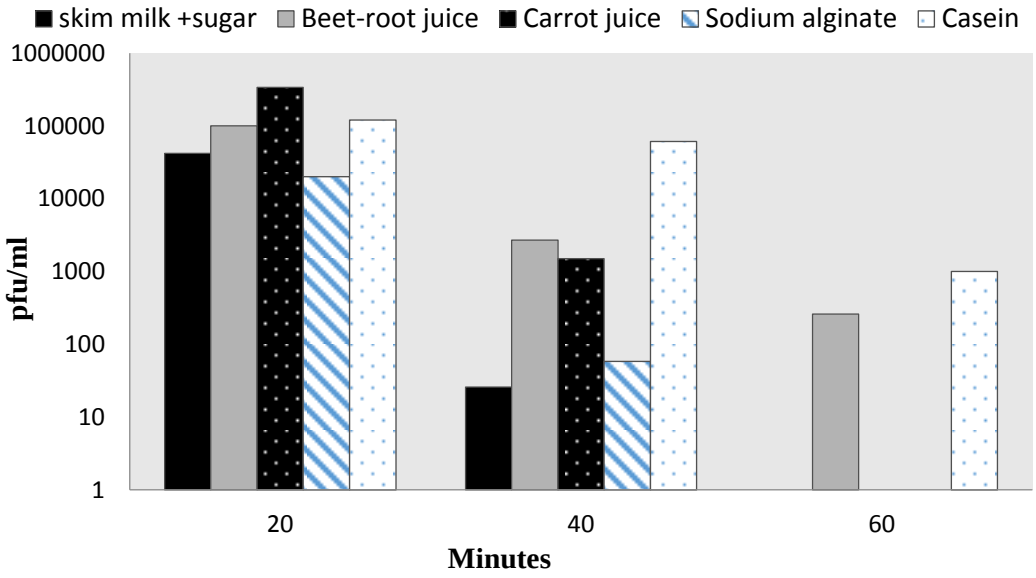

Fig (11): UV stability of BS3 phage treated with different formulas

As shown in Table (5) the statistical analysis of the treatments showed that no significant difference between *B.subtilis* +BS1 and *B.subtilis* +BS2 treatments, In addition no significant difference between *B.subtilis* +BS3, *B.subtilis* cocktail + phage cocktail treatments, *B.subtilis* cocktail+ phage cocktail and BS2+ *B.subtilis* and BS3+ *B.subtilis* treatments. The statistical analysis of the time showed that there is no significant difference between 30 and 60 min. and significantly different with zero time was observed. The statistical analysis of the treatments showed that no significant difference between *B.subtilis* +BS1 and *B.subtilis* +BS2 treatments, In addition no significant difference between *B.subtilis* +BS3, *B.subtilis* cocktail + phage cocktail treatments, *B.subtilis* cocktail+ phage cocktail and BS2+ *B.subtilis* and BS3+ *B.subtilis* treatments. The statistical analysis of the time showed that there is no significant difference between 30 and 60 min. and significantly different with zero time was observed.

As shown in table (6), *B.subtilis* and BS1, BS2 and BS3 phages in the *In vitro* experiment were detected using NA medium for *B.subtilis* and spot test for the phages. In treatment of BS1 phage, *B.subtilis* was isolated in all treatments except in the prevention treatment after 30min and 1hr, BS1 phage exhibited positive results in all treatments except preservation after 30min and 1hr. In BS2 phage treatments, *B.subtilis* was isolated in all treatments except 1hr in prevention experiment; BS2 phage was found spot only in zero-time in preservation and in 30min and 1hr in prevention treatments. In case of BS3 phage, *B.subtilis* was isolated in all treatments in preservation and only in zero-time of prevention while BS3 was detected only in prevention experiment after 30min and 1hr.

Table (5): Number of dead plants treated with *B.subtilis In vitro*

Treatment	Number of dead treated plants					
Control	0/5					
BS1	0/5					
BS2	0/5					
BS3	0/5					
Phage cocktail	0/5					
B.subtilis	4/5					
Cocktail of *B.subtilis* isolates	5/5					
Antibiotic	**10 mg/L**	**20 mg/L**	**100 mg/L**	**200 mg/L**	**400 mg/L**	**1000 mg/L**
	4/5[a]	**4/5 [a]**	**2/5 [b]**	**2/5 [b]**	**1/5 [b]**	**1/5 [b]**
	Zero time		**30min**		**1hr**	
***B.subtilis* +BS1**	3/5^{aI}		3/5^{aII}		4/5^{aII}	
***B.subtilis* +BS2**	2/5^{aI}		4/5^{aII}		4/5^{aII}	
***B.subtilis* +BS3**	3/5^{bI}		4/5^{bII}		4/5^{bII}	
***B.subtilis* cocktail + phage cocktail**	3/5^{bI}		4/5^{bII}		5/5^{bII}	
BS1+ *B.subtilis*	3/5^{cI}		2/5^{cII}		2/5^{cII}	
BS2+ *B.subtilis*	3/5^{dI}		2/5^{dII}		1/5^{dII}	
BS3+ *B.subtilis*	2/5^{dI}		2/5^{dII}		2/5^{dII}	
phage cocktail + *B.subtilis* cocktail	3/5^{bI}		4/5^{bII}		4/5^{bII}	

Table (6): Detection of *B.subtilis* and its phages in *In vitro* treatment

Treatment	***B.subtilis* isolation of on NA**		**Spot test**			
Control	-		-			
BS1	-		+			
BS2	-		+			
BS31	-		+			
Phage cocktail						
B.subtilis	+		-			
***B.subtilis* cocktail**						
	Zero time		**30min**		**1hr**	
	***B.subtilis* isolation**	**Spot test**	***B.subtilis* isolation**	**Spot test**	***B.subtilis* isolation**	**Spot test**
Preservation						
***B.subtilis* +BS1**	+	+	+	-	+	-
***B.subtilis* +BS2**	+	+	+	-	+	-
***B.subtilis* +BS3**	+	-	+	-	+	-
***B.subtilis* cocktail + Phage cocktail**	+	-	+	-	+	-
prevention						
BS1+ *B.subtilis*	+	+	-	+	-	+
BS2+ *B.subtilis*	+	-	+	+	-	+
BS3+ *B.subtilis*	+	-	-	+	-	+
Phage cocktail + *B.subtilis* cocktail	+	+	+	+	+	+

Discussion

Date palm that tropical and subtropical fruit tree is the most economical plants in Africa and in the Arabian region. In this study, work was conducted to isolate and identify some bacterial-disease that infect date palm in Egypt. Moreover, Attempts were made to control these diseases using bacteriophages as bio-agents. In addition, the different characteristics of the isolated bacteriophages were studied to determine the suitable conditions for each phage and to protect phages against any adverse conditions as long time as possible. The shape of *B.subtilis* bacteriophages named BS1, BS2 and BS3 that isolated from free soil were examined using transmission electron microscope. The phage particles were 110x85nm in head and 191nm in tail length in phage BS1, 85x79nm in head and 245nm in tail in phage BS2 and 109x110nm in hexagonal head and 149nm in the length of the tail in BS3. Similar results were reported by Takahashi (1963), Bradley (1965), Nagai and Yamasaki (2009), El-Arabi *et al.* (2013) and Flounlacker *et al.* (2017). Classification of *B.subtilis* phages was determined from the micrograph of phages that obtained by TEM. The phages BS1 and BS3 belong to *Myoviridae* with dsDNA while BS2 phage belongs to *Siphoviridae* family with dsDNA. Similar results were reported by Takahashi (1963), Bradley (1965), Belyaeva and Azizbekyan (1968), Steensma and Blok (1979), Maratea *et al.* (1985), Nagai and Yamasaki (2009), El-Arabi *et al.* (2013), Elmaghraby *et al.* (2015), Anna *et al.* (2015), Mohamed *et al.* (2016) and Flounlacker *et al.* (2017). Family *podoviridae* comprises phages of hexagonal head and short tail with dsDNA that isolated and reported by Shimizu *et al.* (1970) and Ito *et al.* (1973). While *Cystoviridae* family comprises phages that have icosahedral head without tail with ds-RNA as isolated and mentioned by Marei (2013) and Kusmiatun *et al.* (2015). Thermal inactivation point (TIP) for *B.subtilis* phages BS1, BS2 and BS3 were found to be 58±1, 46±1 and 54±1, respectively. These results were in agreement with those reported by Mutsuko and Romig (1964), Anna and Romig (1965), Eli and Marmur (1969), Abo-Sinna (2004), Nagai and Yamasaki (2009), and Marei (2013). Stability of isolated *B.subtilis* bacteriophages for pH range was tested. pH range for isolated phages was 6-8, 6-9 and 5-8 for phages BS1, BS2 and BS3, respectively and the optimum is pH 7 for all phages. These results are in agreement with the results mentioned by Price and Frato (1975), Anna *et al.* (2015) and Elmaghraby *et al.* (2015). Total protein for each phage was determined quantitatively using colorimetric Bradford method. The obtained results indicated that the total proteins were 0.29, 0.35, 0.28, 0.23, 0.14 and 0.28 mg/ml for phages SP1, SP2, SS1, BS1, BS2 and BS3, respectively. These results are in agreement with those of Morrical *et al.* (1996) and Feisal (2013).

Conclusion

Bacteriophages considered the save alternative for bacterial-cides or antibiotics. From the results of this study, the isolated of Bacillus phages (3 different phages) were effective and specific for *B.subtilis* in case of prevention and preservation *In vitro*. In addition to that, the exposure of those phages to many effects (heat stability, pH stability, UV irradiation, longevity *In vitro*, freezing and thawing) showed that they did not have a severe effect on the phages.

References

Abdalla, M.Y. (2001). Sudden decline of date palm trees caused by *Erwinia chrysanthemi*. Plant Dis. Vol. 85:24-26.

Abo-Sinna, A.S.M. (2004). Studies on some viruses occurred under wheat cultivations in some Egyptian soils. Ph.D. Thesis, Faculty of science, Al-Azhar University, Cairo, Egypt.

Anna M. Brodetsky and Romig, W.R. (1965). Characterization of *Bacillus subtilis* Bacteriophages. Journal of Bacteriology. Vol. 90 (6): 1655-1663.

Anna, Krasowska; Anna, Biegalska; Augustyniak, D.; Los, M.; Richert, M. and Aukaszewicz, M. (2015). Isolation and Characterization of Phages Infecting *Bacillus subtilis*. BioMed Research International. http://dx.doi.org/10.1155/2015/179597.

Basdew, I.H. and Laing, M.D. (2014). Stress sensitivity assays of bacteriophages associated with Staphylococcus aureus, causal organism of bovine mastitis. African Journal of Microbiology Research. Vol. 8(2):200-210.

Belyaeva, N.N. and Azizbekyan, R.R. (1968). Fine structure of new *Bacillus subtilis* phage AR9 with complex morphology. J. Virology. Vol. 34(1): 176-179.

Born, Y.; Bosshard, L.; Duffy, B.; Loessner, M. J. and Fieseler, L. (2015). Protection of *Erwinia amylovora* bacteriophage Y2 from UV-induced damage by natural compounds. Bacteriophage. Vol. 5 (4): 1-5

Borrego, J.J.; Morifiigo, M.A.; de Vicente, A.; Cornax, R. and Romero, P. (1987). Coliphages as an indicator of faecal pollution in water. Its relationship with indicator and pathogenic microorganisms. War. Res. 21, 1473-1480

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. Vol. 72: 248-254.

Bradley, D.E. (1965). The isolation and morphology of Some new bacteriophages Specific for Bacillus and Acetobacter species**.** J. gen. Microbiol. Vol. 41: 233-241.

Campos, B.O.; Domènech, M.; Baena, M.; Balmaña, J.; Sanz, J.; Ramírez, A.; Alonso, C. and Baiget, M. (2003). RNA analysis of eight BRCA1 and BRCA2 unclassified variants identified in breast/ovarian cancer families from Spain. Hum Mutat, 22(4):337.

Clark, W.A.; Horneland, W. and Klein, A.G. (1962). Attempts to Freeze Some Bacteriophages to Ultralow Temperatures. Appl Microbiol. Vol. 10(5): 463–465.

El-Arabi, T.F.; Griffiths, M.W.; She, Y.M.; Villegas, A.; Lingohr, E.J. and Kropinski, A.M. (2013). Genome sequence and analysis of a broad-host range lytic bacteriophage that infects the *Bacillus cereus* group. Virology Journal. Vol. 48 (10): 1-11.

Eli, C. Siegel and Julius Marmur (1969). Temperature-Sensitive Induction of Bacteriophage in *Bacillus subtilis* 168. J. of Virology. Vol. 4(5): 610-618.

Elmaghraby, I., Carimi, F.; Sharaf, A.; Marei, E. M. and Hammad, A. M. M. (2015). Isolation and Identification of *Bacillus megaterium* Bacteriophages via AFLP Technique. Current Research in Bacteriology. Vol. 8 (4): 77-89.

Feisal, A. Abeer (2013). Employment of some bacterial viruses for improvement of quality and safety of some food products. PhD, Ain Shams University. pp167.

Flounlacker, K.; Miller, R.; Marquez, D.; and Johnson, A. (2017). Complete Genome Sequences of Bacillus Phages DirtyBetty and Kida. Genome Announcements, 5 (10), e01385–16. http://doi.org/10.1128/genomeA.01385-16

Hadeel, T. AL hadithi; ALutbi, S.D. and Zainab J. Madhi (2007). Isolated bacteria from contaminated callus and healthy date palm offsets. Basrah Journal of Agricultural Sciences. Vol. 20 (1): 166-176.

Hernández, I. (2017). Bacteriophages against Serratia as Fish Spoilage Control Technology. Frontiers in Microbiology. Vol. (8): 1-8

Ito, J.; Menke, W.; Hathaway, G. and Spizizen, J. (1973). Studies on *Bacillus subtilis* 4615. Virology. Vol. 56 (1):110-122.

Koskella, B. and Meaden, S. (2013). Understanding bacteriophage specificity in natural microbial communities. Viruses. Vol. 5:806-823

Kusmiatun, A.; Iman, Rusmana and Budiart, S. (2015). Characterization of Bacteriophage Specific to *Bacillus pumilus* from Ciapus River in Bogor, West Java, Indonesia. HAYATI Journal of Biosciences. Vol. 22 (1):27-33

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

Lewaa, L.M; Othman, B.A; Metwaly, A.M and El-Dougdoug, K.A. (2023). Molecular and Physical characterization of the phage specific for bacterial-pathogen causing pink-rot inflorescence disease in date-palm. Egyptain International Journal of palms. Vol. 3(1), p. 53-68.

Lidia, Ruz; Moragrega, C. and Montesinos, E. (2008). Evaluation of four whole-plant inoculation methods to analyze the pathogenicity of *Erwinia amylovora* under quarantine conditions. International microbiology J. Vol. 11:111-119.

Lima-Mendez, G.; Toussaint, A. and Leplae, R. (2007). Analysis of the phage sequence space: the benefit of structured information. Virology Vol. 365:241-249.

Maniatis, T.; Fritsch, E .F. and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual.New York, Cold Spring Harbor Laboratory.

Maratea, D.; Zsigray, R.M. and Balkwill, D.L. (1985). Characterization of *Bacillus subtilis* Phage 41c. Current microbiology. Vol. 12: 261-266.

Marei, M. Eman (2013). Isolation and Characterization of *Bacillus subtilis* Phage from Soil Cultivated with Liquorices Root. International Journal of Microbiological Research. Vol. 4 (1): 43-49.

Matsushita, K.; Uchiyama, J.; Kato, S.; Ujihara, T.; Hoshiba, H.; Sugihara, S.; Muraoka, A.; Wakiguchi, H. and Matsuzak, S. (2009). Morphological and genetic analysis of three bacteriophages of *Serratia marcescens* isolated from environmental water. FEMS Microbiol Lett.Vol. 291: 201-208

Matsuzaki, S.; Rashel, L.; Uchiyama, J.; Sakurai, S.; Ujihara, T.; Kuroda, M.; Ikeuchi, M.; Tani, T.; Fujieda, M.; Wakiguchi, H. and Imai, S. (2009). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. Journal of Infection and Chemotherapy, Vol. 11 (5): 211-219.

McGrath, S. and Sinderen, D.V. (2007). Bacteriophage Genetics and Molecular Biology. Caister Academic Press. 344pp

Mohamed, H. Sonya, Gado, E.A.; Gomaa, H.A. and Sadik, A.S. (2016). Characterization of bacterial soft rot strains and their specific phages isolated from soil at taif. Pak. J. Biotechnol. Vol. 13 (1): 111-116.

Morrical, W.S.; Beernink, H.T.H.; Dash, A. and Hempstead, K. (1996). The Gene 59 Protein of Bacteriophage T4. The journal of biological chemistry. Vol. 271 (33): 20198 –20207.

Mutsuko, N. and Romig, W.R. (1964). Temperature-sensitive mutants of *Bacillus subtilis* bacteriophage sp3: *In vivo* complementation studies. Journal of bacteriology. Vol. 88(5): 1230-1239.

Nagai, T. and Yamasaki, F., (2009) *Bacillus subtilis* (*natto*) bacteriophages isolated in Japan. Food Science and Technology Research, Vol. 15(3):293‑298.

Price, A.R. and Frato, J. (1975). Bacillus subtilis Deoxyuridinetriphosphatase and Its Bacteriophage PBS2-induced Inhibitor. The Journal of Biological. Chemistry. Vol. 250(22): 8804-8811.

Riaz, M.; Kumar, V.; Eiman Mansoury; Fatma Al-Kandari , Eman Al-Kandari; Eman Al-Attar and Fatma Al-Ameer (2009). Pink rot of inflorscence: a new disease of date palm in Kuwait. Mycopath journal. Vol. 7 (1):1-4

Rohwer, F. (2003). Global phage diversity. Cell, Vol. 113, No. 2, pp. 141

Sethuraman, S.; Arunachalam, A.; Karthikeyan, M.; Kumar S. A., Manidipa, S. and Senthilraj, R. (2011). Antimicrobial sensitivity profile *of serratia marcescens* strains isolated in government general hospital, nagapattinam, tamilnadu, india. International Journal of Preclinical and Pharmaceutical Research. Vol. 2 (1): 7-11.

Setlow, R. and Boyce, R. (1960). The ultraviolet light inactivation of (φx174 bacteriophage at different wave lengths and ph's. Biophysical journal. Vol. 1:29-41.

Shamloul, A.M.; Hadidi, A.; Madkour, M.A. and Makkouk, K.M. (1999). Sensitive detection of banana bunchy top and faba bean necrotic yellows viruses from infected leaves, in vitro tissue cultures, and viruliferous aphids using polymerase chain reaction. Canadian Journal of Plant Pathology.vol 21: 4, 326-337.

Shimizu, N.; Miura, K. and Aoki, H. (1970). Characterization of *Bacillus subtilis* Bacteriophage: Isolation and Morphology of Phage Nf and Properties of Its DNA. J. Biochem. Vol. 68 (3): 277-286.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods . 7thed. The Lowa State Univ. Press, Ames.,Lowa, USA. 75-78.

Steensma, H.Y. and Block, J. (1979). Effect of calcium ions on the infection of *Bacillus subtilis* by bacteriophage SF6. Journal of General Virology. Vol 42: 305-314.

Taj, M.K.; Ling, J.X.; Bing, L.L.; Qi, Z.; Taj, I.; Hassani, T. M.; Samreen, Z. and Yunlin, W. (2014). Effect of dilution, temperature and pH on the lysis activity of t4 phage against *E.coli* bl21. The Journal of Animal & Plant Sciences. Vol. 24 (4): 1252-155.

Takahashi, I. (1963). Transducing Phages for *Bacillus subtilis*. J. gen. Microbiol. Vol. 31 (31): 211-217.

Westra, E.R.; Swarts, D.C.; Staals, R.H.; Jore, M.M.; Brouns, S.J. and van-der-Oost, J. (2012). The CRISPRs, they are A-Changin': how prokaryotes generate adaptive immunity. Annu. Rev. Genet. 46:311-339.

Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Yoshida, T.; Takashima, Y.; Tomaru, Y.; Shirai, Y.; Takao, Y.; Hiroishi, S. and Nagasaki, K. (2006). Isolation and characterization of a cyanophage infecting the toxiccyanobacterium *Microcystis aeruginosa*. Applied Environ. Microbiol. Vol. 72: 1239-1247.

استخدام فيروسات بكتيريا Bacillus في مقاومة بكتيريا *Bacillus subtilis* المسببة لتشوهات نبيتات نخيل البلح في مرحلة زراعة الأنسجة

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الملخص العربي

نخيل البلح يعتبر من أهم المحاصيل في المنطقة العربية. تعتبر بكتيريا *Bacillus subtilis* من ضمن الميكروبات التي تسبب مشاكل في النخيل حيث أنها تسبب تشوهات لنباتات النخيل في مرحلة زراعة الأنسجة. تم عزل ٣ عزلات مختلفة من البكتيريوفاج المتخصصة لبكتيريا *B.subtilis* وأخذت أسماء BS1، BS2و BS3. من خلال الشكل الظاهري للفيروسات بإستخدام الميكروسكوب الألكتروني حيث أوضحت النتائج أن الفيروسات تنتمي لعائلات *Myoviridae,* و *Siphoviridae* لكل من BS1، BS2و BS3. من خلال تقدير كمية البروتين في الفيروسات بإستخدام طريقة Bradford أوضحت أنها ٠.٢٣، ٠.١٤، ٠.٢٨ مجم/مل للفاجات BS1، BS2و BS3. في حين تقدير الأوزان الجزيئية للبروتينات في كل فاج بإستخدام تقنية SDS-PAGE فأوضحت النتائج أنها كانت ٩٧، ٧٠، ٣٧، ٣٥ كيلو دالتون في فاج BS1، وكانت ١٢٠، ٩٧، ٧٠، ٣٥، ٢٥ كيلو دالتون في فاج BS2، وفي حالة فاج BS3 كانت ٩٧، ٧٠، ٣٥، ٢٧ كيلو دالتون. في دراسة الخواص الجزيئية لجينوم الفاجات بإستخدام تقنية RAPD-PCR فأوضحت النتائج ان فاج BS1 تكون معها ٣ قطع بقيمة ١٠٠٠، ٦٠٠، ٥٠٠ قاعدة زوجية. في حالة فاج BS2 فأنها قطعتين فقط بقيمة ١٠٠٠، ٦٠٠ قاعدة زوجية. أما في حالة فاج BS3 فقد اعطت قطعتين بقيمة ١٠٠٠، ٥٠٠ قاعدة زوجية. بإستخدام ٥ مركبات طبيعية مختلفة تم إستخدامها لحماية الفاجات من الظروف الصعبة التي يتعرض لها ولا سيما أشعة ال UV وظهرت من النتيجة أن أفضل المركبات كانت عصير البنجر و محلول الكازين.

الكلمات الدالة: نخيل البلح، بكتيريا *Bacillus subtilis*، البكتيريوفاج، المكافحة الحيوية.