Molecular and Physical characterization of the phage specific for bacterial-pathogen causing pink-rot inflorescence disease in date-palm

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Abstract

Pink rot inflorescence disease in date palm (*Phoenix dactylifera* L.) is considered important disease in date palm in Egypt. The causal agent was isolated from trunk using specific media. The causal agent was identified as *Serratia marcescen*s using 16s rRNA gene sequencing and was recorded in GenBank. Three different specific lytic phages of *S. marcescens* were isolated from soil and trunk of the diseased date palm tree using single plaque isolation technique and named SP1, SP2 and SS1. The morphological characters revealed that two bacteriophage isolates belong to *Myoviridae* family and one belongs to *Siphoviridae* family. Thermal inactivation point (TIP) of the different phages was 60, 80 and 60°C for SP1, SP2 and SS1 respectively. Stability to freezing and thawing were 3, 3 and 2 times respectively. pH range stability was 7-9, 6-9 and 5-9. Longevity *In Vitro* (LIV) of all phages appeared active more than 60 days. Sensitivity of the phages for UV were 20, 25 and 20min. respectively. SDS-PAGE showed molecular weight of protein of SP1 was ~66, 35 and 29 KDa. SP2 was~ 38, 35, 30 and 29 KDa. SS1 was ~97, 38, 35, 30 and 29 KDa. RAPD-PCR showed SP1 amplified to 4 fragments with 2000, 1500, 1000 and 600 bp, phage SP2 amplified to 4 fragments with 1500, 1000, 700 and 600 bp, SS1 phage amplified to 5 fragments with 1500, 1000, 800, 700 and 600 bp.

Keywords: Pink rot inflorescence, date palm, *Serratia marcescens*, bacteriophages, bio-control

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Introduction

Date palm (*Phoenix dactylifera* L.), a tropical and subtropical fruit tree is one of man-kind's oldest cultivated plants in Egypt and in the Arabian region (Ahmed *et al*., 1995). Date palms are monocotyledon, dioecious and can grow up to an altitude of 1500 m in well-drained soils (Al-Shahib and Marshall, 2003). The annual production of dates is 7600315 tones. In Egypt, the annual production is 1465030 tones (FAO, 2014). Several diseases in date palm cause damage like *Fusarium oxysporum*, *Phytopathora* sp., *Ceratocystis paradoxa* (Mansoori and Kord, 2006 and El-Hassni *et al.*, 2007). Pink rot inflorescence in date palm that caused by *Serratia marcescens* was first reported in Kuwait (Riaz *et al.*, 2009). *Serratia marcescens* is a Gram-negative, facultatively anaerobic, rod-shaped bacterium belonging to the family *Enterobacteriaceae* (Grimont and Grimont, 1981). Phages 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 bacteriophage is a virus that replicates and infects within a main host bacterium (Matsushita *et al.,* 2009) .The aim of this paper is isolation the bacterial causal and it's specific lytic phages from date palm in Egypt.

Materials and methods

1. Source of the bacterial pathogen

Source of bacterial pathogen was taken from date palm tree showed distinctive symptoms of disease (dark pink and black spots on trunk, the flowers were colored with pink and in later stages flowers were colored black) from Agricultural research center (ARC) Giza, Egypt.

2. Isolation of the pathogen

The bacterial pathogen was isolated from trunk of date palm trees according to Riaz *et al.* (2009). The inoculum was streaked on Nutrient Agar (NA) and incubated at 25°C/ 24 hrs. After incubation, single colony was streaked on XLD and EMB media and incubated again at 25°C/ 24 hrs.

3. Identification of the isolated bacteria

The isolated bacteria grown on media were identified using MicroSeq 500 16S rDNA Bacterial Identification Kit at CliniLab (colors lab, Maadi, Cairo, Egypt).

4. Source of bacteriophage

Bacteriophages specific to the isolated bacterial pathogen were isolated from the infected trunk of date palm and also from free soil from Agricultural research center (ARC). Giza, Egypt.

5. Phage assaying

5.1. Qualitatively

Bacteriophages that isolated from soil and plant was assayed qualitatively using the spot test according to Borrego *et al.* (1987).

5.2. Quantitatively

Bacteriophages were assayed quantitatively using the plaque assay method according to Markel and Eklund (1974).

6. Isolation of single plaque isolates of lytic phage

Bacteriophages were isolated from the plaques resulting from the plaque assay method using single plaque isolation method according to Markel and Eklund (1974).

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. Phage morphology

The Purified phages were examined using transmission electron microscopy (TEM) Jeol JEM-J1400 in Faculty of Agriculture Research Park (FARP), Cairo University. Using negative staining with phosphotungestic acid (PTA 2%) to determine the shape and size of the particles.

9. Thermal stability of the phages

Thermal stability point of the phages was carried out according to Basdew and Laing (2014) by exposure phage suspension to different degrees of temperature 30, 40, 50, 60, 70, 80, 90 and 100°C for 10 min. then the phages were cooled immediately under the tap water. Finally, each treated phage was assayed using spot test technique.

10. Freezing and thawing

Effect of freezing (-16°C) and thawing (room temperature) on the phages was assayed to investigate the stability of phages according to Clark *et al.* (1962)

11. pH stability of the phages

Stability of the phages to pH degrees was assayed according to Taj *et al.* (2014) by exposure the phages to 4, 5, 6, 7, 8 and 9 degrees of pH that adjusted by 0.1M of NaOH or HCl for overnight. Then the pH was adjusted to the optimum degree (7.2) and the infectivity of phages was assayed qualitatively.

12. Longevity *In Vitro*

Longevity of the isolated phages at room temperature was determined according to Yoshida *et al.* (2006).

13. UV radiation stability

Stability of the isolated phages to the UV radiation was assayed qualitatively after exposure to UV lamp (254 nm) for 5, 10,15,20,25 and 30 min at length of 6cm from the UV lamp. according to Born *et al.* (2015) the infectivity of the exposure particles was determined qualitatively.

14. Quantitation of the phages' protein colorimetrically

The proteins of the isolated phages were determined for its quantity using the colorimetric method as used by Bradford (1976).

15. Molecular characterization of isolated phages

15.1. Extraction of total nucleic acid

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).

15.1.1. RAPD PCR

Random amplified polymorphic DNA–PCR (RAPD-PCR) was used to detection the polymorphism among between phage's DNA 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.

15.1.2. DNA amplification cycles:

RAPD-PCR was amplified using the following program (Table 1) according to Shamloul *et al.* (1999) and using the annealing temp:

$$Tm\ (°C) = 81.5 + 0.41(\%\ GC) - (675/N)$$

(Annealing temp. = Tm – 3)

The program was carried out using thermo cycler MJ Research (PTC-200):

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.	40 cycles
Annealing	36ºc for 30 sec.	
Extension	72ºc for 45 sec.	
Extension	72ºc for 7 min.	1 cycle

15.1.3. Gel electrophoresis

The PCR product was electrophoresed in 1% agarose gel in 1x TBE buffer at 120 V for 1 hour and stained with ethidium bromide (1mg/ml) (Sambrook *et al*., 1989). The fragments were photographed using UV lamp in gel-documentation (Digimage, Gel Doc Digimage system G15).

15.2. Protein assay

15.2.1. Electrophoretic separation of proteins

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).

15.2.2. Preparation of SDS-Polyacrylamide Gel

Two gels were prepared as separating gel containing 12% acrylamide monomer and stacking gel 4% (as shown in table 2). Ammonium persulphate and TEMED were used as initiators for crosslinking and polymerization. The components of the separating gel solution were placed in 100ml flask, stoppered flask and apply for several minutes. Ammonium persulphate and TEMED were added and the flask was mixed gently to avoid the generated bubbles. The solution was pipetted into the assembled vertical slab gel unit in the casting mode to level 1.5cm from the top. N-butanol was layered on the top of the solution. The gel was left to polymerize at room temperature for 1hr. Prior to addition of stacking gel, the N-butanol was poured from the surface of the gel and the surface was washed once with overlay buffer. The 4% stacking gel was prepared by mixing its components well, TEMED and ammonium persulphate were added and gently swirling the mixture. Stacking solution was added to the top of the separating gel, comb was inserted and the gel was allowed to polymerize for at least 30 min. After polymerization the comb was removed carefully from the gel and each well was rinsed with running buffer.

15.2.3. Preparation of the phage's proteins:

The proteins of phages were analyzed by SDS-PAGE. The phage suspensions were centrifuged at 16000rpm at 4°C for 90min and the Pellets were collected and washed with dH_2O, then with 1 ml of 1mM NaCl containing 5mM EDTA. One volume of the solution was mixed with one volume of 2X of boiling buffer (Laemmli buffer) and boiled in a water bath for 90 seconds then quickly transferred to ice water and kept until loading the gel.

Table (2): Preparation of separating and stacking gel of SDS-PAGE.

Solution	Separating gel 12%	Staking gel 4%
Acrylamide stock (30%)	12 ml	1.25 ml
Distilled water	9.8 ml	5.4 ml
1.5M Tris (pH 8.8)	7.6 ml	ـــ
0.5M Tris (pH 6.8)	ـــ	1 ml
10% SDS	0.3 ml	80 µl
10% (w/v) APS	0.3 ml	80 µl
TEMED	20 µl	15 µl

15.2.4. Protein electrophoresis

The gel apparatus was assembled and the lower and upper chambers were filled with the running buffer. A drop of Bromophenol blue was added as a tracking dye. A micropipette was used to load equal amounts of proteins (25µl) in each well. High range molecular

weight protein marker was used. Electrophoresis was carried out at about 120 volts (25 mA) in 1x Tris/glycine- SDS-running buffer. After electrophoresis, the gel was stained in 50 ml of staining solution. The gel was placed between two sheets of cellophane membrane and dried on gel drier for 2hrs and photographed.

Results

Identification of bacteria

The causal pink rot inflorescence pathogenic bacteria was isolated on specific media (XLD and EMB) that gave positive growth. This result indicated that bacteria belong to *enterobacteriaceae* family. The 16s rRNA sequencing of the isolated bacteria comparing with those in GenBank that indicated bacteria was *Serratia marcescens*. (as shown in table 3 and Fig.1). Sequence of *S.marcescens* 505 nt was deposited into the GenBank under the accession number MG266440.

Table (3): 16s rRNA gene sequence for S.*marcescens* isolates collected from GenBank, used for nucleotide sequence comparison

Accession number	Isolate source	Organism	References	Country	Identity
KM206786	Rhynchophorus ferrugineus	*Serratia sp.*	Scrascia *et al.* (Unpublished)	Italy	**99%**
LM652337	Baltic Sea young ice	*Uncultured bacterium*	Eronen-Rasimus *et al.* (Unpublished)	Finland	**99%**
AY043386	Respiratory secretions	*Serratia sp.*	Coney *et al.* (2002)	USA	**99%**
LT691102	Human head and neck cancer tissue	*Serratia sp.*	Wang *et al.* (Unpublished)	USA	**99%**
KX500120	Oil industry waste water	*Serratia sp.*	Smita and Sharma (2016)	India	**99%**
GU193982	Pulp, paper mill effluent sludge"	*Serratia sp.*	Chandra (Unpublished)	India	**98%**

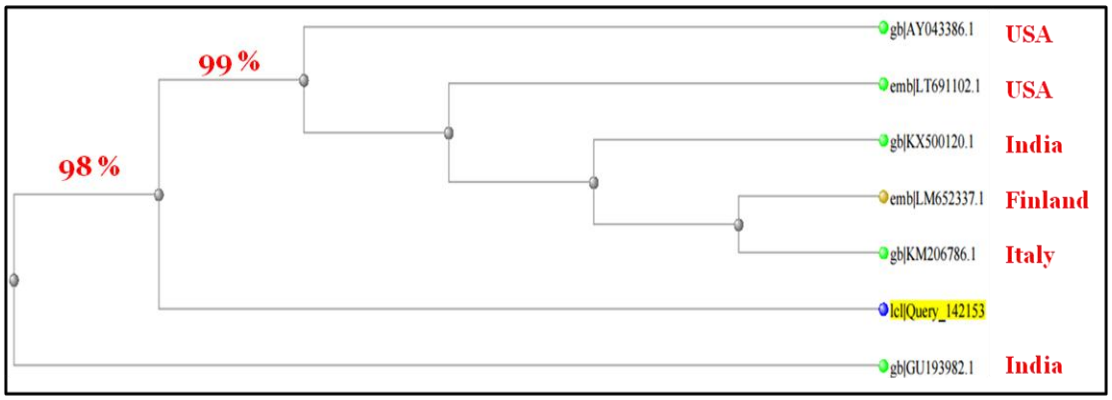


Fig. (1): phylogenetic tree for 16s rRNA of the isolated pathogenic bacteria (Egyptian isolate) with 98% similarity with S. marcescens (Indian isolate) and with 99% similarity with S. marcescens (USA, USA, India, Finland and Italian isolates).

Phage assaying

1. Qualitatively

Positive results of the spot test were shown in the case of infected plant and soil for S.marcescens (fig. 2).

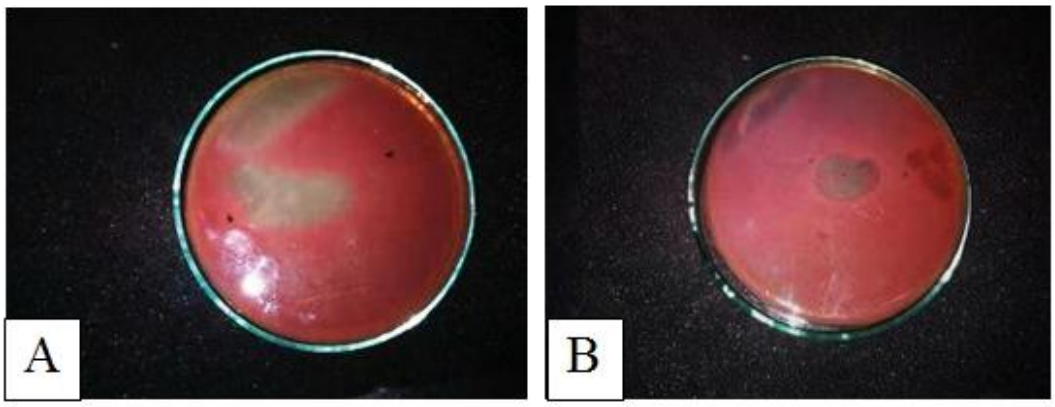


Fig. (2): Macroscopic lysis from spot test for *S.marcescens* from infected plant (A) and from soil (B)

2. Quantitatively

Three phages were isolated from using single plaque isolation method that named Serratia plant 1 (SP1) and serratia plant 2 (SP2) from infected trunk and serratia soil 1 (SS1) from soil. Plaques were isolated according to morphology of plaques. Fig. 3 and table 4 showed that plaque SP1 was clear, circular with 0.5 mm in diameter, plaque SP2 was clear, circular with1 mm in diameter and plaque SS1 was turbid, circular with about 0.3-0.5 mm in diameter.

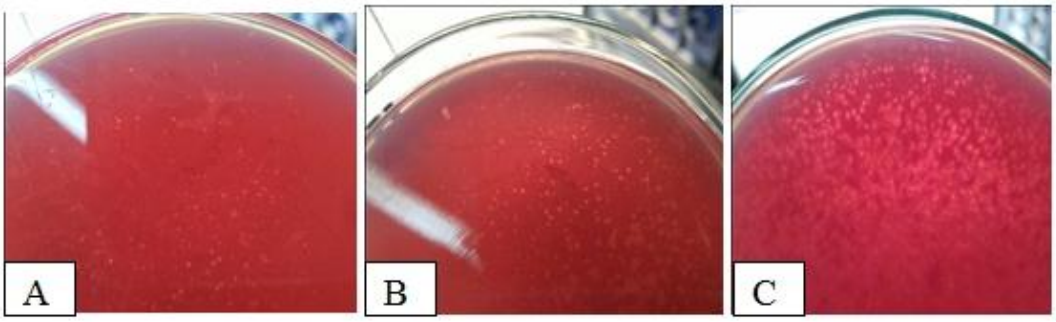


Fig. (3): Morphology of plaques of phages from single plaque isolation. Plaques are clear, circular with 0.5 mm in diameter in SP1 (A), clear, circular with1 mm in diameter in SP2 (B) and turbid, circular with about 0.3-0.5 mm in diameter in SS1 (C).

Table (4): Morphology of isolated plaques

Phage	Plaque diameter (mm)	Plaque morphology
SP1	0.5	Clear and circular
SP2	1	Clear and circular
SS1	0.3-0.5	Turbid and circular

Morphology of phages

Morphology of 3 isolated of bacteriophages specific to *S. marcescens* were examined using the transmission electron microscope. As shown in fig.4 the examination revealed that 3 phages (SP1, SP2 and SS1) belong to *Myoviridae* family and *Siphoviridae* family. The size of phage particles were 98x 90 nm in head and 213 nm in tail in phage SP1, 86x104 nm in head and 128 nm in the tail in phage SP2 (isolated from trunk) and 111x 112nm in head and 236 nm in tail in phage SS1 (isolated from soil).

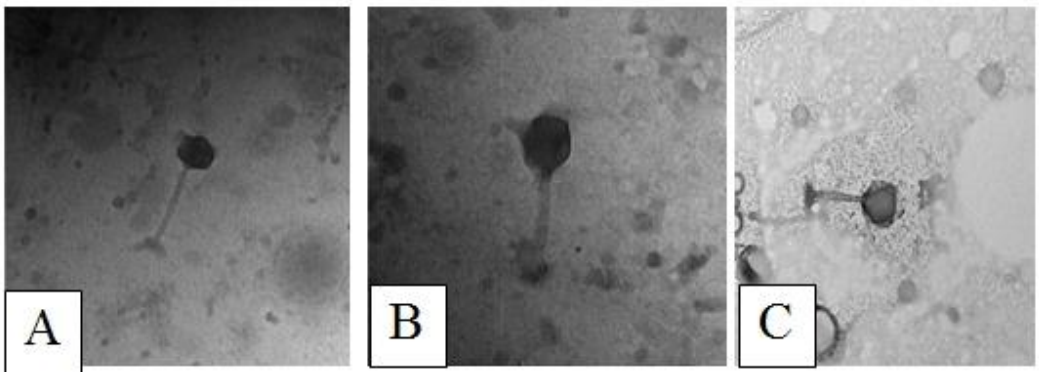


Fig. (4): Transmission electron microscopey of S. marcescens phages using negative staining by PTA 2%. Sizes were 98x 90 nm in head and 213 nm in tail for SP1**(A)**, 86x104 nm in head and 128nm in thetail for SP2 **(B)** (isolated from trunk) and 111x 112nm in head and 236 nm in tail for SS1 **(C)** (isolated from soil). Photos with scale bar

Thermal stability of phages

Thermal inactivation point (TIP) of phages was tested to determine the stability of phages to different temperature degrees. Data in table (5) showed that TIP was 60, 80 and 60°C for phages SP1, SP2 and SS1 respectively.

pH stability of phages

pH range stability of phages were estimated to determine the phages stability to pH range. Data in table (3) showed that pH range of phages were 7-9, 5-9 and 5-9 for phages SP1, SP2 and SS1 respectively.

Table (5): Stability *S. marcescens* phages for TIP and pH range

Phage	TIP	pH range
SP1	60°C	7-9
SP2	80°C	6-9
SS1	60°C	5-9

Freezing and thawing

Stability of the isolated phages for freezing and thawing for many times were assayed. Data in table (6) reveal that the phages still active for 3, 3 and 2 times of freezing and thawing for phages SP1, SP2 and SS1 respectively.

Table (6): Stability of phages for times of freezing and thawing

	Qualitative assay (spot test)		
No. of time	**Phage SP1**	**Phage SP2**	**Phage SS1**
1	+	+	+
2	+	+	+
3	+	+	-

+ = Lysis - = No Lysis

Longevity *In Vitro*

Longevity *In Vitro* (LIV) for *S. marcescens* phages at room temperature were tested qualitatively. The results showed that all phages were stable at room temperature for more than 60 days.

UV radiation stability

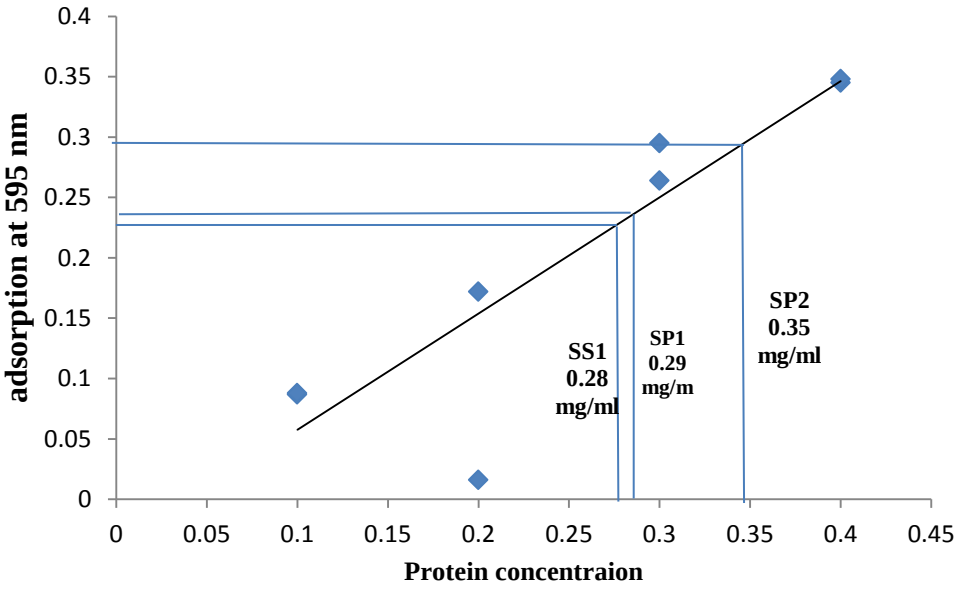
Data in table (7) showed that sensitivity of *S. marcescens* phages to UV lamp for 20, 20 and 25 min for phages SP1, SP2 and SS1 respectively.

Table (7): Stability time of phages for UV

Phage	Time (min.)
SP1	25
SP2	20
SS1	25

Protein quantity in *S.marcescens* lytic phage particles

The amount of protein in viral particle was determined by Bradford method. The viral protein of S.marcescens lytic phages SP1, SP2 and SS1 was red at 595 nm using the spectrophotometer and the amount of protein were calculated from the standard curve (fig 5) that prepared by bovine serum albumin. The amount of protein in phages SP1, SP2 and SS1 was 0.29, 0.35 and 0.28 mg/ml, respectively.



Fig(5): Quantitation of Serratia phages protein using Bradford method.

RAPD-PCR analysis:

RAPD-PCR was used in this study to recognize 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 6 fragments (Fig 21, table 17) of amplified *Serratia* phages' DNA. The results showed that phage SP1 amplified to 4 fragments with 2000, 1500, 1000 and 600 bp, phage SP2 amplified to 4 fragments with 1500, 1000, 700 and 600 bp, SS1 phage amplified to 5 fragments with 1500, 1000, 800, 700 and 600 bp, Monomorphic bands among *Serratia* phages was 1 out of 6 monomorphic bands with percentage 16.67 %. Polymorphic bands (specific bands for each phage) were 3 out of 6 with percentage 50 %. Each SP1 and SS1 phages appeared unique bands (genetic marker) 1 out of 6 for each phage with percentage 33.33 %. As showed in Table, 8 and illustrated in Fig. (6). Monomorphic indicted that the 3 phage isolates belong to *Serratia* phages. Polymorphic showed that genome variability among the 3 isolates.

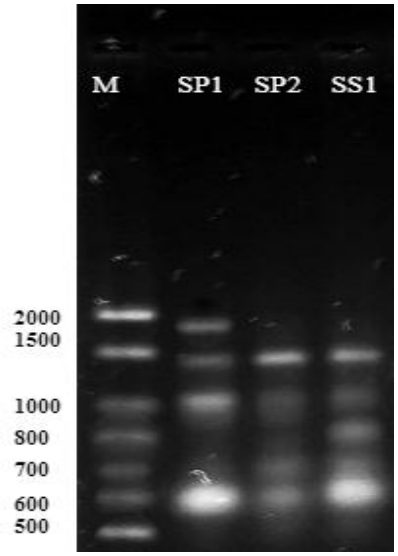


Fig. (6): RAPD-PCR for *Serratia* phages` DNA

Table (8): Genetic polymorphism among *Serratia* phages

bp \ Phage	SP1	SP2	SS1	Polymorphism*
2000	++	-	-	Unique
1500	++	++	++	Monomorphic
1000	++	+	+	Polymorphic
800	-	-	++	Unique
700	-	+	+	Polymorphic
600	+++	++	+++	Polymorphic
Total	4	4	5	6

(-) no amplified fragment,(+) low density, (++) moderate density, (+++) high density, *Monomorphic = common bands 16.67 % , Polymorphic = specific bands 50 % and Unique = Genetic marker 33.33 %.

Protein pattern of Serratia marcescens lytic phages

The structural protein of the lytic phage specific for Serratia marcescens were determined using 12% SDS-polyacrylamide gel electrophoresis. The results showed that in fig (7) showed that the number of the structural proteins and their molecular weight of the phages SP1, SP2 and SS1. As shown in fig (31) phage SP1 has 3 structural protein with molecular weight about 66, 35 and 29 KDa. SP2 phage has 4 structural proteins with molecular weight about 38, 35, 30 and 29 KDa. SS1 phage had 5 major structural proteins with molecular weight about 97, 38, 35, 30 and 29 KDa. Data in table (9) showed the

polymorphism among SP1, SP2 and SS1 phage's protein. SP1 phage has unique band with molecular weight 66 KDa and 2 monomorphic bands with molecular weight 35 and 29 KDa. The phage SP2 has 2 polymorphic bands with molecular weight 38 and 30 KDa and 2 monomorphic bands with molecular weight 35 and 29 KDa. SS1 phage has unique band with molecular weight 97 KDa and 2 polymorphic bands with molecular weight 38 and 30 KDa and 2 monomorphic bands with molecular weight 35 and 29 KDa. On basis of the obtained results, it can be concluded that S.marcescens lytic phages are different in their content of the structural proteins qualitatively and quantitatively.

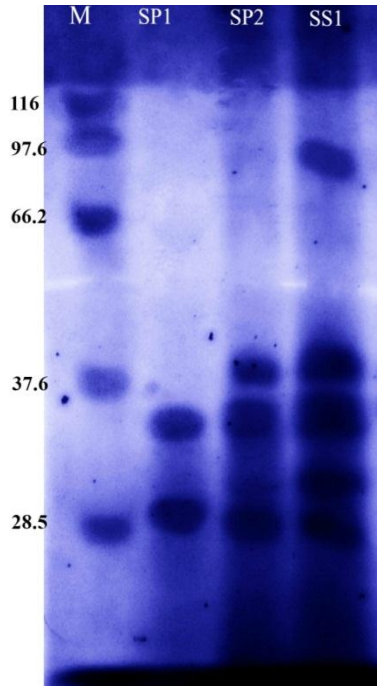


Fig. (7): SDS-PAGE 12% of purified serratia phages

Table (9): Protein polymorphism among *S.marcescens* phages

Phage / MW (KDa)	SP1	SP2	SS1	polymorphism
97	-	-	+++	Unique
66	+	-	-	Unique
38	-	+++	+++	Polymorphic
35	+++	+++	+++	Monomorphic
30	-	+++	+++	Polymorphic
29	+++	+++	+++	Monomorphic
Total	3	4	5	6

Monomorphic = common bands 33.33%
Polymorphic= specific bands 33.3%
Unique= Genetic marker 33.33%
(+++) high density
(-) No band
(+) Low density
(++) moderate density

Discussion

In this study, the causal agent of pink rot inflorescence disease that showed on date palm trees and flowers as dark pink and black spots on trunk, pink color on flowers was isolated and identified on specific media and using 16s rRNA sequencing . Identification showed the causal agent of this disease was *S. marcescens*. The symptoms of this disease and causal agent were similar only with that recorded in Kuwait Riaz *et al.* (2009). *S. marcescens* bacteriophages were isolated from trunk of infected date tree (2 phages) and from soil (1 phage). The morphology of the purified 3 phages were studied on TEM. TEM examination showed that the 3 phages belong to *Myoviridae* and *Siphoviridae* families. This morphology result similar to Matsushit *et al.* (2009) and Bertani and Six, (1988) while the isolated phages of *S. marcescens* belong to *Siphhoviridae* family (Iino and Mitani, 1967 and Yu *et al*., 2008). On the other hand, some isolated phages of *S. marcescens* were reported that belonged to *Podoviridae* family (Matsushita *et al.*, 2009). Bacteriophage stability for temperature and pH range was studied. It was found that TIP of the three phages were 60, 80 and 60°C for phages SP1, SP2 and SS1. Friman *et al.* (2011) revealed that TIP of phage of *S. marcescens* was 37°C. pH range stability of phages was 7-9, 6-9 and 5-9 for phages SP1, SP2 and SS1 respectively. This result was agreement with (Janovska, 1964; Frederick and Lloyd 1995). Protein properties of the isolated phages were determined using SDS- poly acrylamide gel electrophoresis (SDS-PAGE). The results showed that the major structural protein of SP1 phage contain of 3 proteins with molecular weight ~66, 35 and 29 KDa. major structural protein of SP2 phage contain of 4 proteins with molecular weight~ 38, 35, 30 and 29 KDa. In SS1 phage results showed that contain of 5 major structural proteins with ~97, 38, 35, 30 and 29 KDa. Similar results were reported by Vinas *et al.* (1985), Matsushita *et al.* (2009), Evans *et al.* (2010), Hua *et al.* (2013).

Conclusion

Pink-rot inflorescence disease in date palm that caused by *Serratia marcescens*. Three different phages for *S.marcescens* were isolated from free soil and from infected plant. The molecular and physical properties of isolated phages were studied and then these phages will used as antibacterial for this bacteria.

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الخواص الجزيئية والفيزيائية للفاجات المتخصصة للبكتيريا المسببة لمرض عفن النورات الوردي على نخيل البلح

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الملخص العربي

يعتبر مرض عفن النورات الوردي في نخيل البلح من الامراض البكتيرية الهامة التي تصيب النخيل في مصر. تم عزل المسبب المرضي من جذع نخلة بلح مصابة بالمرض على بيئة متخصصة. المسبب المرضي تم تعريفه باستخدام تقنية 16s rRNA gene sequencing وتم التأكيد بانه بكتيريا *Serratia marcescens* وتم تسجيل هذه العزلة على بنك الجينات. تم عزل عدد ٣ فيروسات بكيتريا (بكتيريوفاج) محللة للبكتيريا الممرضة وذلك من جذع النخلة المصابة وكذلك من التربة باستخدام تقنية عزل البلاكات المنفردة وهذه الفاجات اخذت اسماء SP1, SP2, SS1. من خلال الدراسات الظاهرية على الفاجات تبين ان فيروسين منهم ينتميان الى عائلة *Myoviridae* بينما الفيروس الثالث ينتمي الى عائلة *Siphoviridae*. من دراسة درجة حرارة تثبيط الفاجات تبين ان درجات الحرارة كانت ٦٠،٨٠،٦٠ درجة مئوية وذلك على فيروسات SP1, SP2, SS1 على التوالي. عدد مرات ثبات الفاجات للتجميد والتسييح في قدرة الفاجات على الاصابة كانت ٣،٣،٢. مدى درجة ثبات الحموضة كانت في مدى ٧-٩، ٦-٩، ٥-٩. مدة بقاء الفاجات نشطة في درجة حرارة المعمل استمرت في الفاجات الثلاثة لمدة تزيد عن ٦٠ يوم وبنفس النشاط. في دراسة حساسية نشاط الفاجات في التعرض للاشعة فوق البنفسجية لمدد مختلفة كانت ٢٠،٢٥،٢٠ دقيقة للفاجات الثلاثة على التوالي. في دراسة الهجرة الكهربية لبروتينات الفاجات التركيبية باستخدام تقنية SDS-PAGE وضحت النتيجة ان الوزن الجزيئي لبروتين فاج SP1 كان حوالي ٦٦، ٣٥،٢٩ كيلو دالتون. في فاج SP2 كانت الاوزان الجزيئية حوالي ٣٨،٣٥،٣٠،٢٩ كيلو دالتون. بينما في فاج SS1 كانت الاوزان الجزيئية حوالي ٩٧،٣٨،٣٥،٣٠،٢٩ كيلو دالتون. من خلال دراسة تفاعل البلمرة المتسلسل العشوائي RAPD-PCR باستخدام بادئ عشوائي .اوضحت النتائج ان هناك اختلافات على مستوى الجينوم بين الفاجات المعزولة حيث انها في فاج SP1 اعطت ٤ قطع قيمتهم ٦٠٠،١٠٠٠،١٥٠٠،٢٠٠٠ قاعدة زوجية. وفي فاج SP2 اعطت اربع قطع قيمتهم ٦٠٠،٧٠٠،١٠٠٠،١٥٠٠ قاعدة زوجية. وفي حالة فاج SS1 اعطت خمس قطع قيمتهم ٦٠٠،٧٠٠،٨٠٠،١٠٠٠،١٥٠٠ قاعدة زوجية.

الكلمات الدالة: نخيل البلح، عفن النورات الوردي، فيروسات البكتيريا، البكتيريوفاج