Egyptian J.Virol.3(1),(2006) 89-103

Isolation and characterization of a *Pseudomonas putida* bacteriophages

Mayada A. Abd Elgalel[1]; B.A Othman[2]; Th. Radwan, [1] and Amal S.M. Abo-Sinna[3]

[1] *Botany Dept., Fac. of Science, Cairo Univ. (Al-Faoum Branch), Al-Fauom, Egypt.*
[2] *Agric. Microbiol. Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.*
[3] *Botany and Microbiology Dept., Fac. of Science, Al-Azhar (Girls Branch) Univ., Cairo, Egypt.*

Received October 2006: accepted December2006

Abstract

Two phage isolates for *Pseudomonas putida* were isolated from soil cultivated with potato by the enrichment culture technique and signed as PP_1 and PP_2. The phages were propagated and purified by polyethylene glycol, 6000-sodium dextran sulfate 500 two phase system. The two phages had different ultraviolet absorption spectra. Results of the lytic pattern of both phages showed that, they had a restricted host range. Particle morphology of the isolated phages was determined and it was found that the two viruses have tadpole shape, which phage PP_1 has an isometric head (135 nm) and long non-contractile tail (225 nm) whereas phage PP_2 has an isometric head (121 nm) and long contractile tail (202 nm), the tail possing an outer sheath (135 nm) and neck (27 nm). Protein patterns of the isolated *Ps. putida* phages were analysed by SDS-PAGE and the data revealed that phage PP_1 had 16 structural proteins, 6 are known and the other are extrapolated, but phage PP_2 had 13 structural proteins 5 are known and the others are extrapolated. Both viruses have one molecule of the nucleic acid DNA with molecular weight of 2223 bp and 2559 bp for PP_1 and PP_2 respectively.

Key words : *Pseudomonas putida*, Phages, Isolation, Purification, Electron microscopy, SDS-PAGE, DNA, Lytic pattern.

Introduction

Genus *Pseudomonas* has tremendous importance and that, due to it's widespread distribution in soil and it's ability to utilize a wide range of organic substances as carbon or nitrogen sources. *Pseudomonas putida* is one of the saprophytic fluorescent *Pseudomonas* which comprise an important group of bacteria used for biological control of micro-fungi of the plant rhizosphere (**Bergsma-Vlami *et al.*, 2002**). *Pseudomonas putida* showed significant increase in germination percentage, growth of plants and phosphorus uptake of many plants (**Alok-Sarma *et al.*, 2003**).

Virulent bacteriophages play an important role in decreasing numbers of *Pseudomonas putida* in the soil and subsequently their role which reflects on the plants causing decreasing in yield.

Virulent viruses infecting *Pseudomonas*(*Pseudomonas* phages) were isolated from different sources, i.e. plant leaves, seedlings, seeds **(Miyajima, 1980)**, field free and rhizosphere soils **(Kakutani *et al.*, 1994)**, and raw sewage **(Thomas and Leary, 1983)**. Isolation of *Pseudomonas* phages almost, achieved by enrichment liquid method **(Mosa *et al.*, 1996)**. The most effective method for concentrating and purifying of *Pseudomonas* phages as well as many bacteriophages can be done using polyethylene glycol, 6,000, differential centrifugation and 10-40% sucrose density gradient centrifugation **(Tokarchuk *et al.*, 1975)**. *Pseudomonas* bacteriophages form different plaques ranging from small to large clear **(Kakcutani *et al.*, 1994)**. Some *Pseudomonas fluorescens* phages have an isometric icosahedral head and a short or long contractile or non contractile tail with different diameters **(Keel *et al.*, 2002)**.

Many investigators studied *Pseudomonas* phage/host patterns and reported that phages attack their *Pseudomonas* strains with different degree of activities. The lytic activity of *Pseudomonas* phages is either specific in which the phage lysis only a single strain of *Pseudomonas* or polyvalent in which the phage lyses numerous strains of *Pseudomonas* **(Mosa *et al.*, 1996)**.

Protein structure of *Pseudomonas* phages was determined by SDS-polyacrylamide gel electrophoresis, and it was found that, bacteriophages differ between them in their protein patterns **(Auling, 1978)**.

Because of the little knowledge about the bacteriophages specific for fluorescens *Pseudo-monas* particularly *Pseudomonas putidae* in the Egyptian soil. This investigation was planned to isolate, purify and characterize some phage isolates occur in the soil, depending upon the lytic pattern, electron microscopy, protein patterns and serology.

Materials and Methods

Source of bacteria:

Bacterial strains of *Ps. putida* and *Ps. fluorescens* were kindly obtained from Plant Pathology Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt. They were grown on the King's medium (10.0 g peptone, 0.75 g K_2HPO_4, 0.75 g $MgSO_4$, 7.5 ml glycerol and 1.5% agar).

Isolation of bacteriophages :

Clay loamy and sandy soil samples were collected from, soil cultivated with potato plants at Qalubia Governorate (Farm of Fac. Agric. Shoubra El-Khima and Basos) and Sharkia Governorate (Dahshour). Phages were isolated using the enrichment culture technique **Eayre *et al.* (1995)** as follows :

Five g of each sieved soil sample, 0.01 of $CaCO_3$ and 5 ml broth cultures *Ps.* spp. (24 hr old) were added into 250 ml Erlenmeyer flasks containing 100 ml of King's medium.

After shake- culturing on a rotary shaker for 72 h at 30°C, the cultures were centrifuged at 6000 rpm for 15 min. Supernatants were decanted in test-tubes and mixed with chloroform (1:10 v/v), vortexes and centrifuged for 15 min at 6000 rpm. The resulting upper phase supernatants (crude phage lysates) were separated from the chloroform, and stored in sterile vials at 4°C.

Assaying of bacteriophages:

Qualitative and quantitative assaying of the isolated two phages were carried out using the spot test and double layer agar plate methods. The phages presumptively responsible for the plaques were purified by three times of single plaque isolation **(Tanaka *et al.* 1990)**, to obtain a single purified plaque type.

Propagation and purification:

Phage lysate of each isolated phage was propagated according to **Tanaka *et al.* (1990)** to prepare high titre suspension of the phage.

The propagated high titre phage stock was purified and concentrated according to **Othman (1997)** by sedimentation by polyethylene glycol (PEG 6000), dextran sulphate two phase system 65.0, 2.0 and 17.0 g of PEG, sodium dextran sulfate and NaCl) were added to 1000 ml phage lysate in separating funnel and mixed well, then left to stand for overnight at 4°C. The heavily turbid bottom layer was collected slowly and centrifuged at 2000 rpm for 15 min. The clear top and bottom phase were removed and the remaining inter-phase layer was suspended in dextran sulphate. The mixture was allowed to stand and then centrifuged (at 6000 rpm for 15 min) and supernatant containing phages was collected followed by centrifugation at 15000 rpm for 2 h. The pellets were re-suspended in saline solution and dialyzed against NaCl solution (0.85%) for 48 hr.

Determination of phage particle morphology:

Phage suspension (10^8 pfu/ml) were negatively stained with 4% uranyl acetate and examined with transmission electron microscope (Beckman 1010) operated at 60 KV at the Regional Centre for Mycology and Biotechnology, El-Azhar University, Cairo, Egypt. Electron micrographs were taken and phage dimensions were measured.

Ultraviolet extinction spectra of purified phages :

Purified phage preparations were diluted to 100-fold and measured at range of 230 to 300 nm. ultraviolet waves (Unico – UV – 2100 spectrophotometer) MIRCEN, Faculty of Agriculture, Ain Shams University, in order to determine the optical properties, evaluate purity and yield of the purified phages.

Lytic pattern of *Ps. putida* phages :

Two bacteriophage isolates of *Ps. putida* were tested against 7 bacterial *Ps. putida* and *Ps. fluorescens* isolates illustrated in Table (1) obtained from the Department of Microbiology, Faculty of Agriculture, Ain Shams University.

Tasted bacteria were suspended in King's medium to a density of about 10^8 cfu/ml. Aliquot (0.5 ml) of suspension of each bacterial isolate was mixed with 2 ml melted medium (0.8% agar) and then overlaid on plates of king's solid medium.

Twenty ul of phage suspension were spotted on the agar overlayers and the plates were incubated at 30°C overnight. Clear confluent lysis or turbid confluent lysis were recorded as positive reaction, while extremely faint zones were considered negative reaction, according to **Eayre *et al.* (1995).**

Protein patterns of phages :

Proteins of phage particle were fractionated by SDS-pAGE described by **Laemmli (1970),** slab gels were formed between two glass plates (16 x 16 cm separated by 1.0 mm thick Teflon spacers). Gels were formed with 12% separating gel and a 4% stacking gel. After complete polymerization, the comb was removed and aliquots of 50 ul of the purified phage preparations were dissociated by heating for 5 to 10 min. in 50 ul of Laemmli buffer containing 2-mercaptoethanol protein samples and protein markers were electrophoresed at 200volt (25 mA) for 3-5 h.

The molecular weights of capsid proteins were estimated by comparison with those of protein markers with molecular wt (116.0, 66.2, 45.0, 35.0, 25.0, 18.4 and 14.4 KDa). Gels were stained overnight in 200 ml of 0.1% comassie brilliant blue R250 and destained according to **Hames and Rhichwood (1985).** Data were obtained by gel documintation quantity 1 (Bio-Rad).

Analysis of phage nucleic acid :

DNA nucleic acid of phages was separated according to the method of **Mayer *et al.* (1973)** with slight modification of **Maniatis *et al.* (1982).** The nucleic acid extraction was carried out with phenol saturated with TE buffer (10 mM tris-HCl (pH 8.0), 1 mM EDTA). The agarose gel electrophoresis was carried out using the tris-borate EDTA buffer (TBE) **(Peacock and Bingman, 1968).** Electrophoresis was carried out at 60 Volts for 3h, the gel was stained in 1 ug/ml of ethidium bromide for 30 min. **(Dillon *et al.*, 1985).** The stained gels were examined using an ultraviolet lamb and the DNA molecular weights were estimated by comparison with those of DNA markers with molecular weight (1, 517, 1,200, 1,000, 900, 800, 700, 600, 500, 400, 200 and 100 bp.). Data were obtained by gel documintation quality 1 (Bio-Rad).

Results and Discussion

Isolation and characterization of two phage isolates of *Ps. putida* isolated from the clay loamy soil cultivated with potato plants (Farm of Fac. Agric., Ain Shams Univ.Shoubra El-Khaima, Qalubia) which was the only one that give positive for phage isolation. In contrast, no phages were isolated from other types of soil. Phages were isolated by enrichment culture technique and phages were designated as PP_1 and PP_2. Numerous studies decument that phages capable of infecting fluorescent Pseudomonads can be isolated from different environments **(Ackermann, 2001; Jensen *et al.*, 1998; Erkan and Saygl, 1987; Mosa *et al.*, 1996 and Park *et al.*, 2000).**

From the plates derived from the overlayer assaying technique and containing the different morphological characters plaques. A plaque of each isolates was picked up and used for preparing purified phage lysate by single plaque isolation method. After repeating the technique three times, the purified phage lysate was propagated to obtain large scale production of the phages specific for *Ps. putida* to use in purification procedure. **Mosa *et al.* (1996)** used enrichment culture tehcnique for *Ps. solanacearum* phage isolation; **Park *et al.* (2000)** used also the enrichment method for isolation of phages of *Pseudomonas plecoglosicida*, The two phage isolates produced different types of plaques, PP_1 isolate produced circular, clear plaque (2 mm in diameter) without a halo while PP_2 isolate produced a circular plaques with central clear area surrounded by opaque area forming a halo, with 7.0 mm in diameter.

Propagation and purification of *Ps. putida* phages:

A large amount (1000 ml) with high titer of phage lysates (PP_1 and PP_2) was prepared using liquid culture technique. The titer of the propagated phages was 1.6×10^{12} and 1.1×10^{12} for PP_1 and PP_2 respectively.

Polyethylene glycol dextran sulfate two phase system was used to obtain partial purified phage preparations. Twenty three ml for PP_1 and twenty eight ml for PP_2 phage of turbid phase were collected from separating funnel after precipitation with PEG (Fig. 1A) and about 2 ml of the intermediate phase (cake) were collected after centrifugation of the turbid phase (Fig. 1B). Intermediate phases were obtained and centrifuged at 3000 rpm for 30 minutes, then the supernatant was centrifuged at 15.000 rpm for 120 min. The pellets were resuspended in saline solution and dialyzed against NaCl solution (0.85%).

Investigators usually prepare high titer phage lysates either from plates demonstrating confluent lysis

(Greer, 1982) or by liquid enrichment cultures **(Othman, 1997)**. Purification of some phages was accomplished using a two-phase water soluble polymer system by polyethylene lycol, 600 (4%) and sodium dextran sulfate, 500 (0.22%) followed by differential centrifugation **(Hu' *et al.*, 1981; Othman *et al.*, 2004 and Thomas and Leary, 1983).**

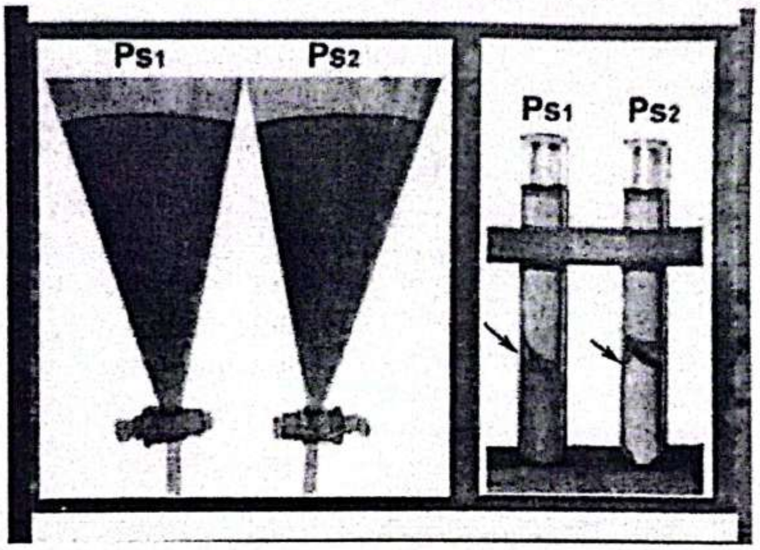

Fig. (1): Separating funnel containing viruses Pp_1 and Pp_2 in turbid bottom after precipitation (A) and intermediate phase containing viruses PP_1 and PP_2 after centrifugation of the turbied phase (B).

Characterization of phage particles :

Morphological properties of the isolated phages :

The particle morphology of the isolated phages PP_1 and PP_2 was determined by examination of the negatively stained preparations loaded onto carbon coated copper grids. Fields in the electron microscope screen were repeated many times to confirm the homogeneity of the preparations and/or the presence of more than view or identity. Electron micrographs (Fig. 2A and B) showed that, the two viruses have tadpole shape PP_1 virus ,with isometric head (135 nm in diameter) and long non-contractile tail (225 nm) whereas, PP_2 virus having isometric head (121 nm) and long contractile tail (202.9 nm), the tail posing an outer sheath (135 nm) and neck (27 nm). The phage PP_1 can be classified as member of siphoviridae family, while phage PP_2 can be classified as member of styloviridae family. **Miyajama (1980)** examined phages of *Pseudomonas, fuscovaginae* and found that, the all phage

A_{max}/A_{min} was 1.075. The absorption curves of protein and nucleic acid give rise to conclusions that the ratio A_{260}/A_{280} will be higher with viruses containing more protein and A_{max}/A_{min} will be higher with viruses containing more nucleic acid. Therefore, phage PP_1 had protein content more than phage PP_2 because A_{260}/A_{280} of PP_1 less than (1.72) that of PP_2 (2.15), and it had nucleic acid content less than Pp_2 because the ratio A_{max}/A_{min} of PP_1 (0.76) less than that of PP_2 (1.075).

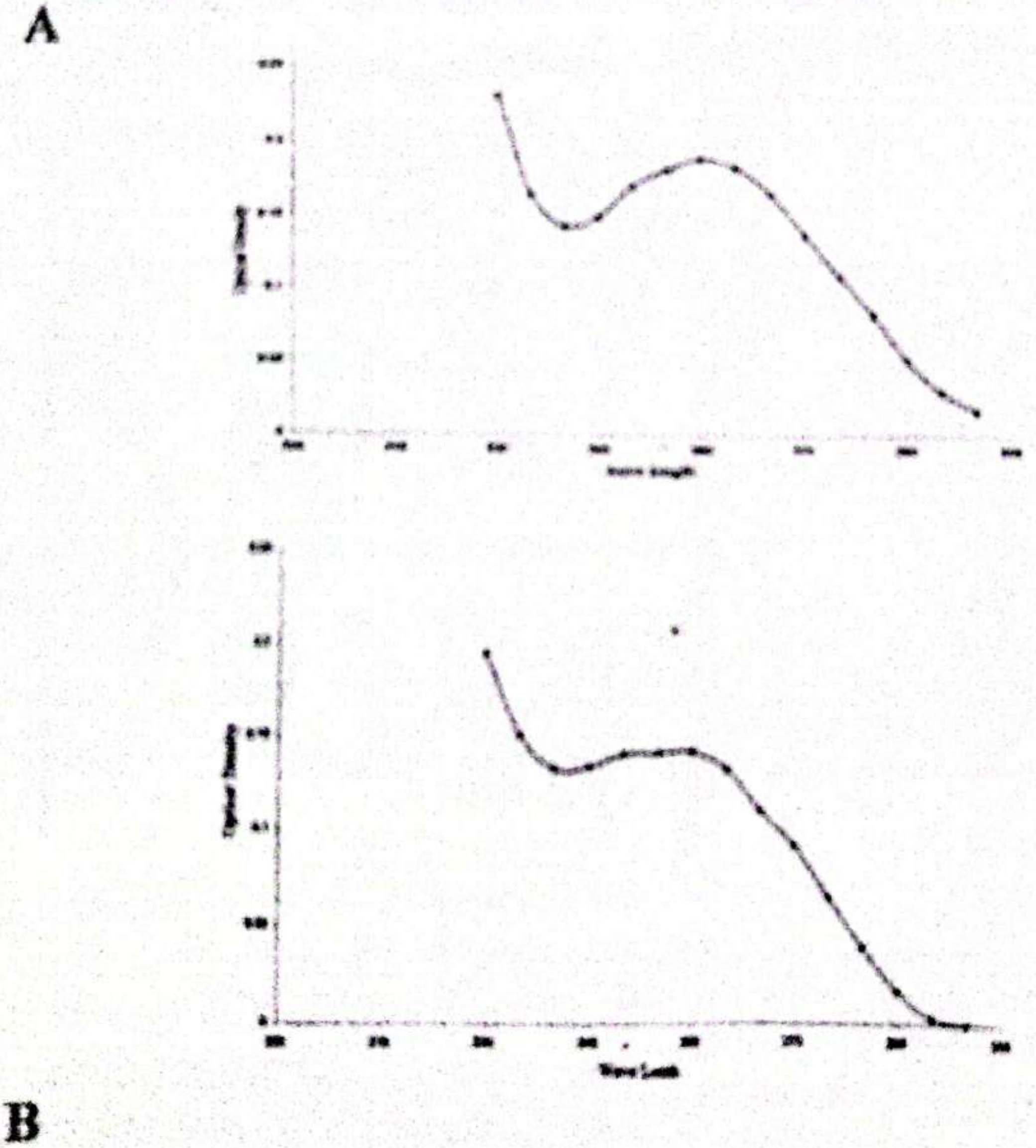

Fig. (3): U.V. absorption spectrum of *Pseudomonas* phage PP_1 (A) and *Pseudomonas* phage PP_2 (B).

Protein patterns of *Pseudomonas* phages :

SDS-PAGE has been more widely used to compare structural differences between virus strains **(Maillard *et al.*, 1996).**

The molecular weight of the proteins of PP_1 and PP_2 were determined comparing with marker proteins. As shown in Fig. (4), *Pseudomonas putida* PP_1 virus had 16 structural proteins with molecular weights known and extrapolated of 112.41, 86.78, 84.18, 72.29, 65.60, 55.96, 42.87, 36.84, 33.56, 29.21, 23.55, 17.48, 7.03, 5.92, 2.78 and 1.73 KDa while *Pseudomonas putida* Pp_2 virus had 13 structural proteins with molecular weights known and extrapolated of 117.66, 113.38, 83.38, 65.60, 57.11, 42.87, 34.19, 28.72, 23.55, 17.48, 7.94, 6.60 and 3.23 KDa. Data also showed that there were four known structural protein presented in the two phages with molecular weights of 65.60, 42.87, 23.55 and 17.48 KDa .Data also indicated that the two phage isolates PP_1 and PP_2 differed quantitatively and qualitatively in their structural proteins and they are considered according to these two phages. **Auling (1978)** reported that *Pseudomonas pseudofalva* temperate phages give 5 major & 10 minor bands when their proteins were determined by SDS-PAGE, the molecular weight of proteins ranged from 6.5 to 145 KDa.

Electrophoresis of DNA of *Pseudomonas putidae* viruses :

The purified preparations of nucleic acid of PP_1 and PP_2 (*Pseudomonas putida*) viruses were electrophoresed (1%) by agarose slab gel. Fig. (5), Illustrates that there is one molecule in each phage, comparing with marker DNA. The molecular weight of Ps_1 and Ps_2 viruses were 2223.885 and 2559.069 bp. Respectively.

Thomas and Leary (1983) reported that either the nucleic acid was RNA or DNA and wether it is single or double stranded, they were determined by acrylamid gel electrophoresis and enzyme digestion. They determined the molecular wt of 20 *Pseudomonas syringae* pv. *glycinea* bacteriophages, they were double stranded DNA with molecular weights varying from 5.6 to 8.0 x 10^6 bp by comparison with standards also **Keel *et al.*, 2002** reported also that the genome size of *P. fluorescens* strain CNAO was approximately 50 kbp.

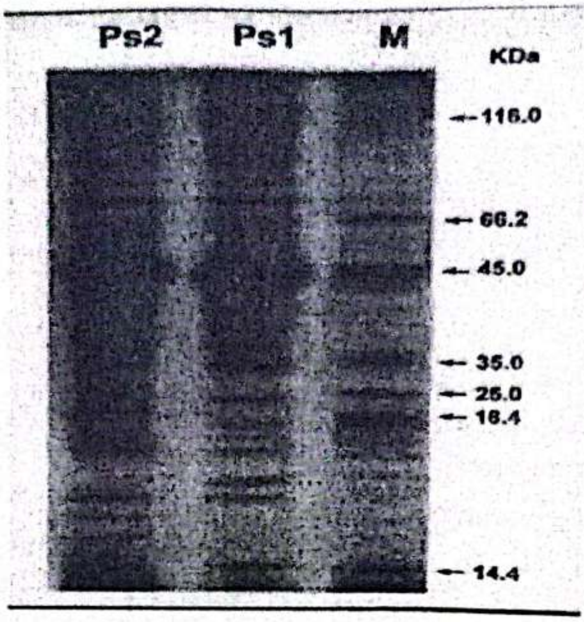

Fig. (4):Protein patterns of *Pseudomonas* viruses (PP_1 and PP_2) as determined by 12%SDS-PAGE.

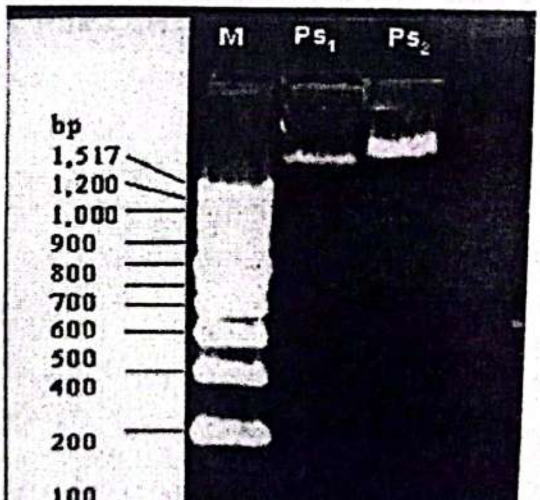

Fig. (5): Molecular weight of the PP_1 and PP_2 genome viruses, as detrermined on 1% agarose gel electrophoresis.
- Lane 1 PP_1 genome; - Lane 2 PP_2 genome; - Lane M marker DNA.

Lytic patterns of *Pseudomonas* phages :

Pseudomonas phages have been purified and their infectivity on various *Pseudomonas* host strains is being studied qualitatively by the spot test technique. Data represented in Table (2) showed that *Pseudomonas* phages PP_1 and PP_2 have a host range restricted to three or four of the 7 tested strains respectively. The two isolates of Pseudomonas phages differ in their reaction with the bacterial host strains, as Pp_1 caused lysis to four strains (Pf8, 50, Pf1, andPs_4 while phage PP_2 reacted positively with three isolates (Pf8, 50, and Pf1). Many investigators studied the *Pseudomonas* phages host patterns and reported that phages attack their *Pseudomonas* strains with different degrees of activity **Kropinaki and Warren (1970)** reported that the lytic bacteriophage QW-14 specific for *Pseudomonas acidovorans* showed limited host range, lysing only four of seven different strains. While **Aria and Uehara (1982)** reported that phi phage of *Ps. syringae* attacked 18 isolates of *Ps. syringae*. The lytic activity of *Pseudomonas* phages is either specific in which phage lysis only a single strain of *Pseudomonas* or polyvalent in which the phage lyses numerous strains of *Pseudomonas*.

Table (2): Lytic pattern of the isolated phases specific for *Pseudomonas putida*.

	Indicator bacteria	Spot test results*	
		Phage PP_1	Phage PP_2
1	*Pseudomonas putida* PF1	+	+
2	*Pseudomonas putida* 50	+	+
3	*Pseudomonas putida* P55	-	-
4	*Pseudomonas putida* PP	-	-
5	*Pseudomonas fluorescence* B	±	±
6	*Pseudomonas fluorescence* PS4	+	-
7	*Pseudomonas fluorescence*	-	-

* Result of three replicates for each treatment.

References

Ackermann, H.W. (2001). Frequency of morphological phage descriptions in the year 2000. Arch. Virol. 146: 843-857.

Alok-Sharma; Johri, B.N. and Sharma, A. (2003). Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays*L.) under iron limiting conditions. Microbiological- Research, 158: 3, 243-248.

Arai, K. and Uehara, K. (1982). Studies on bacteriophages of *Pskleudomonas syringae* pv. theae (Hori 1915) young, Dye and Wilkie. Bulletin of the Faculty of Agriculture, Kagoshima University (32): 63-68.

Auling, G. (1978). Biological, serological and biochemical investigations of three closely related temperate bacteriophages specific for *Pseudomonas pseudoflava.* J. of Gen. Virol., 40: 615-622.

Bergsma-Vlami, M.; Prins, M.; Staats, M.; Raaijnarkers, J.; Elad, Y. (ed.); Kohl, J. (ed.) and Shtienberg D. (2002). Diversity and host specificity of *Pseudomonas* spp. producing the antibiotic 2,4-diacetylphloroglucinol (DAPG) IOBC-WPRS Working Group "Biological control of Fungal and bacterial plant pathogens. Proceedings of the 7th working group meeting, influence of abiotic and biotic factors on biocontrol agents at Pine Bay, Kusadasi, Turkey Bulletin-OILB-SROP. 25: 10, 45-48.

Dillon, J.R.; Nasim, A. and Nestmann, E.R. (1985). Recombinant DNA Methodology. John Wiley & Sons. U.S.A.

Eayre, C.G.; Bartz, J.A. and Concelmo, D.E. (1995). Bacteriophages of *Erwinia carotovora* and *Erwinia ananas* isolated from fresh water lakes. Plant Disease 79: 801-804.

Erkan, S. and Saygl, H. (1987). The preliminary studies on the isolation of the bacteriophages of some phytopathogenic bacteria. Journal of Turkish Phytopatho-logy, 16 (2): 71-76.

Hames, B.D. and Richwood, D. (1985). Gel electrophoresis of proteins a practical approach. IRL Press Ltd., Britain 4th ed.

Jensen, E.C.; Schrader, H.S.; Rieland, B.; Thompson, T.L.;. Lee, K.W; Nickerson, K. and Kokjohn, T.A. (1998). Prevalence of broad- host-range lytic bacteriophages of

Sphaerotilus natans, Escherichia coli, and *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 64: 575-580.

Kakutani, K.; Toyoda, H. and Ooto, S. (1994). Some chracteristics of virulent bacteriophages isolated from *Pseudomonas solanacearum* infested soil. Annals of the Phytopathological Society of Japan. 60 (4): 531-534.

Keel, C.; Ucurum, Z.; Michaux, P.; Adrian, M. and Haas, D. (2002). Deterions impact of a virulent bacteriophage on survival and biocontrol activity of *Pseudomonas fluorescens* strain CHAO in natural soil. Molecular Plant Microb. Interactions. 15 (6): 567-576.

Kropinski, B.M.A. and Warren, J.A.R. (1970). Isolation and properties of *Pseudomonas acidovorans* bacteriophage. J. of gen. Virol. 6: 85-93.

Laemmli, U.K. (1970). Cleavage of the structural proteins during assembly of the head of bacteriophage T_4. Nature 227: 680-685.

Maillard, J.Y.; Beggs, T.S.; Day, M.; Hudson, R.A. and Russell, A.D (1996). The effect of biocides on proteins of *Pseudomonas aeruginosa* PAG bacteriophage F116. Journal of Applied Bacteriology, 80, 291-295.

Maniatis, T.; Frilsch, E.F. and Sambrook, K.J. (1982). Molecular cloning a laboratory amnnual. New York. Col. Spring Harbor Laboratory.

Mayer, F.; Lotz, W. and Long, D. (1973). Electron microscope study of length and partial denaturation of *Rhizobium* bacteriophage DNA. J. Virology, 11: 946-952.

Miyajiam, K. (1980). Isolation and some properties of bacteriophages specific for *Pseudomonas fuscovaginae*, the causal bacterium of sheath brown rot of rice plant. Annals of the Phytopathological Society of Japan. 46 (2): 132-139.

Mosa, A.A.; Ab El-Ghafar, N.Y. and Othman, B.A. (1996). Bacteriophages of *Pseudomonas solanacearum* and their potential for biological control of potato bacterial wilt. Zagazig Journal of Agricultural Research. 23 (6): 1053-1063.

Othman, B.A.; El-Dougdoug, Kh.A.; Abdel-Ghaffar, M.H. and El-Arabi, T.F. (2004). Interaction between *Rhizobium meliloti* and it's specific viruses in clover plants. Egyptian J. Virol. 1, 367-381.

Othman, B.A.A. (1997). Isolation of lambdoid bacteriophage β_4EC from sewage polluted drinking water. PP. 78-88. Proceeding of the 10th Conference of Microbiology. March 25-27, Cairo, Egypt.

Park, S.C.; Shimamura, I.; Fukunaga, M.M.; Mori, K.I. and Nakai, T. (2000). Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. Appl. Environ. Microbiol. 66: 1416-1422.

Peacock, A.C. and Dingman, G.W. (1968). Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gel. Biochem. 7: 668-674.

Tanaka, H.; Negishi, H. and Maeda, H. (1990). Control of tobacco bacterial wilt by an a virulent strain of *Pseudomonas solanacearum* M45 and H5 bacteriophage. Annals of the Phytopathological Society of Japan. 56 (2): 243-246.

Thomas, M.D. and Leary, J.V. (1983). Bacteriophages from sewage specific for fluorescent phytopathogenic *Pseudomonas*. Phytopathology, 73 (3): 403-406.

Tokarchuk, L.V.; Popenko, G.G.; Zhovnovata, V.L and Samoilenko, V.I. (1975). Production, concentration and purification of *Pseudomonas vignae*. Mkorobiologichnii Zhurnal 37 (3): 387-390.

عزل وتوصيف بكتريوفاجات بسيدوموناس بيوتيدا

ميادة عبد السلام عبد الجليل [1] ، بدوى عبد السلام عثمان [2] ، ثروت دسوقى رضوان [1] ، وأمل سعيد ابو سنه[3]

1- قسم النبات ، كلية العلوم ، جامعة القاهرة ، فرع الفيوم ، مصر .
2 – قسم الميكروبيولوجيا الزراعية ، كلية الزراعة ، جامعة عين شمس ، شبرا الخيمة ، مصر
3 – قسم النبات والميكروبيولوجى ، كلية العلوم ، جامعة الأزهر فرع بنات ، القاهرة ، مصر

من تربة زراعية منزرعة بطاطس تم عزل إثنين من البكتريوفاجات سميا PP_1 و PP_2 ثم أجريت لهما عملية إكثار فى مزارع مقواه سائلة وأتبعت بإجراء تقنية للفيروسات (البكتريوفاجات) بنظام الطبقتين السائلتين فى وجود البولى إثيلين جليكول والدكستران سلفات.

درست خواص الفاجات وأوضحت النتائج المتحصل عليها أن للفاجين خواص إدمصاصية للأشعة الفوق بنفسجية مختلفة ولكليهما مجال عوائلى ضيق ومحدود لسلالات النوع بيوتيدا . أوضح الفحص بالميكروسكوب الإلكترونى أن الشكل الظاهرى للفاجين يشبه شكل فرخ الضفدع ، حيث يمتلك فاج PP_1 رأساً أيزومترية قطرها حوالى 135 نانوميتر ونيلاً طويلاً غير منضغط بطول 225 نانوميتر ، فى حين يمتلك فاج PP_2 رأساً أيزومترية قطرها 121 نانوميتر ونيلاً طويلاً منضغطاً طوله 202 نانوميتر ويغطى النيل غلافاً خارجياً قابلاً للإنضغاط طوله 135 نانوميتر .

أوضحت تحليلات البروتينات بإستخدام الـ SDS-PAGE أن الفاجين مختلفين فى أعداد البروتينات التركيبية حيث يمتلك فاج PP_1 0بناء على برامج التحليل بالحاسب الآلى للجل) على ستة عشر نوعاً من بينهم ستة أنواع معلومة الوزن الجزيئى والباقى متوقع الوزن الجزيئى ، ويمتلك فاج PP_2 ثلاثة عشر نوعاً من بينهم خمسة أنواع معلومة الوزن. الجزيئى والباقى متوقع الوزن الجزيئى . أستخلص الحامض النووى للفاجين وقدر الوزن الجزيئى للأحماض وبينت النتائج أن كل فيروس يحتوى على جزىء واحد من الحامض إلا أنهما يختلفان فى وزنهما الجزيئى حيث جاء الوزن ألفين ومائتين ثلاثة وعشرين زود قاعدة وألفين وخمسمائة تسعة وخمسين زوج قاعدة للفاجين على التوالى .