Egyptian J.Virol.3(1),(2006)19-34

Isolation and characterization of a *Pseudomonas aeruginosa* bacteriophage from agriculture drain water

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Received October 2006; accepted December2006

Abstract

Five bacteriophages infecting *Pseudomonas aeruginosa* PAO1 strain were isolated from agricultural drain water of Zagazig city. One of them designated as PA1 has been characterized. The ability of phage PA1 to form plaques with strain PAO1 was examined. Two kinds of plaques (turbid and clear) have been observed. The average number of turbid plaques was 2.52 x 10^9 pfu/ml whereas of clear plaques was 9.96 x 10^7 pfu/ml. The phage was able to lysis 13 out of 20 strains of *Pseudomonas aeruginosa*, this result shows that phage PA1 has a broad host range. The phage was able to lysogenized three strains (PAO1, PU21, MAM2) and was also able to release spontaneously from these lysogenic strains. The phage particles which released spontaneously from lysogens were used for transducing different genes from donor ATC43 to recipient ATC114. The phage was able to transduce the same marker with nearly the same frequency. When phage was treated by different temperature, the phage was stable informing plaques with 5, 15°C whereas the pfu/ml 6.8, 6.9 x 10^9, but the ability of phage to form plaques was declined with low and high temperature (1.8 at 0°C and 0.18 at 45°C x 10^9 pfu/ml). The ability to transduce antibiotic resistance genes was also influenced. When phage was treated with 5 different Ph values (2, 3, 7, 10, 12), no plaques or transductants were observed at acid Ph values (2, 3). But with Ph7 resulting high titration and subsequently high number of transductants. This study demonstrates that the bacteriophages as a mediated generalized transducing vectors of genetic material play role in natural ecology.

Key words: Isolation, Characterization, Bacteriophage, Host range, Titration, Transduction.

Introduction

Bacteriophages are ubiquitous in nature, and it has been suggested that they are environmentally important both in controlling bacterial numbers and in facilitating bacterial gene transfer (**Bergh *et al.*, 1989, Bratbak *et al.*, 1990, and Kokjohn & Miller, 1992**). By their vary nature, phages are likely to be most prevalent in environments where there is a high density of metabolically active bacteria. One such environment is the agricultural drain water in Zagazig

City, Sharkia Governorat, which is known to contain a wide diversity of bacterial species. A high level of viral particles in many habitats has been revealed **(Wiggins & Alexander, 1985 ; Sunny *et al.*, 1998; Amin *et al.*, 2004).**

The agricultural drain water may contain a wide variety of chemicals, detergents, fats pesticides, heavy metals and others including different phages and host bacterial species **(Katyal and Satake, 1996).** A considerable efforts has been devoted to understand how cells cope with environmental stresses. The occurrence and distribution of bacteriophages and their bacterial hosts in different ecosystems, still, have not been extensively investigated. Moreover, little attention has been given to the factors which influence the association between them. There have been no assessments of the effects of these factors on the activities of bacteriophages in natural environments.

The aim of this work is to isolate several bacteriophages infecting *Pseudomonas aeruginosa* from different agricultural drain waters of different sources from Zagazig city to characterize them.

Materials and Methods

1- Bacterial strains:

Pseudomonas aeruginosa bacterial strains PAO1, PU21 and MAM_2 that have been used in this study were obtained from M. Day, UWIST University, Wales, UK. The isolates found in Table (1) (ATC1, ATC11, ATC17, ATC37, ATC43, ATC45, ATC50, ATC58, ATC 68, ATC 70, ATC 76, ATC77, ATC78, ATC 87, ATC111, ATC113, and ATC114) were obtained from M. EL-Hosyny, Faculty of Pharmacy, Zagazig University, Zagazig Egypt.

Table (1): The bacterial strains and isolates of *Pseudomonas aeruginosa* that were used in this study.

Strains and isolates	Genotype	Reference
PAO1	Prototrophic, Str^s, Tet^s	Holloway and Morgan (1986)
PU21	Str^s, Tet^s, Val^-	Amin and Day (1988)
MAM2	Str^s, Tet^s, Met^-	Amin et al., (2004)
ATC1	Prototrophic, Str^s, Tet^s, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC11	Prototrophic str^s, Tet^s, Amp^s, Chl^r	Faculty of Pharmacy, Zagazig University
ATC37	Prototrophic, Str^s, Tet^s, Ampr, Chl^r	Faculty of Pharmacy, Zagazig University
ATC43	Prototrophic, Str^r, Tet^r, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC45	Prototrophic, Str^s, Tet^s, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC50	Prototrophic, Str^r, Tet^r, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC58	Prototrophic, Str^r, Tet^r, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC68	Prototrophic, Str^r, Tet^r, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC70	Prototrophic, Str^s, Tet^r, Amp^s, Chl^r	Faculty of Pharmacy, Zagazig University
ATC76	Prototrophic, Str^s, Tet^s, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC77	Prototrophic, Str^s, Tet^s, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC78	Prototrophic, Str^r, Tet^r, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC87	Prototrophic, Str^r, Tet^s, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University

Strains and isolates	Genotype	Reference
ATC111	Prototrophic, Str^r, Tet^r, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC113	Prototrophic, Str^s, Tet^s, Amp^r, Chl^r	Faculty of Pharmacy, Zagazig University
ATC114	Prototrophic, Str^s, Tet^s, Amp^s, Chl^s	Faculty of Pharmacy, Zagazig University

Str^r= Streptomycin resistance
Str^s= Streptomycin sensitive
Tet^r= Tetracycline resistance
Tet^s= Tetracycline sensitive
Chl^s= Chloramphenichol sensitive
Amp^r= Ampicillin resistance
Amp^s= Ampicillin sensitive
Chl^r= Chloramphenichol resistance
Val^- = Valine auxotroph
Met^- = Methionine auxotroph

2- Growth media:

Nutrient agar (NA) and nutrient broth (NB) media were prepared according to manufacture's instructions. Phosphate buffer was prepared from 1/15 M potassium phosphate (KH_2PO_4) and 1/15 M disodium phosphate ($Na_2 HPO_4$. 2 H_2O). The antibiotics were added as sterilized solution after filtration through 0.2 µm filter membrane to the media after autoclaring. The concentration of antibiotics were 500 µg/ml for streptomycin, tetracycline and 1500 µg/ml for ampicillin, chloramphenichol.

3- Isolation of phage from agricultural drain water:

10 ml of water have been centrifugated at 10000 rpm for 30 min. Supernatant was filtred trough membrane filter of 0.2 µm (**Whatman**) according to **Sunny *et al.*, 1998.** One ml of sterilized supernatant was mixed with 1.0 ml of potential host culture of *Pseudomonas aeruginosa* strain (PAO1) in a tube containing 3 ml of soft agar kept at 47°C. The mixture was vortexed and poured over a NA plate. After incubation for 12 h. at 30°C, turbid and clear single plaques were picked from the plates and each individual plaque was reisolated three times to ensure the purity of the phage isolate. Phage lysate was produced by eluting the top agar overlay plates. Stock samples were stored at 4°C after filtration.

4- Study of host range of phage:

Many strains of *Pseudomonas aeruginosa* have been used to determine the host range of phage. Phage host range was established by using the spot test method **(Rodolphe *et al.*, 2002),** 3 ml of soft agar was mixed with 100 µl of an overnight culture of bacterial host, vortexed and spread onto the surface of NA plate. Single drops of phage lysate was spotted onto the inoculated NA plates, and the plates were incubated overnight at 30°C. Bacterial sensitivity to a bacteriophage was established by bacterial lysis at the spot where the phage lysate drop was deposited. Positive spot tests were confirmed by titration assay by using diluted phage preparation.

5- Titration assay:

The overlay mothod of **Adams (1959)** was used. Serial hunderd - fold dilutions of phage were prepared in phosphate buffer (pH 7.0). Equal volumes (0.1) ml of phage and host

cells(grown overnight in NB at 30°C)were mixed in 3 mls of soft molten agar kept at 45°C. The mixture was vortxed and poured immediately onto NA plate . Plates were incupated at 30°C for 24h. Plques were counted and the number of plaques forming units (pfu/ml)was calculated.

6- Ability of phage to lysogenize strains:

The ability of phage to lysogenize many strains of *P. aeruginosa* has been investigated. Putative lysogenic strains were isolated from the centers of individual plaques and, after purification were assessed for phage production in order to confirm their identities. The lysogenic colonies were then checked for sensitivity to the phage to confirm their immunity to further infection by the same phage. Overnight cultures of the lysogenic strains and a wild- type strain were used to inoculate separate overlay agar plates, which were then challenged with lysate. After overnight incubation at 30°C the ability of phage lysate to lyse each strain was assessed **(Kevin *et al* ., 1999).**

7- Single plaques isolation for study the burst size:

Five individual plaques were picked from plates and each one was put in 1.0 ml of NB, incubated at 30°C overnight. The suspensions were centrifugated at 5000 rpm for 30 min and filtered through 0.2 µm filter membrane. The phage particles were assayed, the process was performed 10 times.

8- Reproducipilty:

The 3 lysogenic strains which prepared in this study were subcultured 10 times. The spontaneous released phage was assayed.

9- Transduction by lysate:

Phage lysate was prepared and titrated by using streptomycin, tetracycline, ampicillin and chloramphenichol resistant isolate ATC 43 as the donor. Nutrient broth culture of isolate ATC 114 was mixd with transducing lysate at a multiplicity of infection (ratio of phage to bacteria) 1: 10 **(Kevin *et al.*, 1999).** The mixture was kept for 30 min at room temperature, to allow phage adsorption. Serail dilutions have been prepared and placed onto selective media. Number of transductants were recorded and transduction frequency was calculated.

10- Effect of temperature and pH on survival and ability of phage to transduce different markers.

The phage lysate samples were treated with different temperatures (0 , 5, 15, 30, 40, 45) and pH (2, 3, 7, 10, 12) for 24 h . Phage titration was performed and the ability to transduce was assayed **(Amin *et al* ., 2004).**

11- Effect of UV irradiation on prophage induction from lysogenic *Pseudomonas aeruginosa strain* :

The lysogenic strains were grown at 30°C in nutrient broth media for overnight, 10 ml samples have been removed into Petri dishes **(Amin and Abdel Basit, 2000).** Samples have been exposed to UV for 0.5, 1.0, 4,

16, 20, 25 and 30 min. After treatment the samples were centrifuged and filtered,then phage titre was performed. The phage particles which released from lysogenic cells were used to transduction assay.

Results and Discussion

1- Isolation of bacteriophage capable of infecting the original strain PAO1 of *Pseudomonas aeruginosa* :

Five bacteriophages infecting *Pseudomonas aeruginosa* original prototrophic PAO1 strain have been isolated from the agricultural drain water of Zagazig
city. One of them designated PA1 has been fully characterized. Data in Table (2) show the ability of phage PA1 to form two kinds of plaques, turbid and clear with strain PAO1. The average number of turbid plaques was $25.24x10^9$ pfu/ml, whereas, the average number of clear, plaques reached up to 9.96×10^7 pfu/ml. This means that PA1 is said to be a lytic bacteriophage since the phage was able to form about 0.39% clear plaques among the total number of formed plaques. Moreover, when several turbid plaques has been identified to detect the lysogenicity ability of PA1, the lysogenicity percent was 85% among the tested plaques.

Table (2): Titration of phage PA1 using *P. aureginosa* strain PAO1 as an sensitive host .

No. Trails	Turbid plaques Pfu/ml 10^9	Clear plaques Pfu/ml 10^7
1	1.2 ± 0.01	25 ± 0.7
2	57 ± 0.5	20 ± 0.9
3	34 ± 0.7	2 ± 0.7
4	1.9 ± 0.03	1.1 ± 0.03
5	32.1 ± 0.9	1.7 ± 0.04
Mean	25.24 ± 0.4	9.96 ± 0.5

Each value is the mean of 3 replica ± SD.

2- Host range of phage PA1:

20 strains of *Pseudomonas aeruginosa* have been used to assess the host range of phage PA1. The phage was able to lysis 13 out of 20 strains with percentage of 65%.

Table (3): Host range of phage PA1.

Host strain of *Pseudomonas aeruginosa*	Spot test
PAO1	+
PU21	+
MAM2	+
ATCI	-
ATC11	-
ATC 17	-
ATC 37	+
ATC 43	+
ATC 45	-
ATC 50	+
ATC 58	+
ATC 68	-
ATC 70	+
ATC 76	+
ATC 77	-
ATC 78	+
ATC 87	-
ATC 111	+
ATC 113	+
ATC 114	+

+ producing lysis. - not producing lysis

These results clearly show that phage PA1 has a broad host range. Many phages have been characterized principally by host range **(Chopin *et al.*, 1976; Eric & Betty, 1978; and Jensen *et al.*, 1998).** Phages fall into two categories, those with a limited host range (often only one strain) and, those with a multistrain range **(Eric & Betty, 1978; and Jensen *et al.*, 1998).** These two host range groups correspond roughly to a natural morphological classification of the phages. However, some phages have the ability to interact with a wide range of host species. These would be significant in the control of the composition and genetic diversity of microbial communities as well as the processes of transductional gene

exchange and the transfer of antibiotic resistance genes through those communities. **(Harshey, 1988; Yarmolinsky & Sternberg, 1988 ; and Jensen *et al.*, 1998,).**

3- Ability of phage PA1 to lysogenize *Pseudomonas aeruginosa* strains:

Phage PA1 was capable in lysogenizing three strains of *Pseudomonas aeruginosa*, PA01, PU21, and MAM2. The phage was also able to release spontaneously from these lysogenic strains (Table 4). However PA1 phage was able to lysogenize 3 different host cells, but the average number of phage released spontaneously from each lysogenic strains was nearly constant (pfu/ml=2.41, 2.10 and 2.15 x 10^9 for PA01, PU21, MAM2 lysogenic strains, respectively).

Table (4): Reproducibility of releasing phage PA1 spontaneously from lysogenic strains.

No. trials	10^9 pfu/ml of lysogens		
	PAO1	PU21	MAM2
1	2.15 ± 0.1	0.2 ± 0.01	2.16 ± 0.7
2	6.62 ± 0.3	1.4 ± 0.3	6.30 ± 0.8
3	0.17 ± 0.01	4.76 ± 0.3	2.21 ± 0.5
4	2.11 ± 0.5	2.88 ± 0.1	2.91 ± 0.6
5	2.41 ± 0.6	0.23 ± 0.07	2.10 ± 0.09
6	2.12 ± 0.7	6.36 ± 0.7	0.09 ± 0.01
7	2.61 ± 0.6	0.59 ± 0.03	2.10 ± 0.7
8	3.9 ± 0.4	1.65 ± 0.01	2.40 ± 0.6
9	0.15 ± 0.09	0.39 ± 0.04	0.18 ± 0.01
10	1.86 ± 0.07	1.54 ±0.02	1.01 ± 0.02
Mean	2.41 ± 0.3	2.1 ± 0.3	2.15 ± 0.4

Each value is the mean of 3 replica ± SD.

4- Ability of phage PA1 released spontaneously from lysogenic strains informing plaques and transducing different genes:

In order to assess the ability of phage released spontaneously from lysogenic strain informing plaques and transducing different genes. The 3 phage lysates have propagated on strain ATC43 as donor host cells. The prepared lysates have been used in titration (Table 5) and transduction (Table 6). Phage PA1 was capable in forming plaques on the three tested host cells with high efficiency, PAO1 (13.8 x 10^9 pfu/ml), PU21 (11.6 x 10^9 pfu/ml) and MAM2 (11.54 x 10^9 pfu/ml).

Phage PA1 which released spontaneously from 3 different

lysogenic strains then allowed to propagate on the donor strain ATC43, that carrying 4 antibiotic resistance genes, transduced the 4 genes into the recipient strain ATC114 (Table 6). The phage was able to transduce the same gene marker with nearly the same frequency. In the case of streptomycin, the frequency ranged from 8.2 up to 9.6 x 10^{-6}. However, the transduction frequency of each marker was varied. This may due to the location of the marker on the chromosomal map of the donor strain and subsequently the number of transducing particles carrying each marker was varied resulting in the differences of transduction frequency of each gene.

A physiological feature which is of evolutionary importance has transducing ability. Gene transfer mediated by bacteriophages could be of great significance to the environment **(Ashelford *et al.*, 1999; and Day & Marchesi, 1996).** Transduction frequencies recorded in this study using lysates prepared from lysogen do agree with other studies **(Ashelford *et al.*, 1999).** Usually, the transfer frequencies were around 10^7 to 10^9 for phage lysates and 1 to 2 orders of magnitude less when lysogens were involved **(Ashelford *et al.*, 1999)**

Table (5): Titration of phage PA1 prepared on *P. aeruginosa* strain ATC43.

No. trials	Titration pfu/ml 10^9		
	PAO1	PU21	MAM2
1	11.25 ± 0.1	12.5 ± 0.1	5.1 ± 0.7
2	16.9 ± 0.2	14.1 ± 0.3	15.3 ± 0.9
3	16.1 ± 0.3	15.2 ± 0.7	15.7 ± 0.8
4	14.5 ± 0.3	10.3 ± 0.8	13.6 ± 0.9
5	13.3 ± 0.7	13.4 ± 0.9	23.4 ± 0.9
6	21.7 ± 0.9	6.3 ± 0.9	0.4 ± 0.03
7	30.1 ± 0.9	21.4 ± 0.9	25.8 ± 0.9
8	0.2 ± 0.01	5.3 ± 0.9	0.5 ± 0.03
9	6.1 ± 0.04	7.8 ± 0.8	3.1 ± 0.07
10	7.4 ± 0.9	9.4 ±0.7	12.5 ± 0.9
Mean $\bar{X}$	13.8 ±0.415	11.6 ±0.7	11.54 ±0.73

Each value is the mean of 3 replica ± SD.

Table (6): Transduction by lysate prepared from lysogens.

Lysogen	Transducing antibiotic resistance gene							
	Str		Tet		Amp		Chl	
	No. of transductants 10^4	Transduction frequency	No. of transductants 10^4	Transduction frequency	No. of transductants 10^4	Transduction frequency	No. of transductants 10^4	Transduction frequency
PA1ϕ PAO1	13.43	8.2×10^{-6}	18.20	1.12×10^{-5}	5.61	3.4×10^{-6}	113.43	7.1×10^{-5}
PA1ϕPU21	15.69	9.6×10^{-6}	20.66	1.27×10^{-5}	4.34	2.6×10^{-6}	117.54	7.25×10^{-5}
PA1ϕMAM2	14.53	8.9×10^{-6}	16.73	1.03×10^{-5}	6.94	4.2×10^{-6}	110.78	6.83×10^{-5}

Strain ATC114 (1.62×10^{10} cfu/ml) was used as recipient host cells.
Str = streptomycin, Tet = tetracycline, Amp = ampicilin, Chl = chloramphenicol.
Each value is the mean of 10 experiments.

5- The burst size of phage PA1:

In order to estimate the burst size of phage PA1, five different single turbid plaques were chosen randomly, and pfu/ml of each one was calculated as average of 10 experiments (Table 7).

Table (7): Burst size of phage PA1.

No. trials	Titration pfu/ml 10^9				
	(1) pfu/ml 10^9	(2) pfu/ml 10^9	(3) pfu/ml 10^9	(4) pfu/ml 10^9	(5) pfu/ml 10^9
1	1.3 ± 0.1	2.11 ± 0.1	0.25 ± 0.3	12 ± 0.7	15 ± 0.7
2	0.61 ± 0.07	1.76 ± 0.2	1.83 ± 0.5	0.97 ± 0.01	0.7 ± 0.1
3	5.6 ± 0.04	2.82 ± 0.3	11.97 ± 0.7	1.92 ± 0.1	2.19 ± 0.2
4	2.64 ± 0.2	13.11 ± 0.3	10.88 ± 0.7	8.36 ± 0.1	13.68 ± 0.3
5	5.68 ± 0.3	6.24 ± 0.3	12.71 ± 0.3	3.56 ± 0.1	14.51 ± 0.4
6	21.4 ± 0.4	36.51 ± 0.9	21.12 ± 0.4	3.19 ± 0.2	19.85 ± 0.4
7	0.37 ± 0.01	7.25 ± 0.9	14.26 ± 0.3	6.52 ± 0.1	11.96 ± 0.5
8	12.14 ± 0.2	0.58 ± 0.01	16.59 ± 0.2	30.17 ± 0.1	12.35 ± 0.5
9	12.51± 0.2	10.88 ± 0.3	9.96 ± 0.1	10.21 ± 0.1	11.30 ± 0.7
10	10.96±0.3	21.37 ±0.9	14.71 ± 0.1	12.16 ± 0.1	10.69 ± 0.9
Mean $\overline{X}$	7.32±0.142	10.16±0.421	11.43±0.36	8.91±0.061	11.22±0.47

Each value is the mean of 3 replica ± SD.

The burst size ranged from 7.32 up to 11.22 x 10^9 pfu/ ml.

Previous research revealed the importance of burst size and the ability to produce lysogens as strategies by which phage might optimize its ability to survive in nature (Abedon, 1989; Stewart &Levin, 1984; and Wang *et al.*, 1996).

6- Effect of the temperature on phage PA1:

Phage PA1 was treated with six temperature 0, 5, 15, 30, 40 and 45°C, then its ability to produce plaque and transducion was assessed (Table 8). The phage was stable in forming plaques when treated with temperature 5, 15°C whereas the pfu/ml 6.8, 6.9 x 10^9 pfu/ml. The ability of phage declined dramatically in forming plaques upon treated with low or high temperature (1.8 at 0 °C and 0.18 at 45°C x 10^9 pfu/ml). The ability to transduce antibiotic resistance genes was also influenced. The higher frequency of transfer was observed at temperatures 5, 15°C. Temperature is one of the most obvious environmental factors that controls the interaction between phages and bacterial cells in the nature (Primorse *et al.*, 1982).

Table (8): Effect of temperature on phage PA1.

Temp. °C	Titration 10^9 pfu/ml	Transducing antibiotic resistance gene							
		Str		Tet		Amp		Chl	
		No. of transductants 10^4	Transduction frequency	No. of transductants 10^4	Transduction frequency	No. of transductants 10^4	Transduction frequency	No. of transductants 10^4	Transduction frequency
0	1.8±0.07	15.6±0.1	$5.7x10^{-5}$	8.2±0.9	$3.1x10^{-5}$	17.9±0.3	$6.6x10^{-5}$	7.9±1.1	$2.9x10^{-5}$
5	6.9±0.3	34.9±0.2	$1.3x10^{-4}$	21.6±0.9	$7.9x10^{-5}$	38.9±1	$1.4x10^{-4}$	17.8±1.9	$6.5x10^{-5}$
15	6.8±0.4	69.6±0.1	$2.6x10^{-4}$	35.6±0.8	$1.3x10^{-4}$	39.8±2	$1.5x10^{-4}$	27.3±0.9	$1.1x10^{-4}$
30	4.3±0.01	68.9±0.7	$2.5x10^{-4}$	35.1±0.7	$1.3x10^{-4}$	38.1±0.9	$1.4x10^{-4}$	7.9±1.2	$2.9x10^{-5}$
40	3.3±0.2	61.3±0.3	$3.2x10^{-4}$	33.8±0.9	$1.2x10^{-4}$	32.2±0.9	$1.2x10^{-4}$	5.1±1.1	$1.9x10^{-5}$
45	0.18±0.001	11.8±0.9	$2.3x10^{-5}$	9.3±0.9	$3.4x10^{-5}$	31.9±0.8	$1.1x10^{-4}$	1.7±0.9	$6.2x10^{-6}$

Recipient strain, ATC114 was 2.72 x 10^9 cfu/ml.

7- Influence of pH:

Phage PA1 was treated with five different pH values, 2, 3, 7, 10 and 12. No plaques or transductents have been observed at acid pH values, 2 and 3 (Table 9). When phage PA1 was treated with pH 7, resulting in high titration 2.5 x 10^9 pfu/ml and subsequently high number of transductents (9.6 up to 68 x 10^4 transductents/ml). The acidity or alkalinity as indicated by pH is a factor that profoundly affects all organisms. pH was able to induce c-mutants in phage ϕ 63 of *Bacillus* cells (Amin, 1999), and lambda phage of *E. coli* (Konevega, &Kalinin,

1985; and Povirk &Goldberg, 1986).

Table (9): Effect of pH on phage PA1.

	Transductants				
pH	Pfu/ml 10^9	Str 10^4	Tet 10^4	Amp 10^4	Chl 10^4
2	0.0	-	-	-	-
3	0.0	-	-	-	-
7	2.5	68.1 ± 2	19.7 ± 0.9	41.5 ± 2	9.6 ± 0.9
10	0.47	23.3± 1	10.7 ± 0.1	20.7 ± 3	5.1± 0.7
12	0.07	1.9 ± 0.7	2.7 ± 0.3	3.9 ± 0.3	2.3 ± 0.9

8- Induction of prophage PA1 form lysogenic strains by ultraviolet radiation:

The lysogen PA1 in it's lysogenic strains(PAO1, PU21, and MAM2) was exposed to different doses of UV radiation. Phage lysates were collected and checked their abilities in forming plaques and transducing different genetical markers. Low or high does of UV were not capable of increasing the induced prophage. Doses ranging from 4-16 min have induced the prophage PA1 from the 3 lysogenic strains with fold increasing between 1.7 up to 6.7 (Table 10). Phages induced by UV have been propagated on strain ATC43 and then used with ATC 114 as recipient (Table 11).

Phage induced by exposure of UV in doses ranged from 1-16 min was able to increase the transduction frequency of the four antibiotic resistance genes that have been used in this study. Higher exposure times (20-30 min) and lower exposure time (0.5min.) have no influence in gene transfer. The significant increases in transduction frequency may due to the influence of UV on DNA damage and subsequently increased the number of transducing particles resulting in significant increases in observed transduction frequency.

The phage can release from the lysogen either spontaneously **(Reyrolle *et al.*, 1982; Germida, 1984; and Amin &El Adaway, 1991)** or after treating the lysogen with an inducing agents such as mitomycin C, irradiation with UV and mutagenic and carcinogenic compounds **(Heinemann, 1971; and Terzaghi & Sandine, 1981).**

Table (10): Induction of prophage PA1 by UV radiation.

Time of exposure (min)	Titration					
	PAO1		PU21		MAM2	
	Pfu/ml 10^9	Fold increase	Pfu/ml 10^9	Fold increase	Pfu/ml 10^9	Fold increase
0	2.6 ± 0.1	-	1.5± 0.03	-	1.6 ± 0.01	-
0.5	0.61±0.01	-	1.3± 0.04	-	1.1 ± 0.02	-
1	1.4 ± 0.07	-	1.5 ± 0.3	-	1.7 ± 0.3	1.1
4	17.5 ± 0.9	6.7	8.2 ± 0.7	5.5	9.4 ± 0.4	5.9
8	11.4 ± 0.9	4.4	7.8 ± 0.8	5.2	4.3 ± 0.03	2.7
16	4.4±0.9	1.7	3.2 ± 0.4	2.1	3.6± 0.04	2.3
20	1.9± 0.05	-	0.92±0.01	-	1.5 ± 0.01	-
25	1.7±0.06	-	0.61±0.02	-	1.3±0.01	-
30	1.3 ±0.03	-	0.21±0.03	-	0.95±0.01	-

Each value is the mean of 3 replica ± SD.

Table (11): Transduction ability of phage PA1 induced by UV from lysogen PA1ϕ PAO1.

Time of exposure (min)	Antibiotic resistance gene											
	Str			Tet			Amp			Chl		
	No. of transductants 10^4	Transduction frequency	Fold increase	No. of transductants 10^4	Transduction frequency	Fold increase	No. of transductants 10^4	Transduction frequency	Fold increase	No. of transductants 10^4	Transduction frequency	Fold increase
0	2.2	8.8×10^{-6}	-	1.8	7.2×10^{-6}	-	8.1	3.2×10^{-5}	-	62.8	2.5×10^{-4}	-
0.5	1.6	6.4×10^{-6}	-	0.7	2.8×10^{-6}	-	7.3	2.9×10^{-5}	-	61.1	2.4×10^{-4}	-
1	4.6	1.8×10^{-5}	2.1	11.3	4.5×10^{-5}	6.3	15.4	6.2×10^{-5}	1.9	69.4	2.8×10^{-4}	1.1
4	10.8	4.3×10^{-5}	4.9	11.5	4.6×10^{-5}	6.4	10.5	4.2×10^{-5}	1.3	71.3	2.9×10^{-4}	1.2
8	2.9	1.2×10^{-5}	1.3	12.7	5.1×10^{-5}	7.1	21.3	8.5×10^{-5}	2.6	74.4	3.0×10^{-4}	1.2
16	2.3	9.2×10^{-6}	1.1	3.2	1.3×10^{-5}	1.8	14.5	5.8×10^{-5}	1.8	65.6	2.6×10^{-4}	1.1
20	1.9	7.6×10^{-6}	-	1.6	6.4×10^{-6}	-	2.9	1.2×10^{-5}	-	51.4	2.1×10^{-4}	-
25	1.2	4.8×10^{-6}	-	0.9	3.6×10^{-6}	-	2.6	1.1×10^{-5}	-	39.6	1.6×10^{-4}	-
30	1.1	4.4×10^{-6}	-	0.3	1.2×10^{-6}	-	2.1	8.4×10^{-6}	-	3.9	1.6×10^{-5}	-

Recipient cells of ATC 114 = 2.5×10^9 cfu/ml.

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عزل و توصيف سيدوموناس اريجنوزا بكتريوفاج من مياه الصرف الزراعي

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تهدف هذه الدراسة إلى عزل بكتريوفاجات متخصصة لبكتريا *Pseudomanas aeruginosa* من مياه الصرف الزراعي بمدينة الزقازيق – محافظة الشرقية ، وقد تم بالفعل عزل خمسة بكتريوفاجات, و تم إختيار واحد فقط منها والذي تم تسميته PA1 وذلك لدراسة بعض خصائص هذا الفاج, وكانت أول خاصية تم دراستها هي قدرة فاج PA1 على تكوين plaques على السلالة الأصلية PAo1 , وقد وجد أن هذا الفاج يكون نوعين من plaques وهى: عكرة بمعدل 2.25×10^9, ورائقة بمعدل 9.96×10^7. وبدراسة المدى العوائلي لهذا الفاج وجد أنه يستطيع أن يحلل 13سلالة من 20 سلالة لنفس البكتريا السابق ذكرها, وهذه النتيجة توضح أن فاج PA1 له مدى عوائلي فسيح. كذلك تم دراسة قدرة هذا الفاج على عمل lysogenization لبعض السلالات, وقد وجد أنه يستطيع أن يعمل ذلك لثلاث سلالات هي PAo1, PU21, MAM_2 وقد وجد أيضاً أن هذا الفاج يستطيع أن ينطلق من السلالات الليسوجينية بطريقة تلقائية دون إستخدام أي عامل خارجي.و أيضا تم دراسة قدرة فاج PA1 على نقل جينات المقاومة لأربع مضادات حيوية هى الأستريتومايسين, التتراسيكلين, الأمبسللين, الكلورامفينيكول من إحدى السلالات التي كانت مقاومة لهذه المضادات الحيوية إلى سلالة أخرى حساسة, وقد وجد أنه يستطيع نقل هذه الجينات بمعدلات متقاربه. بدراسة بعض العوامل التي يمكن أن تؤثر على الفاج نفسه والتي توجد في البيئة الطبيعية للبكتريوفاجات وهى الحرارة, pH, وجد أن الفاج يستطيع أن يكون plaques بمعدل عالى عند درجات حرارة 5, 15°م ولكن تقل مقدرته عند درجات حرارة صفر, 45°م, كما أن قدرة الفاج على نقل الجينات الأربعه قد تأثرت أيضاً. وعند دراسة تأثير pH وجد أنه عند pH الحامضي (2, 3) لم تتكون plaques , بينما عند pH 7 كان أعلى معدل لـ plaques وبالتالي زيادة عدد Transductants.

هذه الدراسة أوضحت أن البكتريوفاجات كعوامل ناقلة للمادة الوراثية تلعب دورا هاما في التأثير على البيئة التي توجد بها بما فيها من كائنات حية مثل البكتريا.