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MICROBIAL GENETICAL STUDIES OF *Pseudomonas aeruginosa* BACTERIA ISOLATED FROM HUMAN.

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ABSTRACT

Pseudomonas aeruginosa bacterial isolates which isolated from human, were used to study some microbial genetical properties. Nutritional requirements were tested, the results showed that all the 17 isolates were prototrophs. Lysogenicity test demonstrated that all 17 isolates were lysogenic with one prophage,since every phage released from a lysogenic isolate was not able to lysis the same host cells. Host range of phage released from a lysogenic isolates was varied, and reached up to 65% (ϕ 43). The phage released spontaneously from lysogens has been titered, phage (ϕ50) resulted in high titration (3.56 x 10^7 Pfu/ ml). The ability of phage released from lysogen to lysis of the standard strains (PA01, PU21, MAM2) was tested. Phage (ϕ50) had the ability to make lysis on the three strains.

Sensitivity of the bacterial isolates to some antibiotics (streptomycin, tetracycline, ampicillin, penicillin and chloramphenichol) was tested. Sensitivity was varied, depended on type, concentration of antibiotic and type of isolate. The effect of temperature on efficiency of plating for five phage lysates had been investigated. No plaques have been detected at low temperatures (5 and15°C) or at high temperature(45°C).Transducing ability of five phage lysates spontaneously released from lysogens was assessed. The ϕ37 and ϕ78 were able to transduce successfully the streptomycin, tetracycline, ampicillin and chloramphenicol resistance genes ($5.0x10^{-6}$,$1.73x10^{-7}$,$1.43x10^{-4}$,$8.8x10^{-3}$and$7.2x10^{-5}$,$4.6x10^{-5}$,$1.25x10^{-5}$,$6.2x10^{-3}$ respectively).

The phage induced by ultraviolet irradiation was used in transduction assay. The Pfu/ml was increased. Transduction by lysogenic bacterial isolates was assessed. The results showed that transduction frequency was lower than in transduction by lysate.

Keywords: Bacteria, bacteriophage, transduction, titration, antibiotics.

INTRODUCTION

Pseudomonas species are plant, animal, and human pathogens, exhibit plant pathogen-suppressing properties useful is biological control, or express metabolic versatilities valued in biotechnology and bioremediation (Widmer *et al.*, 1998). Specific detection of *pseudomonas* species in the environment could help us gain a more complete understanding of the ecological significance of these microorganisms.

The genus *pseudomonas*, was described as a genus of gram-negative, rod shaped microorganisms (Palleroni, 1984) and had been subject to repeated taxonomic revisions (Palleroni, 1993).

The genus *pseudomonas* includes species with functions of ecological, economic, and health-related importance. Some species are pathogenic for plants (Stead, 1992), while others are pathogenic for animals or human (Gilligan, 1991; Palleroni, 1992; Hobden, 2002). Some species exhibit plant

growth promoting and pathogen suppressing functions and may be exploited for use in biological control (Keel *et al.*, 1996), bioremediation (Palleroni, 1993) and biodegradation a variety of compounds (Guerin and Boyd, 1995; Grimberg *et al.*, 1996). Investigation of new *Pseudomonas aeruginosa* isolates would represent a valuable tool in ecological, genetical and diagnostic studies of this genus.

The objective of this study was to study some microbial genetical properties of some *Pseudomonas aeruginosa* bacteria isolated as human pathogens. The isolation was carried out in the Hospital of Zagazig University and fully microbiologically characterized in the Dept. of Microbiology Fac of Pharmacy, Zagazig University. This study was carried out in Microbial Lab., Genetics Dept., Agric.Fac.,Zagazig Univ.

MATERIALS AND METHODS

1- Bacteria and bacteriophages:

Pseudomonas aeruginosa strains (PA01, PU21 and MAM2) were obtained from M. Day, University of Wales, College of Cardiff, UK and the bacterial isolates of *Pseudomonas aeruginosa* that have been used in this study were obtained from M.EL-Hussieny, Faculty of Pharmacy, Zagazig University, (Table 1). The generalized F116 and AMSE 2000 bacteriophages have been used in this study.

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

Strains and isolates	Genotype	Reference
PA 01	Prototrophic, Str^s, Tet^s.	Holloway and Morgan (1986)
PU 21	Str^s, Tet^s, Val^-	Amin and Day (1988)
MAM2	Str^s, Tet^s, Met^-	Amin *et al.*, (1987)
ATC 1	-	Fac.pharmacy,Zagazig Univ.
ATC 11	-	Fac.pharmacy,Zagazig Univ.
ATC 17	-	Fac.pharmacy,Zagazig Univ.
ATC 37	-	Fac.pharmacy,Zagazig Univ.
ATC 43	-	Fac.pharmacy,Zagazig Univ.
ATC 45	-	Fac.pharmacy,Zagazig Univ.
ATC 50	-	Fac.pharmacy,Zagazig Univ.
ATC 58	-	Fac.pharmacy,Zagazig Univ.
ATC 68	-	Fac.pharmacy,Zagazig Univ.
ATC 70	-	Fac.pharmacy,Zagazig Univ.
ATC 76	-	Fac.pharmacy,Zagazig Univ.
ATC 77	-	Fac.pharmacy,Zagazig Univ.
ATC 78	-	Fac.pharmacy,Zagazig Univ.
ATC 87	-	Fac.pharmacy,Zagazig Univ.
ATC 111	-	Fac.pharmacy,Zagazig Univ.
ATC 113	-	Fac.pharmacy,Zagazig Univ.
ATC 114	-	Fac.pharmacy,Zagazig Univ.

Str^s = Streptomycin sensitive. **Val^- = Valine auxotroph.**
Tet^s = Tetracycline sensitive. **Met^- = Methionine auxotroph**
- = The genotype is unknown.

2- Growth media:

The nutrient agar (NA), nutrient broth (NB) and minimal media (MM) were used. Soft agar (0.8% w/ v agar) was prepared in distilled water and

kept at 45°C on waterbath. Phosphate buffer was prepared from 1/ 15 M potassium phosphate (KH_2PO_4) and 1/15 M disodium phosphate ($Na_2HPO_4.2H_2O$).The antibiotics (streptomycin, tetracycline, ampicillin, pencillin and chloramphenichol) were added as sterilized solutions by filtration through 0.2 µm filter membrane to the media after autoclaving.

3- Nutritional requirements:

This experiment was performed by streaking the bacterial isolates on complete and minimal media.

4- Lysogenesity test:

Each isolate cells were inoculated into 10 ml of NB medium, then placed on a shaker incubator overnight at 30°C. A few drops of chloroform were added and centrifugation was carried out at 10000 rpm for 15 min. the supernatant was removed and passed through a sterile membrane (0.2 µm). the supernatant was assayed by using the spot test method (Barrangou *et al.*, 2002).The plate inoculum consisted of 3 ml of soft agar mixed with 100µl of an overnight host culture. This mixture was briefly vortexed and spread onto the surface of an agar plate. Single drops of each supernatant were spotted onto inculated agar plates, and the plates were incupated overnight at 30 °C. Bacterial sensitivity of a bacteriophage was establish by bacterial lysis at the spot where the supernatant drop was deposited.

5- Phage titration:

The double-agar-layer method(Park *et al.*, 2000 and Sharma *et al.*,2002)was used. Serial hundred-fold dilutions of phage lysate were prepared in phosphate butter (PH 7.0). Equal volumes (0.1 ml) of phage lysate and host cells (grown overnight in NB at 30°C) were mixed in 3 ml of soft molten agar. The mixture was vortexed and poured immediately onto NA plate. Plates were incubated at 30°C for overnight. Plaques were counted and the number of plaque forming units (pfu/ ml) was calculated.

6- Isolation and purification bacteriophage from lysogen:

After titration, five phages (ϕ37, ϕ43, ϕ 50, ϕ78 and ϕ111) were isolated and purified by single-plaque isolation. Well-isolated plaques were cut from agar plates, placed in sterile diluent and used to produce new phage lysates. This procedure was repeated through three cycles or until produced only a single-plaque morphology(Jensen *et al.*, 1998 and Chakrabarti *et al.*, 2000).

7- Phage host range:

Many strains and isolates of *Pseudomonas aeruginosa* have been used to study host range of phages released spontaneously from lysogenic isolates. The phage lysates were spotted onto layer of bacterial host .

8- The efficiency of plating(EOP):

This experiment was performed by titration of five phage isolates (ϕ37, ϕ43, ϕ 50, ϕ78 and ϕ111) on NA plates and incubated at different temperatures (5, 15, 19, 25, 30, 35, 40 and 45). The number of plaques were recorded and the pfu/ml was calculated.

9- Sensitivity to antibiotics:

The five antibiotics that have been used in this experiment were streptomycin, Ampicillin, tetracycline, penicillin and chloramphenichol. Different concentrations of each antibiotic (100, 250, 400, 500, 1000, 1200

and 1500 µg/ ml) have been added to NA plates. All 17 isolates were streaked onto the plates which contain antibiotic and plates without antibiotic and incubated at 30°C for 2- 3 days.

10- Transduction by lysate of released phages spontaneously from lysogenic isolates:

The lysogenic isolates (ATC37, ATC 43, ATC 50, ATC 78 and ATC 111) were inoculated into 10 ml of NB and incubated at 30°C for overnight. The cultures were centrifuged at 10000 rpm for 15 min and filtered through 0.2 µm filter membrane. The phage lysates were titered and equal volumes (1.0 ml) of phage lysate and recipient cells were mixed. The mixture was kept for 20 to 30 min at room temperature to allow phage adsorption (Toth *et al.*, 2003). Serial dilutions have been prepared and placed onto selective media, The plates were incubated at 30°C. Number of transductants were recorded and transduction frequency was calculated. In other experiment, the plates were incubated at different temperature (5, 15, 19, 25, 30, 35, 40 and 45°C).

11- Transduction by induced phage by ultraviolet irradiation:

10 ml of overnight cultures were placed on petri dish. The plates were exposed to ultraviolet lamb for 25 min, centrifuged, filtered and titered. The induced phages were used to transduce different markers.

12- Transduction by lysogenic isolates:

The donor and recipient cells were grown independently in NB overnight at 30°C. One ml of donor and recipient was layered onto separate nitrocellulose filter membrane (0.2 µm). The two membranes were placed face-to-face on NA plate and incubated at 30°C for 24h. After incubation time, the membrane filters were vortexed for 60 sec in 10 ml phosphate buffer (pH 7.0). Serial dilutions have been prepared and placed onto selective media to count the donor, recipient and transductants. The phage particles was also calculated.

RESULTS AND DISCUSSION

1- Nutritional requirements:

Data in Table 2 show that all the pathogenic bacterial isolates of *P. aeruginosa* that have been isolated from human are prototrophs. Since all the 17 isolates were able to grow on complete or minimal media.

2- Lysogenicity ability:

Data is Table 3 represent the lysogenicity ability of the 17 isolates of *P. aeruginosa*, it seems that all the isolates were lysosenic with one prophage. That is because every phage released spontaneously from the same lysogenic was not able to lysis the same host cells because of lysogenic immunity.

3- Host range of phage released form lysogens:

The host range of phage released from the lysogens was varied (Table 4). Phage lysate (ϕ43) was able to induce lysis in 11 isolates of 17 with high percentage of host range reached up to 65% of tested isolates. In addition, the same host isolate (ATC 43) had been infected by 13 phage lysates of 17 lysates (77% of the tested phage lysate). However, phages, ϕ 76 and ϕ 83 have a narrow host range, since they were able to induce lysis in three host

only (With a percentage of 18% of isolates tested). The phage released form lysogens were selectively lytic against some bacterial isolates but were not observed to lyse others, this observation is common in phage isolated from environments. A temperate phage isolated from sewage does form plaque on strain H of *E. coli*, but was not able to form plaque on neither strain C and K12 of *E. coil*, nor on strain L12 of *Salmonella spp* (Dhillon *et al.*, 1998).

Table 2: Nutritional requirements of *P. aeruginosa* bacteria isolated from human.

Media / Strain	CM	MM
ATC 1	+	+
ATC 11	+	+
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	+	+

+ =Growth.

4- Titration of released phage:

The ability of phage released spontaneously from lysogens has been assessed using one proper host bacterial cells (Table 5). Phage (ϕ50) results in high titration ($3.56x10^7$ pfu/ml) upon using with isolate ATC 68. These results clearly show that the occurrence of naturally lysogenic bacteria among *Pseudomonas aeruginosa* isolates from human among the tested ones reached up to 100%.

5-Lysis of standard strains of *Pseudomonas aeruginosa* by phage released from isolated lysogens.

All the phage lysates that released from the pathogenic isolates have been allowed to induce lysis with three standard strains of *P. aeruginosa* (Table 6) phage (ϕ50) had the ability to make lysis in the three standard strains, PA01, PU21, and MAM2.

6- Lysis of isolated *P. aeruginosa* bacterial cells by standard phage particles:

Data in Table 7 illustrate the ability of two standard bacteriophages of *P. aeruginosa,* F116 and AMSE2000 to form lysis in the human *P. aeruginosa* isolates. Bacteriophages F116 and AMSE2000 were not able to lysis the lysogenic isolates ATC: 1, 45, 70, 78, 87, and 114. This means that the phages that are lysogenized these isolates may be belong to the same group of the standard phages, F116 and AMSE2000 because of the lysogenic immunity property.

Table 3 : Lysogenesity test of *P. aeruginosa* bacterial cells isolated from human.

Host \ Phage	ϕ1	ϕ11	ϕ17	ϕ37	ϕ43	ϕ45	ϕ50	ϕ58	ϕ68	ϕ70	ϕ76	ϕ77	ϕ78	ϕ87	ϕ111	ϕ113	ϕ114
ATC 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATC 11	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
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	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+	+	-

+ = Lysis. - = Not lysis.

Table 4: Host range of released phage spontaneously from lysogen.

Host \ Phage	ϕ1	ϕ11	ϕ17	ϕ37	ϕ43	ϕ45	ϕ50	ϕ58	ϕ68	ϕ70	ϕ76	ϕ77	ϕ78	ϕ87	ϕ111	ϕ113	ϕ114
ATC	37	43	43	58	11	43	43	17	43	43	43	17	43	37	17	43	11
ATC	43	58	50	76	37	58	45	43	45	50	78	58	58	43	43	68	43
ATC	68	76	58	77	45	70	58	45	87	58	87	68	70	113	45	78	45
ATC	113	113	70	78	58	76	68	50	113	68		70	76		58	87	58
ATC			87	87	70	77	77	76		76		76	77		70	114	70
ATC			113	113	76	78	78	77		77		78	87		78		76
ATC				114	77	87	113	113		78		113	113		87		77
ATC					78	113				87		114			113		78
ATC					87	114				113					114		87
ATC					111												113
ATC					114												
No. lysed host	4	4	6	7	11	9	7	7	4	9	3	8	7	3	9	5	10
% of host range	24	24	35	41	65	53	41	41	24	53	18	47	41	18	53	29	59

Table 5: Titration of phage released with proper host cells.

phage	Host	Pfu/ ml
ϕ 1	ATC 68	2.32×10^5
ϕ11	ATC 43	4.0×10^3
ϕ 17	ATC 43	8.0×10^3
ϕ 37	ATC 87	9.2×10^4
ϕ 43	ATC 58	7.0×10^3
ϕ 45	ATC 58	3.4×10^5
ϕ 50	ATC 68	3.56×10^7
ϕ 58	ATC 45	2.7×10^4
ϕ 68	ATC 70	5.5×10^4
ϕ 70	ATC 78	8.0×10^3
ϕ 76	ATC 11	1.42×10^3
ϕ 77	ATC 37	6.0×10^3
ϕ 78	ATC 77	1.76×10^5
ϕ 87	ATC 76	8.5×10^4
ϕ 111	ATC 43	1.3×10^4
ϕ 113	ATC 1	9.6×10^4
ϕ 114	ATC 113	1.73×10^5

However, phages F116 and AMSE2000 were able to lysis isolates, ATC: 11, 17, 37, 43, 50, 58, 68, 76, 77, 111 and 113 (with about 65% of isolates). So, these released phage particles may be do not belong to the same group of phages F116, and AMSE2000. These results need further molecular analysis to find the genetic relationship between the isolated phages and the two standard.

Table 6: Lysis of standard strains.

Host / Phage	PA 01	PU 21	MAM2
ϕ 1	-	+	-
ϕ11	-	-	-
ϕ 17	-	-	-
ϕ 37	-	-	-
ϕ 43	-	+	+
ϕ 45	-	-	-
ϕ 50	+	+	+
ϕ 58	-	-	-
ϕ 68	-	-	-
ϕ 70	-	-	-
ϕ 76	-	-	-
ϕ 77	-	-	-
ϕ 78	+	-	-
ϕ 87	-	-	-
ϕ 111	-	+	+
ϕ 113	+	-	-
ϕ 114	-	-	-

+ = Lysis . - = Not lysis.

7- Sensitivity to antibiotics:

Data in Table 8 show the sensitivity of the bacterial isolates to five different antibiotics. The same concentrations have been used with each antibiotic.

Table 7: Lysis of standard phages.

Host \ Phage	F 116	AMSE 2000
ATC 1	-	-
ATC 11	+	+
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	-	-

+ = Lysis . - = Not lysis.

Table 8: Sensitivity to antibiotics.

Antibiotic / Strain	Str							Tet							Amp							Pen							Chl						
	100	250	400	500	1000	1200	1500	100	250	400	500	1000	1200	1500	100	250	400	500	1000	1200	1500	100	250	400	500	1000	1200	1500	100	250	400	500	1000	1200	1500
ATC 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
ATC 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-

+ = Resistance . - = Sensitive .

8- Effect of temperature on the efficiency of plating:

The influence of temperature on the efficiency of five phage lysates plating with bacterial host has been investigated (Table 9). No plaques have been detected at low temperatures, 5 and 15°C or at high temperature, 45°C. The optimum temperature of plating phages ϕ 43, 50, 78, and 111 was 35 °C (pfu/ml ranged from 0.01 up to 3.11×10^{10}).

Table 9: Effect of temperature on efficiency of plating.

Phage	Pfu/ ml + Temperature (°C)							
	5	15	19	25	30	35	40	45
ϕ37	-	-	3.0×10^{7}	7.0×10^{7}	2.9×10^{8}	1.6×10^{8}	1.4×10^{8}	-
ϕ43	-	-	3.72×10^{9}	6.0×10^{7}	1.78×10^{10}	3.11×10^{10}	2.53×10^{10}	-
ϕ50	-	-	2.0×10^{7}	3.0×10^{5}	7.0×10^{7}	0.01×10^{10}	1.2×10^{8}	-
ϕ78	-	-	7.1×10^{7}	8.2×10^{8}	1.56×10^{10}	2.36×10^{10}	1.82×10^{10}	-
ϕ111	-	-	3.1×10^{8}	4.0×10^{7}	4.2×10^{9}	0.73×10^{10}	5.68×10^{9}	-

9- Effect of temperature on transduction:

No transductants have been formed on temperatures, 5, 15 and 45 °C. The optimum temperature for transduction ranged from 30-35°C. This may depend on the phage lysate and transduced marker (Table 10).

Table 10: Effect of temperature on transduction mechanism.

Phage	Recipient cfu/ ml (10^8)	Marker	(5°C) No. transductants	(15°C) No. transductants	(19°C) No. transductants	(25°C) No. transductants	(30°C) No. transductants	(35°C) No. transductants	(40°C) No. transductants	(45°C) No. transductants
ϕ 37	ATC 77 = 1.46	Str	-	-	2.0×10^{1}	8.0×10^{1}	2.7×10^{2}	1.2×10^{2}	1.1×10^{2}	-
		Tet	-	-	-	-	-	-	-	-
		Amp	-	-	2.4×10^{1}	2.4×10^{4}	2.23×10^{5}	8.5×10^{4}	6.7×10^{4}	-
	ATC 114 = 2.2	Chl	-	-	1.3×10^{5}	1.3×10^{5}	9.1×10^{5}	6.8×10^{7}	3.8×10^{5}	-
ϕ 43	ATC 114	Str	-	-	-	-	3.0×10^{1}	-	-	-
	= 2.2	Tet	-	-	-	-	-	-	-	-
		Amp	-	-	1.37×10^{3}	1.42×10^{3}	1.43×10^{3}	6.2×10^{2}	4.3×10^{2}	-
		Chl	-	-	2.47×10^{2}	4.5×10^{4}	1.78×10^{7}	8.3×10^{5}	6.1×10^{5}	-
ϕ 50	ATC 77	Str	-	-	1.1×10^{2}	1.2×10^{2}	1.5×10^{2}	7.0×10^{1}	5.0×10^{1}	-
	= 1.46	Tet	-	-	-	-	-	-	-	-
		Amp	-	-	1.7×10^{3}	4.8×10^{3}	7.3×10^{4}	6.1×10^{4}	5.3×10^{4}	-
ϕ 78	ATC 114	Str	-	-	1.0×10^{1}	1×10^{1}	5×10^{1}	2×10^{1}	1.0×10^{1}	-
	= 2.2	Tet	-	-	-	-	-	-	-	-
		Amp	-	-	2.72×10^{3}	1.19×10^{4}	2.5×10^{4}	1.7×10^{3}	1.49×10^{3}	-
		Chl	-	-	3.5×10^{5}	2.1×10^{5}	6.8×10^{7}	8.9×10^{5}	7.2×10^{5}	-
ϕ 111	ATC 114	Str	-	-	5.7×10^{2}	2.97×10^{3}	1.9×10^{4}	7.2×10^{3}	5.0×10^{3}	-
	= 1.46	Tet	-	-	-	-	-	-	-	-
		Amp	-	-	2.12×10^{3}	3.1×10^{3}	1.8×10^{4}	1.37×10^{3}	5.1×10^{2}	-
		Chl	-	-	2.16×10^{2}	3.1×10^{4}	7.9×10^{5}	8.0×10^{5}	6.9×10^{5}	-

10- Transduction by lysate of phages released from lysogenic isolates:

The phages released spontaneously from lysogenic isolates ATC37, ATC43, ATC50, ATC78 and ATC111 were used. The ϕ37 was able to transduce successfully the streptomycin, tetracycline, ampcillin and chloramphenicol resistance genes to strains ATC76, ATC77, ATC87 and

ATC114 respectively. The transduction frequency was 5.0×10^{-6}, 1.73×10^{-7}, 1.43×10^{-4} and 8.8×10^{-3}. The ϕ78 also was able to transduce the same markers (7.2×10^{-5}, 4.6×10^{-5}, 1.25×10^{-5} and 6.2×10^{-3}). The two phage isolate ϕ50 and ϕ111 were not able to transduce tetracycline resistance gene. Data in Table 11 shows that the five phage isolates were able to transduce more than one marker at range from 1.73×10^{-7} to 2.3×10^{-3}, so it may be generalized transducing phages.

Table 11: Transduction by spontaneously released phages from lysogenic isolates.

Phage Pfu/ml (10^7)	Recipient Cfu/ ml (10^9)	Marker	No. transductants	Transduction frequency
ϕ 37	ATC 76 = 2.72	Str	1.38×10^4	5.0×10^{-6}
	ATC 77 = 9.48	Tet	1.64×10^3	1.73×10^{-7}
7.28	ATC 87 = 1.34	Amp	1.92×10^5	1.43×10^{-4}
	ATC 114 = 6.9	Chl	6.1×10^7	8.8×10^{-3}
ϕ 43	ATC 78 = 1.6	Amp	5.3×10^3	3.31×10^{-6}
2.79	ATC 58 = 1.78	Chl	3.9×10^2	2.2×10^{-7}
ϕ 50	ATC 43 = 1.48	Str	7.88×10^5	5.32×10^{-4}
3.56	ATC 77 =9.26	Tet	-	
		Chl	5.2×10^3	5.6×10^{-7}
ϕ 78	ATC 43 = 1.48	Tet	1.08×10^5	7.2×10^{-5}
	ATC 77 = 9.48	Str	4.38×10^5	4.6×10^{-5}
2.96	ATC 70 = 3.16	Amp	3.96×10^4	1.25×10^{-5}
	ATC 114 = 6.9	Chl	4.3×10^7	6.2×10^{-3}
ϕ 111	ATC 114 = 6.9	Str	3×10^3	4.35×10^{-7}
		Tet	-	-
3.62		Amp	1.56×10^7	2.3×10^{-3}
	ATC 58 = 1.78	Chl	1.71×10^4	9.6×10^{-6}
	ATC 78 = 1.6	Amp	4.6×10^3	2.88×10^{-6}

11-Transduction by ultraviolet irradiation induced phages:

The results of this study showed that the number of induced phage upon exposure to ultraviolet irradiation was increased (8.12×10^7, 3.11×10^7, 3.78×10^7, 3.7×10^9 and 3.96×10^7 for phages ϕ37, ϕ43, ϕ50, ϕ78 and ϕ111 respectively) (Table 12) comparing with phages spontaneously released (7.28×10^7, 2.79×10^7, 3.56×10^7, 2.96×10^7 and 3.62×10^7).Taeok *et al.*, (2006) found that phages that released spontaneously from *Staphylococcus aureus* , were artificially increased by adding mitomycin C. Prophage induction can be provoked by factors stimulating the SOS response. One could imagine that conditions in bioreactors stimulate the SOS response. The SOS induced Rec A protein triggers self – cleavage of the repressor, causing prophage induction (Czyz *et al.* 2001).

12- Transduction by lysogenic bacterial isolates:

The same previous lysogenic isolates were used as donor to compare transduction frequency by lysogen and by lysate. Table 13 shows that no any isolate was able to transduce tetracycline resistance gene. Also,

transduction frequencies were lower upon using phage lysate. These results come agree with the results of Ashelford *et al.*, (1999).

Table 12: Transduction by induced phages by ultraviolet irradiation.

Phage Pfu/ml (10^7)	Recipient Cfu/ ml (10^9)	Marker	No. transductants	Transduction frequency
ϕ 37	ATC 76 = 2.72	Str	2.7×10^3	9.9×10^{-7}
	ATC 77 = 9.48	Tet	-	-
8.12	ATC 87 = 1.34	Amp	5.1×10^3	3.8×10^{-6}
	ATC 114 = 6.9	Chl	4.3×10^4	6.2×10^{-6}
ϕ 43	ATC 78 = 1.6	Amp	1.5×10^3	9.4×10^{-7}
3.11	ATC 58 = 1.78	Chl	6.7×10^2	3.76×10^{-7}
ϕ 50	ATC 43 = 1.48	Str	3.0×10^1	2.02×10^{-8}
3.78	ATC 77 = 9.26	Str	-	-
		Tel	-	-
		Chl	3.6×10^2	3.88×10^{-8}
ϕ 78	ATC 43 = 1.48	Tet	-	-
	ATC 77 = 9.48	Str	2.0×10^2	2.1×10^{-8}
37.0	ATC 70 = 3.16	Amp	1.6×10^2	5.06×10^{-8}
	ATC 114 = 6.9	Chl	5.1×10^4	7.4×10^{-6}
ϕ 111	ATC 114 = 6.9	Str	-	-
		Tet	-	-
		Amp	5.6×10^2	8.1×10^{-8}
3.96	ATC 58 = 1.78	Chl	1.8×10^4	1.01×10^{-5}
	ATC 78 = 1.6	Amp	1.1×10^2	6.9×10^{-8}

Table 13: Transduction by lysogenic isolates.

Donor at 24h. (10^5)	Recipient at 24h. (10^5)	Phage at 24h.	Recipient at zero time. (10^9)	Marker	No. transductants	Transduction frequency.
ATC 37= 2.37	ATC 76 = 3.11	8.7×10^4	2.72	Str	1.0×10^1	3.7×10^{-9}
ATC 37= 0.96	ATC 77 = 2.56	1.13×10^5	9.48	Tet	-	-
ATC 37= 1.19	ATC 87 = 1.99	1.7×10^6	1.34	Amp	1.2×10^2	8.96×10^{-8}
ATC 37= 2.21	ATC114 = 2.6	1.46×10^5	6.9	chl	1.7×10^3	2.5×10^{-7}
ATC 43= 2.43	ATC 78 = 3.17	5.2×10^6	1.6	Amp	-	-
ATC 43 = 0.24	ATC 58 = 6.99	6.5×10^6	1.78	Chl	-	-
ATC50 = 9.86	ATC 43 = 1.1	2.2×10^6	1.48	Str	3.0×10^1	2.03×10^{-8}
ATC 50 = 3.56	ATC 77 = 2.13	4.6×10^6	9.26	Str	-	-
				Tet	-	-
				Chl	5.0×10^1	5.4×10^{-9}
ATC 78 = 3.46	ATC 43 = 9.7	8.4×10^6	1.48	Tet	-	-
ATC 78 = 9.65	ATC 77 = 1.06	2.5×10^4	9.48	Str	-	-
ATC 78 = 4.25	ATC 70 = 1.24	6.6×10^4	3.16	Amp	1.5×10^2	4.7×10^{-8}
ATC 78 = 3.71	ATC 114= 3.28	8.9×10^4	6.9	Chl	2.6×10^2	3.8×10^{-9}
ATC111= 3.98	ATC 114= 2.46	1.22×10^5	6.9	Str	1.0×10^1	1.44×10^{-9}
				Tet	-	-
				Amp	2.2×10^3	3.2×10^{-8}
ATC 111= 4.59	ATC 58 = 6.68	1.48×10^7	1.78	Chl	2.6×10^3	1.46×10^{-8}
ATC 111= 3.98	ATC 78 = 7.39	1.33×10^7	1.6	Amp	3.2×10^3	2.0×10^{-8}

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دراسات وراثية ميكروبية لبكتريا *Pseudomonas aeruginosa* معزولة من الإنسان

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استخدمت بكتريا *Pseudomonas aeruginosa* التي عزلت من الإنسان لدراسة بعض الخصائص الوراثية الميكروبية.

تم دراسة المتطلبات الغذائية لهذه العزلات وقد أوضحت النتائج أن كل ١٧ عزلة كانت Prototrophs. اختبار lysogenicity test اظهر أن كل ١٧ عزلة كانت lysogens، كل عزلة كانت lysogen بفاج واحد حيث أن الفاج المنطلق من كل عزلة لم يستطيع تحليل نفس العزلة بسبب مناعة الليسوجين. أظهرت النتائج أيضا أن المدى العوائلي للفاجات المنطلقة من العزلات الليسوجينية كان متباين وقد وصلت النسبة المئوية للمدى العوائلي إلى ٦٥% كما في ϕ43، الفاجات المنطلقة تلقائيا من العزلات الليسوجينية تم معايرتها، وكان أعلى Pfu/ml لفاج ϕ50 حيث وصلت إلى 3.56×10^7. قدرة هذه الفاجات على تحليل سلالات قياسية من بكتريا *P. aeruginosa* (PA01, PU21, MAM2) تم اختبارها، فاج ϕ50 استطاع أن يحلل الثلاث سلالات.

تم اختبار حساسية العزلات البكتيرية لبعض المضادات الحيوية (استربتومايسين، تتراسيكلين، أمبسللين، بنسلين، كلورامفينيكول)، وكانت الحساسة متباينة اعتمادا على نوع وتركيز المضاد الحيوي وكذلك على نوع العزلة.

تم اختيار ٥ بكتريوفاجات من الفاجات المنطلقة من العزلات الليسوجينية لدراسة تأثير الحرارة على efficiency of plating، لم يلاحظ تكوين Plaques على درجات الحرارة المنخفضة (٥، ١٥°م) أو على الدرجة المرتفعة (٤٥°م).

تم دراسة قدرة هذه الخمس فاجات على نقل بعض جينات المقاومة للمضادات الحيوية.

الفاجات ϕ37، ϕ78 كانت لها القدرة على نقل جينات المقاومة لـ (الاستربتومايسين، التتراسيكلين، الأمبسيلين، الكلورامفينيكول) بنجاح.

تم استحثاث الخمس فاجات عن طريق المعاملة بالأشعة الفوق بنفسجية، لوحظ أن عدد Pfu/ ml قد ازداد بالمقارنة بتلك المنطلقة تلقائيا .

استخدمت أيضا خمس عزلات ليسوجينية لدراسة Transduction by lysogens وقد أوضحت النتائج أن Transduction frequency في هذه الحالة أقل منها في حالة Transduction by lysates.