Horizontal Gene Transfer Events among Different Species of Bacteria

Amina, A. Hassan and M.K. Amin*

Department of Genetics, Fac. Agric. Zagazig Univ. Egypt.

*osammour@hotmail.Com

Abstract: Horizontal gene transfer may play a key role in the evolution of bacterial populations and the adaptation of microbial communities to environment. The genetic exchange of antibiotic resistant determinants between organisms of the same or different species is believed to play a role in resistance among bacteria with a variety of antibiotic. In this study an assessment of a horizontal gene transfer among different species of bacteria by two mechanisms of gene transfer, conjugation and transduction has been investigated. The ability of Pseudomonas aeruginosa bacteriophages to propagate on different bacterial genera has also been addressed. The results of this study showed that the three P. aeruginosa bacteriophages, F116, AMSE2000 and Ø111 propagated successfully on different species of Staphylococcus and Rhizobium beside their original host (P. aeruginosa). The plaque forming units (PFU/ml) of phage F116 on P. aeruginosa strain PAO1 was 2×109 and ranged from 5.4×107 up to 6.9×108 on tested strains. Moreover horizontal gene transfer by conjugation can occur either as inter or intra species gene transfer, where it was occurred among different strains of P. aeruginosa using chloramphenecole (chlr) and ampcillin (ampr) resistance genes. Conjugation frequencies ranged from 1.02×10-3 to 8.8×10-4 for chlr and 9.9×10-4 to 8.9×10-4 for ampr. Furthermore conjugation was occurred between Staphylococcus and Pseudomonanas bacterial species. The three P. aeruginosa bacteriophages that propagated on different genera have been tested to asses their ability in horizontal gene transfer by transduction into different strains of the same species. Transduction frequency ranged from 3.2×10-5 to 2.6×10-3. [Journal of American Science 2010; 6(9):534-544]. (ISSN: 1545-1003).

Key words: Horizontal gene transfer, conjugation, transduction, antibiotic resistance, Pseudomonas, Staphylococcus, Rhizobium, bacteriophage, lysogen, induction.

1. Introduction

Horizontal gene transfer the transmission of DNA fragments between different organismal lineages has studied for decades in laboratory strains of bacteria (Dunn and Gunsalus, 1973; Kapur *et al*., 1992, Kinkle *et al*., 1993). This genetic exchange plays a significant role in the evolution and ecological impact of microorganisms (Amabile and Chicurel, 1992). Studies of horizontal gene transfer have utilized laboratory based or other model systems have carried out to demonstrate that the potential for genetic exchange can exist in nature. Thus, with defined donor and/or recipient microorganisms, exchange can be observed in all environments (Herrick *et al*., 1997). Such studies often infer horizontal transfer by showing that a highly conserved gene is shared by a group of taxonomically diverse hosts. However, that multiple bacterial species coexisting together in different ecosystems might interact, but evidence for interspecies gene transfer still is lacking (Christine *et al*., 2008). In bacteria, gene transfer takes place by transformation, transduction or conjugation. The capacity of microbes to exchange genes through horizontal gene transfer is an important phenomenon with implications for ecology, evolution biotechnology and medicine (Chen *et al*., 2005, Zaneveld *et al*., 2008). Novel sequences may arise in genomes through sequence evolution of adaptive alleles or by the divergence of gene duplication or by the acquisition of alien sequences. Each of these may generate phylogenetic effect (Kurland *et al*., 2003, linz *et al*., 2007). Thousands of coding sequences found in five taxa of photosynthetic bacteria (Meyer and Kuever , 2007) were reduced to a set of 188 lineages that could not be resolved into a single three. So, the aim of this study is to assess the possibility of horizontal gene transfer among different species of bacteria. Two mechanisms of gene transfer have been employed in this study, conjugation and transduction.

2. Materials and Methods:

This study was carried out at Microbial Genetic Laboratory, Faculty of Agricultural, Zagazig University.

1. Bacteriophge and bacterial strains:

The generalized transducing F116 bacteriophage and the original bacterial strains of *Pseudomonas aeruginosa* PAO1, PU21 and MAM2 were obtained from M. Day, university of wales, Cardiff, UK. The *Staphylococcus* isolates were obtained from Oliya H. Attia, Genetics Deprtment Faculty of Agriculture Zagazig University *Rhizobium leguminosarum bv. vicieae* obtained from Howaid M.L. Abd El-Basit, Agriculture Microbiology Department Faculty of Agriculture Zagazig University. The bacteriophages AMSE2000 and Ø111 were isolated in past studies (Amin *et al*., 2004 and Amina *et al*., 2007). The strains that were used listed in Table (1).

2. Growth media:

The nutrient agar (NA), nutrient broth (NB), yeast extract mannitol (YEM) agar and YEM broth media were used. Soft agar (0.8% w/v agar) was prepared in distilled water and kept at 45°C on water bath.

Phosphate buffer was prepared from 1/15 M potassium phosphate (KH_2 PO_4) and 1/15M disodium phosphate (Na_2 HPO_4. $2H_2O$). Streptomycin (10 mg/ml), chloramphenechole (1mg/ml), penicillin (50 µg/ml) and ampcillin (2mg/ml) were added as sterilized solutions by filtration through 0.2 µm filter membrane to the media after autoclaving.

3. Spot test:

Three ml of soft molten agar was mixed with 100µl of an overnight culture of bacterial host, vortexed and spread onto the surface of NA plate. Single drops of phage lysate were spotted onto the inoculated NA plates (Rodolphe *et al*., 2002) and the plates were incubated at 30°C (with *Pseudomonas*), 37°C with *Staphylococcus* and 28°C with *Rhizobium* for overnight.

4. Phage titration:

The double agar layer method (Park *et al*., 2000; Sharma *et al*., 2002 and Payan *et al*., 2005) was used. Serial hundred dilutions of phage 1ysate were prepared in phosphate buffer (PH 7.0). Equal volumes (0.1 ml) of phage 1ysate and host cells (grown overnight in liquid medium) were mixed in 3 ml of soft molten agar. The mixture was vortexed and poured immediately onto complete medium (CM) plate. Plates were incubated at optimal temperature for each genus for overnight. Plaques were counted and the number of plaque forming units (pfu/ml) was calculated.

5. Host range of *Pseudomonas aeruginosa* bacteriophages, F116, AMSE 2000 and Ø111:

Host range was carried out by using the spot test method (Barrangou, *et al*., 2002). Each individual phage lysate was spotted onto a layer of bacterial host cells from different genera (*Pseudomonas*, *Staphylococcus* and *Rhizobium*).

6. Ability of *Pseudomonas aeruginosa* bacteriophages to propagate in liquid medium:

The bacterial hosts were grown in liquid media for overnight. Defined volume of phage lysate (0.5ml) was added to 5ml of host. The cultures were incubated overnight. Serial dilutions were prepared and counts of bacterial cells were recorded.

7. Lysogen preparation:

A sterile needle was inserted into the center of turbid plaque and streaked across the surface of a NA plate in order to get single colonies. The plates were incubated. The single colonies have been tested for phage production spontaneously using spot test and phage titration assays.

8. Stability of lysogenes:

The lysogenic isolates were stored at 4◦C. The phage production was tested after 2, 4, 8 and 16 weeks using the phage titration and spot test assays.

9. Gene transfer by conjugation:

All donor and recipient isolates were inoculated in liquid media and incubated for 24 h. Equal volumes (1 ml) of donor and recipient were added on surface of complete media plates and incubated for 24 h. the growth washed by 10 ml phosphate buffer and removed by spreader to sterile flasks. Serial dilutions were prepared, 0.1 ml spread on selective media for donor, recipient and trans-conjugant's (Amin, *et al*., 2008).

10. Gene transfer by transduction:

10.1. Transduction by lysate:

Recipient cells were grown in NB overnight, equal volumes (1 ml) of phage lysate and recipient cell suspension were mixed. The mixture was kept for 15-30 min at room temperature, to allow phage adsorption. Serial dilutions were prepared and placed onto selective media. Number of transductants and donor were recorded and the frequency of transduction per recipient was calculated.

Table (1): The Strains used in this study.

Strains	Genotype	References
Pseudomonas aeruginosa		
PAO 1	Prototrophic, str^s	Holloway and Morgan (1986)
PAO 1	amp^r	This study
PU 21	amp^r	This study
PU 21	chl^r	This study
MAM 2	chl^r	This study
MAM 2	str^r	This study
LF 62 (PAO1)	amp^r, lysogen (F116)	This study
LF 64 (PAO1)	amp^r, lysogen (F116)	This study
LF 65 (PAO1)	amp^r, lysogen (F116)	This study
LF 66 (PAO1)	amp^r, lysogen (F116)	This study
LF 68 (PAO1)	amp^r, lysogen (F116)	This study
LA 74 (PAO1)	amp^r, lysogen (AmSE2000)	This study
LA 76 (PAO1)	amp^r, lysogen (AmSE2000)	This study
LA 77 (PAO1)	amp^r, lysogen (AmSE2000)	This study
LA 79 (PAO1)	amp^r, lysogen (AmSE2000)	This study
LA 80 (PAO1)	amp^r, lysogen (AmSE2000)	This study
LØ 83 (PAO1)	amp^r, lysogen (Ø111)	This study
LØ 85 (PAO1)	amp^r, lysogen (Ø111)	This study
LØ 86 (PAO1)	amp^r, lysogen (Ø111)	This study
LØ 87 (PAO1)	amp^r, lysogen (Ø111)	This study
LØ 89 (PAO1)	amp^r, lysogen (Ø111)	This study
Staphylococcus		
S. aureus 2	chl^r	This study
S. aureus 4	pen^r	This study
S. aureus 5	str^r	This study
S. saprophyticus	str^r	This study
S. epidermids	str^r	This study
S. cohnii	str^r	This study
S. sp	str^r	This study
LF 1 (*S. aureus 2)*	Chl^r, lysogen (F116)	This study
LF 3(*S. aureus 2*)	Chl^r, lysogen (F116)	This study
LF 5 (*S. aureus 2)*	Chl^r, lysogen (F116)	This study
LF 7 (*S. aureus 2)*	Chl^r, lysogen (F116)	This study
LF 9 (*S. aureus 2*)	Chl^r, lysogen (F116)	This study
LA 11(*S. aureus 2*)	Chl^r, lysogen (AMSE2000)	This study
LA 13 (*S. aureus 2*)	Chl^r, lysogen (AMSE2000)	This study
LA 15(*S. aureus 2*)	Chl^r, lysogen (AMSE2000)	This study
LA 17(*S. aureus 2*)	Chl^r, lysogen (AMSE2000)	This study
LA 20(*S. aureus 2*)	Chl^r, lysogen (AMSE2000)	This study
LØ 23(*S. aureus 2*)	Chl^r, lysogen (Ø111)	This study
LØ 24(*S. aureus 2*)	Chl^r, lysogen (Ø111)	This study
LØ 26(*S. aureus 2*)	Chl^r, lysogen (Ø111)	This study
LØ 27(*S. aureus 2*)	Chl^r, lysogen (Ø111)	This study
LØ 29(*S. aureus 2*)	Chl^r, lysogen (Ø111)	
Rhizobium leguminosarum		
RZ 11	str^r	This study
RZ 13	chl^r	This study

str^r: streptomycin resistance amp^r: ampcillin resistance
chl^r: chloramphenecol resistance pen^r: penicillin resistance

10.2. Transduction by lysogen:

The donor and recipient cells were grown independently in NB overnight. One ml of donor and recipient was layered onto separate nitrocellulose membrane filter (0.2 µm Whatman). The membranes were placed face – to – face on a NA plate. The plates were incubated for 24 h. After incubation time the membrane filters were vortexed for 60 sec in 10 ml phosphate buffer (PH 7.0). Counts of donor, recipient and transductants were recorded. The phage particles were also calculated. The transduction frequency was calculated (Amina, H. *et al*., 2007).

3. Results and Discussion:

1. Ability of *Pseudomanas aeruginosa* bacteriophases to propagate on different bacterial genera:

The host range of bacteriophages F116, AMSE2000, and Ø111 that prepared on *Pseudomanas aeruginosa* has investigated against different strains of *Staphylococcus aureus* and *Rhizobiun leguminosarum* (Table 2). The three bacteriophages propagated successfully on *S. aureus* strains 2, 4, and on *S. saprophytics*, *S. epidermis* and *R. leguminosarum*. The titration of phage F 116 was $2X10^9$ pfu/ml on the host, *Pseudomanas aeruginosa* strain PAO1 and ranged from $5.4X10^7$ up to $6.9X10^8$ on the tested strains. The same observation has detected upon using the other two bacteriophages. So, the three bacteriophages were able to propagate on two different bacterial genera *Staphylococcus* and *Rhizobium* beside *Pseudomanas* their original host cells.

The ability of these three bacteriophages to propagate on different bacterial genera has extended even in the liquid media (Table 3).

Table (2): Broad host range of bacteriophages F116, AMSE2000 and Φ111 prepared on *Pseudomonas aeruginosa* .

Phages / Bacterial genera	F116		AMSE2000		Φ111	
	Spot test	pfu / ml	Spot test	pfu / ml	Spot test	pfu / ml
P.aerugenosa strain PAO1	+	$2 x10^9$	+	$3.1 x10^9$	+	$4.7 x10^9$
S. aureus strain (2)	+	$5.4 x10^7$	+	$5.2 x10^7$	+	$8.7 x10^7$
S. aureus strain (4)	+	$6.1 x10^7$	+	$4.8 x10^7$	+	$5.4 x10^6$
S. saprophytics	+	$6.9 x10^8$	+	$7.1 x10^8$	+	$8.2 x10^8$
S. epidermis	+	$5.3 x10^8$	+	$5.6 x10^7$	+	4.3x107
S . sp.	+	$4.7x10^8$	+	$2.7 x10^8$	+	$1.9 x10^8$
Rhizobium leguminosarum	+	$3.9x10^8$	+	$2.5 x10^7$	+	$7.2 x10^7$

Phage titration: $5.8 x10^{10}$ pfu / ml for F116 .

Phage titration: $5.7 x10^{10}$ pfu / ml for AMSE2000.

Phage titration: $2.9 x10^{10}$ pfu / ml for Φ111.

Table (3): The ability of *Pseudomonas aeruginosa* bacteriophage to propagate in liquid media.

Phage + Host	Counts of bacteria at zero time(cfu/ ml)	Counts of bacteria after 24 h.	
		Without adding Adding phages	Phages (cfu / ml) (cfu / ml)
F116+*P.aeruginosa*	$15.3X10^{11}$	$11.3X10^{11}$	$0.8X10^{10}$
F116+*S.saprophytics*	$20.1X10^{11}$	$14.7X10^{11}$	$1.4\ X10^{10}$
F116+*R.leguminosarum*	$8.4X10^{11}$	$8.1X10^{11}$	$1.7\ X10^{10}$
AMSE2000+*P.aeruginosa*	$15.3X10^{11}$	$11.3X10^{11}$	$0.7X10^{10}$
AMSE2OOO+*S.saprophytics*	$20.1X10^{11}$	$14.7X10^{11}$	$2.3X10^{10}$
AMSE2OOO+*R.leguminosarum*	$8.4X10^{11}$	$8.1X10^{11}$	$2.1X10^{10}$
Φ111+*P.aeruginosa*	$15.3X10^{11}$	$11.3X10^{11}$	$0.6\ X10^{10}$
Φ111+*S.saprophytics*	$20.1X10^{11}$	$14.7X10^{11}$	$3.1\ X10^{10}$
Φ111+*R.leguminosarum*	$8.4X10^{11}$	$8.1X10^{11}$	$2.5\ X10^{10}$

Phage counts pfu / ml = $5.4x10^{10}$ for phage F116 .
Phage counts pfu / ml = $7.6x10^{10}$ for phage AMSE2000.
Phage counts pfu / ml = $9.8x10^{10}$ for phage Φ111
.

The turbidity of the medium measured as colony forming units (cfu/ml) in liquid medium has dropped from 11.3X1011 (F 116 + P. aeruginosa) before adding the phage, to 0.8X1010 after adding the phage. However upon using the some phage with Staphylococcus and Rhizabim bacteria, cfu/ml has been dropped from 14.7x1011 to 1.4X1010 and from 8.1X1011 to 1.7X1010 respectively.

The same phenomenon has been demonstrated with the other two phages. These data show that, the bacteriophages of *Pseudomanas aeruginosa* bacteria were able to lyses different bacterial genera effectively when compared with the original host in liquid culture as well. So, they may have a wide host range.

2. Stability of lysogens:

Data in Table 4 show that, the 3 bacteriaophages have the ability to lysogenize the parental strain PAO1 of *Pseudomanas aeruginosa* the stability of lysogen has extended up to 16 weeks. The pfu/ml ranged from $1.9x10^4$ up to $4.7x10^5$ after incubation time of 16 weeks for phage F116. Moreover, *S. aureus* phage F116 lysogens were also stable up to 16 weeks of incubation (Table 5).

The previous results clearly show that, 3 bacteriophages of *Pseudomanas aeruginosa* have the potential effect to propagate, lyse, and even lysogenize efficiently two different bacterial genera, *Staphylococcus* and *Rhizobium* bacteria.

3. Horizontal gene transfer by conjugation:

Gene transfer through conjugation can occur successfully among different donor and recipient strains of *P. aeruginosa* using two antibiotic resistance genes, chl^r and amp^r (Table 6). Conjugation frequencies ranged from $1.02X10^{-3}$ to $8.8X10^{-4}$ for chl^r and from $8.9X10^{-4}$ to $9.9X10^{-4}$ for amp^r. So gene transfer can occur among different strains of *Pseudomanas aeruginosa*. Furthermore, interspecies gene transfer can occur between different species of *Staphylococcus* bacteria (Table 7). Four different species have used as recipient cells to transfer chl^r resistance gene. Number of trascanjugants reached up to $118x10^2$ upon using *S. epidermids* as recipient cells.

Moreover horizontal gene transfer occurs as intraspecies gene transfer between *Staphylococcus* species as donor to different strains of *Pseudomanas* as recipients. Conjugation frequencies varied from $3.6X10^{-4}$ up to $6.9X10^{-4}$ (Table 8).So, conjugation as a mechanism of horizontal gene transfer can address as inter or intra species gene transfer.

4. Horizontal gene transfer through transduction:

The three bacteriophages of *P.aeruginosa* have allowed to propagate on *S.aureus* strain 2 and *R. leguminosarum* strain RZ11 beside the original strain PU21 of *P.aeruginosa* bacteria. These prepared phages have been tested to asses their ability in horizontal gene transfer by transduction into different strains of the same species (Table 9). Transduction frequency ranged from $5.2X10^{-5}$ up to $7.3X10^{-5}$ and from $3.2X10^{-5}$ up to $4.4x10^{-5}$ upon using *S. epidermids* and *R. leguminosarum* strain RZ 13 as recipient cells.

Table (4): Stability of *Pseudomonas aeruginosa* lysogens.

Time (week) / Lysogen isolates	Zero time	2	4	8	16
	Spot test pfu/ml	Spot test pfu/ml	Spot test pfu/ml	Spot test pfu/ml	Spot test pfu/ml
P.aeruginosa ΦF116 isolates					
LF62	+ $6.8X10^7$	+ $5.2X10^7$	+ $4.3X10^6$	+ $2.9X10^4$	+ $1.9X10^4$
LF64	+ $1.3X10^7$	+ $1.1X10^7$	+ $9.4X10^6$	+ $5.7X10^5$	+ $3.1X10^4$
LF65	+ $5.3X10^7$	+ $4.1X10^7$	+ $2.1X10^7$	+ $9.8X10^6$	+ $4.7X10^5$
LF66	+ $2.4X10^7$	+ $1.1X10^7$	+ $8.7X10^7$	+ $5.1X10^5$	+ $1.1X10^5$
LF68	+ $6.7X10^7$	+ $5.9X10^7$	+ $4.2X10^7$	+ $3.9X10^6$	+ $2.7X10^5$
P.aerugenosa ΦAMSE2000 isolates					
LA74	+ $9.2X10^7$	+ $5.1X10^7$	+ $4.9X10^6$	+ $3.2X10^6$	+ $1.7X10^6$
LA76	+ $5.2X10^7$	+ $3.1X10^7$	+ $2.8X10^6$	+ $7.6X10^5$	+ $5.4X10^5$
LA77	+ $1.8X10^7$	+ $9.8X10^6$	+ $6.7X10^6$	+ $3.8X10^5$	+ $1.1X10^5$
LA79	+ $9.8X10^6$	+ $7.1X10^6$	+ $9.4X10^5$	+ $6.3X10^5$	+ $2.7X10^5$
LA80	+ $9.7X10^7$	+ 3.1X107	+ $2.1X10^7$	+ $8.1X10^6$	+ $2.4X10^6$
P.aerugenosa Φ111 isolates					
LΦ83	+ $3.7X10^7$	+ $1.1X10^7$	+ $8.5X10^6$	+ $7.1X10^5$	+ $5.1X10^5$
LΦ85	+ $6.2X10^7$	+ $4.1X10^7$	+ $2.2X10^7$	+ $1.3X10^6$	+ $9.2X10^5$
LΦ86	+ $5.9X10^7$	+ $1.8X10^7$	+ $7.6X10^6$	+ $4.1X10^6$	+ $2.1X10^6$
LΦ87	+ $9.8X10^7$	+ $8.7X10^7$	+ $3.1X10^6$	+ $2.7X10^5$	+ $2.1X10^4$
LΦ89	+ 9.8 $X10^7$	+ $7.8X10^7$	+ $2.9X10^7$	+ $1.1X10^6$	+ $8.7X10^5$

Table (5): Stability of *Staphylococcus aureus* lysogens .

Time (week) / Lysogen isolates	Zero time	2	4	8	16
	Spot test pfu /ml	Spot test pfu/ml	Spot test pfu/ml	Spot test pfu/ml	Spot test pfu/ml
S.aureus ΦF116 isolates					
LF 1	+ $2.0X10^5$	+ $1.7X10^5$	+ $1.3X10^4$	+ $3.1X10^3$	+ $1.7X10^3$
LF 3	+ $4.1X10^3$	+ $1.5X10^3$	+ $1.1X10^3$	+ $9.2X10^2$	+ $8.7X10^3$
LF 5	+ $3.5X10^5$	+ $1.9X10^5$	+ $1.8X10^4$	+ $2.1X10^4$	+ $9.1X10^3$
LF 7	+ $3.7X10^5$	+ $1.8X10^5$	+ $1.7X10^4$	+ $1.2X10^4$	+ $2.3X10^3$
LF 9	+ $2.9X10^4$	+ $1.6X10^4$	+ $1.5X10^3$	+ $1.1X10^3$	+ $9.5X10^2$
S.aureus ΦAMSE2000 isolates					
LA 11	+ $1.9X10^5$	+ $8.2X10^4$	+ $6.1X10^4$	+ $8.1X10^3$	+ $4.6X10^3$
LA 13	+ $1.7X10^5$	+ $6.3X10^4$	+ $5.1X10^3$	+ $2.1X10^3$	+ $1.7X10^3$
LA 15	+ $3.8X10^5$	+ $2.1X10^5$	+ $7.3X10^4$	+ $5.1X10^3$	+ $2.7X10^3$
LA 17	+ $2.2X10^5$	+ $1.7X10^5$	+ $8.1X10^4$	+ $4.1X10^4$	+ $1.1X10^4$
LA 20	+ $3.9X10^5$	+ $7.9X10^4$	+ $1.7X10^4$	+ $1.9X10^3$	+ $1.2X10^3$
S.aureus Φ111 isolates					
LΦ23	+ $11.2X10^5$	+ $6.7X10^5$	+ $3.4X10^4$	+ $2.9X10^4$	+ $1.3X10^4$
LΦ24	+ $19.2X10^5$	+ $5.9X10^5$	+ $2.7X10^4$	+ $9.2X10^3$	+ $7.8X10^3$
LΦ26	+ $14.7X10^5$	+ $9.3X10^5$	+ $8.1X10^4$	+ $7.8X10^4$	+ $2.3X10^4$
LΦ27	+ $12.8X10^4$	+ $9.8X10^4$	+ $7.1X10^4$	+ $8.1X10^3$	+ $4.7X10^4$
LΦ29	+ $13.7X10^5$	+ $8.7X10^4$	+ $6.1X10^4$	+ $4.2X10^4$	+ $2.3X10^4$

Table (6): Conjugation in *Pseudomonas aeruginosa* .

Donor	Recipient	Selective genetic marker	No. of Transconjugants	Conjugation frequency
PU21 (chl^r) $8.9 \pm 0.1X10^6$	PAO1 (amp^r) $9.2\pm 0.8\ X10^6$	(chl^r)	$9.4\pm 0.3\ X10^3$	$1.02\ X\ 10^{-3}$
PU21 (chl^r) $8.9\pm 0.7\ X10^6$	MAM2 (str^r) $6.9\pm 0.5\ X10^6$	(chl^r)	$6.1 \pm 0.2X10^3$	$8.8\ X\ 10^{-4}$
PU21 (amp^r) $7.6\pm 0.4\ X10^6$	MAM2 str^r) $6.9 \pm 0.5X10^6$	(amp^r)	$6.8 \pm 0.4X10^3$	$9.9\ X\ 10^{-4}$
PAO1 (amp^r) $8.6 \pm 0.6X10^6$	MAM2 (str^r) $6.9\pm 0.5\ X10^6$	(amp^r)	$6.2\pm 0.2X10^3 \pm$	$8.9\ X\ 10^{-4}$

Chl^r : chloramphenicol resistance gene .
amp^r :ampcillin resistance gene .
str^r : streptomycin resistance gene .

Regarding the way of preparing the transducing phage particles either released spontaneously from lysogens (Table 10), induced from lysogen by UV (Table 11) or as in lysogens (Table 12), the horizontal gene transfer by transduction still occur.

The results of this study clarify that horizontal gene transfer can occur between different bacterial species and genera. These data come agree with other investigation. Bhakta *et al*., 2003 found that, the interspecies transfer can occur among different clinical isolates of *S. aureus.* They believed that, such transfer may play a role in the evolution of antibiotic resistant bacteria.

Table (7): Conjugation in *Staphylococcus* species .

Donor	Recipient	No. of Transconjugants	Conjugation frequency
***S. aureus* strain2 (chl^r)** **$1.6 \pm 0.4X10^8$**	*S.aureus* strain5(str^r) $6.9 \pm 0.5X10^8$	$2\pm 0.4X10^2$	$2.0\ X\ 10^{-7}$
	S.saprophyticus (str^r) $2.1 \pm 0.3X10^7$	$23 \pm 0.6X10^2$	$10.09\ X\ 10^{-5}$
	S.epidermids (str^r) $6.4 \pm 0.4X10^8$	$118\pm 0.2\ X10^2$	$1.8\ X\ 10^{-6}$
	S. cohnii (str^r) $8.4 \pm 0.6X10^9$	$4 \pm 0.7X10^2$	$4.7\ X\ 10^{-7}$
	S. sp (str^r) $2.9 \pm 0.2X10^9$	$13\pm 0.2\ X10^2$	$4.4\ X\ 10^{-7}$

Table (8): Transfer through conjugation between *Staphylococcus* and *Pseudomonas* bacteria.

Donor (cfu / ml)	Recipient (cfu/ ml)	Selective marker	No. of transconjugants	Conjugation frequency
S.epidermids (str^r) $8.6 X10^6$	*P.aeruginosa* PU21 (chl^r) $9.6 X10^8$	(str^r)	$3.5\pm 0.2 X10^5$	$3.6 X10^{-4}$
S.epidermids (str^r) $8.6 X10^6$	*P.aeruginosa* PAO1 (amp^r) $6.9 X10^8$	(str^r)	$4.8 \pm 0.4 X10^5$	$6.9 X10^{-4}$
S.epidermids (str^r) $8.6 X10^6$	*P.aeruginosa* MAM2 (chl^r) $9.8 X10^8$	(str^r)	$5.7 \pm 0.2 X10^5$	$5.8 X10^{-4}$
S. aureus strain2(chl^r) $7.2 X10^6$	*P.aeruginosa* PAO1 (amp^r) $6.9 X10^8$	(chl^r)	$3.6 \pm 0.6 X10^5$	$3.8 X10^{-4}$

Table (9): Horizontal gene transfer by transduction .

Phage	Donor	Recipient counts		No.of Transductants	Transduction frequency
		Zero time	After24 h.		
	P.aeruginosa PU21 (amp^r)	*P.aeruginosa* MAM2 (str^r)			
F116		$4.9 X10^{11}$	$1.7 X10^7$	$4.5 X10^4$	$2.6 X10^{-3}$
AMSE2000		$4.9X10^{11}$	$1.1X10^7$	$2.1 X10^4$	$1.9 X10^{-3}$
Φ111		$4.9 X10^{11}$	$2.3 X10^7$	$5.2 X10^4$	$2.2 X10^{-3}$
	S. aureus strain2 (chl^r)	*S.epidermids* (str^r)			
F116		$3.6X10^{11}$	$3.2 X10^7$	$2.1 X10^3$	$6.5 X10^{-5}$
AMSE2000		$3.6 X10^{11}$	$2.6 X10^7$	$1.9 X10^3$	$7.3 X10^{-5}$
Φ111		$3.6 X10^{11}$	$2.1. X10^7$	$1.1 X10^3$	$5.2 X10^{-5}$
	R.leguminosarum StrainRZ11 (str^r)	*R.leguminosarum* StrainRZ13 (amp^r)			
F116		$2.3X10^{12}$	$5.4 X10^7$	$2.4 X10^3$	$4.4 X10^{-5}$
AMSE2000		$2.3 X10^{12}$	$7.7 X10^7$	$3.1 X10^3$	$4.1 X10^{-5}$
Φ111		$2.3 X10^{12}$	$9.2 X10^7$	$2.9 X10^3$	$3.2 X10^{-5}$

Table (10): Horizontal gene transfer through transduction by bacteriophages F116, AMSE2000 and Φ111 released spontaneously from lysogen.

Donor	Recipient	Recipient counts		Titration of phage on recipient (cfu/ml)	No. of Transductants	Transduction frequency
		Zero time	After24 h			
P.aeruginosa PAO1(amp^r)	*P.aeruginosa* MAM2(str^r)	*P.aeruginosa* MAM2 (str^r)				
LF68		$1.36 X10^{11}$	$5.86 X10^7$	$6.32 X10^9$	$1.12 X10^3$	$1.9 X10^{-5}$
LA79		$1.36X10^{11}$	$5.17X10^7$	$1.12X10^{10}$	$1.05X10^5$	$2.03 X10^{-3}$
LΦ87		$1.36 X10^{11}$	$5.43 X10^7$	$9.87X10^{10}$	$1.38X10^5$	$2.5X10^{-3}$
S. aureus strain 2 (chl^r)	*S.epidermids* (str^r)	*S.epidermids* (str^r)				
LF3		$9.95X10^{11}$	$9.27X10^7$	$4.0 X10^6$	$1.0X10^1$	$1.07 X10^{-7}$
LA15		$9.95 X10^{11}$	$7.89X10^7$	$3.8 X10^6$	$5.7 X10^2$	$7.2X10^{-6}$
LΦ24		$9.95 X10^{11}$	$7.97X10^7$	$1.9 X10^7$	$6.1 X10^2$	$7.6 X10^{-6}$

Table (11): Transduction ability of bacteriophages F116, AMSE2000 and Φ111 induced from lysogens by UV.

Donor	Recipient	Recipient counts		Titration of phage on recipient (cfu/ml)	No.of Transductants	Transduction frequency
		Zero time	After24 h.			
P.aeruginosa PAO1(amp^r)	*P.aeruginosa* MAM2(str^r)	*P.aeruginosa* MAM2 (str^r)				
LF68		$1.37 X10^{11}$	$5.98 X10^5$	$6.32 X10^9$	$9.6 X10^3$	$1.6 X10^{-2}$
LA79		$1.37X10^{11}$	$5.21X10^5$	$1.12X10^{10}$	$3.6X10^4$	$6.9 X10^{-2}$
L Φ87		$1.37 X10^{11}$	$5.72 X10^5$	$9.87X10^{10}$	$1.2X10^4$	$2.09X10^{-2}$
S. aureus strain 2(chl^r)	*S.epidermids* (str^r)	*S.epidermids* (str^r)				
LF3		$9.95X10^{11}$	$6.39X10^5$	$4.0 X10^6$	–	–
LA15		$9.95 X10^{11}$	$5.97X10^5$	$3.8 X10^6$	–	–
LΦ24		$9.95 X10^{11}$	$6.86X10^5$	$1.9 X10^7$	–	–

Table (12): Transduction ability of bacteriophages F116, AMSE2000 and Φ111 by lysogen.

Donor	Recipient	Recipient counts		Titration of phage on recipient (cfu /ml)	No. of Transductants	Transduction frequency
		Zero time	After24 h.			
P.aeruginosa PAO1(amp^r)	*P.aeruginosa* MAM2(str^r)	*P.aeruginosa* MAM2 (str^r)				
LF68		$1.37 X10^{11}$	$7.23 X10^7$	$6.9 X10^7$	$1.22 X10^4$	$1.6 X10^{-4}$
LA79		$1.37X10^{11}$	$5.97X10^7$	$5.7X10^8$	$9.6X10^4$	$1.6 X10^{-3}$
L Φ87		$1.37 X10^{11}$	$6.41 X10^7$	$8.8X10^8$	$2.8X10^4$	$4.4X10^{-4}$
S. aureus strain 2 (chl^r)	*S.epidermids* (str^r)	*S.epidermids* (str^r)				
LF3		$9.87X10^{11}$	$9.85X10^7$	$6.8 X10^3$	$1.5X10^4$	$1.5 X10^{-4}$
LA15		$9.87 X10^{11}$	$8.61X10^7$	$3.9 X10^3$	$1.25 X10^3$	$1.4X10^{-5}$
LΦ24		$9.87 X10^{11}$	$8.74X10^7$	$8.4 X10^3$	$3.0X10^2$	$3.4 X10^{-4}$

Moreover, the remarkable spread of resistance among bacteria to multiple antibiotics may have been aided by the transfer of resistance genes within populations and even between species (Frisse *et al*., 2001). However, the recent completion of the sequence of the entire genome of a variety of different bacteria and archaea suggested that, genes have in the past moved from one species to anther. (Suchard *et al*., 2003, Didelot and Daniel, 2007).

Horizontal genetic exchange might facilitate adoption of an optimal genetic profile for survival. Whereas transfer of plasmid pAM 81 between two species, *Streptococcus gordonii* and *Enterococcus Faecalis* was demonstrated in an ex vivo tooth model (Christine *et al*., 2008).

Nucleotide sequence analysis and the whole genome analysis showed that, bacterial evolution has

often proceeded by horizontal gene flow between different species and genera (Davison, 1999). The rise and spread of multiple antibiotic resistance plasmids are consequences of inter-generic gene transfer.

Our understanding of the role of horizontal gene transfer in the environment is essential for the evaluation of the possible consequences of the deliberate environmental release of natural or recombinant bacteria for agricultural and bioremediation purposes. In addition, horizontal gene transfer is a key step in the evolution of bacterial pathogens. It has been demonstrated that conjugative transfer and homologous DNA recombination play a major role in horizontal transfer of a pathogenicity island within the species of *E. coli.*

In nature, many microbial communities exist as biofilms where genetic exchange is facilitated. It has been observed that gene transfer can occur and may modify the phenotypes of biofilm communities giving then specific and desirable functions (Perumbakkam *et al*., 2006).

Finally it has been suggested that horizontal gene transfer is the essence of phylogeny, especially to primitive genomes with important evolutionary consequence (Kurland *et al*., 2003, Dagan and Martin, 2007). In modern organisms, both the range of frequencies of horizontal gene transfer are constrained most often by selective barriers (Choi and Kim, 2007). This may due to in part from a reliance on inadequate methods to identify horizontal gene transfer events.

Corresponding author
M.K. Amin
Department of Genetics, Fac. Agric. Zagazig Univ. Egypt.
osammour@hotmail.Com

4. References:

1. Amabile- Cuevas, C.F. and M.E. Chicurel (1992). Bacterial plasmids and gene flux. Cell 70: 189-199.
2. Amin M.K.; A.A. Mahmoud; E.I Mahgoub and Amina A. Hassan (2004). Characterization of a transducing temperate phage isolated from sewage water. Zagazig Journal of Agricultural Research., 31,4A, 1533-1550.
3. Amin, M.K.; A.S. Hassan; A.A. Mahmoud and Oliya, H. Attia (2008). Gene transfer among different species of *Staphylococcus* isolated from human. Zagazig Journal of Agricultural Research., 35: 293-313.
4. Amina A.H.; M.K. Amin; M.A.H. Youssef and M. El- Hussein (2007). Microbial genetical studies of *Pseudomonas aeruginosa* bacterial isolated from human. Mansoua Univ. Journal. of Agricultural Sciences., 32: 3439-3451.
5. Barrangou, R.; S.S. Yoon; F. Breidt; J.R. Henry; P. Fleming and T.R. Klaenhammer (2002). Characterization of six *Leuconostoc falla* bacteriophages isolated from an industrial sauerkraut fermentation. Applied and Environmental Microbial. 68: 5452-5458.
6. Bhakta Mausumi, Suranjana Arora and Manjust Bal (2003). Intraspecies transfer of a choramphenicol- resistance plasmid of staphylococcal origin. Indian J. Med. Res. 117: 146- 151.
7. Chen, I.; P.J. Christie and D. Dubnau (2005). The ins and outs of DNA transfer in bacteria. Science. 310: 1456- 1460.
8. Choi, I. and Kim, S. (2007). Global extent of horizontal gene transfer. Proc. Natl. Acad. Sci. USA. 104 :4489-4494.
9. Christine M.; Sedgley MDSc; MDS, phD Esther H. Lee BS, Matthew J. Martin BA and Susan E. Flannagan MA. (2008). Antibiotic resistance gene transfer between *Streptococcus gordonii* and *Enterococcus faecalis* in root canals of teeth ex vivo. Journal of Endodontics. 34: 570- 574.
10. Dagan, T. and Martin, W. (2007).Ancestral genome size specify the minimum rate of lateral gene transfer during prokaryote evolution. Proc. Natl. Acad. Sci., USA. 104 :870- 675.
11. Davison, J. (1999). Genetic exchange between bacteria in the environment. Plasmid. 42: 73- 91.
12. Didelot, X. and F. Daniel (2007). Inference of bacterial microevolution using multilocus sequence data. Genetics. 173: 1251- 1266.
13. Dunn, N.W. and I.C. Gunsalus (1973). Transmissible plasmid coding early enzymes of naphthalene oxidation in *Pseudomonas putida.* Journal of Bacteriology. 114: 974-979.

14. Frisse, L.; R. Hudson; A. Bartoszewicz and A. Di Rienzo (2001). Gene conversion and different population histories may explain the contrast between polymorphism and linkage disequilibrium levels. American Journal of Human Genetics, 69: 831- 843.

15. Herrick, J.B.; K.G. Stuart; Kell, W.C. Ghiorse and E.l. Madsen (1997). Natural horizontal transfer of a naphthalene dioxygenase gene between bacteria native to a coal tar- contaminated field site. Applied and Environmental Microbiology, 63: 2330-2337.

16. Hollway, B. and Morgan, A. (1986). Genome organization in *Pseudomonas* Annual Review of microbiology, 40: 79-105.

17. Kapur, V.K. Nelson; P.M. Schlievert; R.K. Selander and J.M. Musser (1992). Molecular population genetic evidence of horizontal spread of two alleles of the pyrogenic exotoxin C gene (spe C) among pathogenic clones of Streptococcus pyogenes. Immun. 60: 3513-3517.

18. Kinkle, B.K.; M.J. Sadowsky; E.I. Schmidt and W.C. Kosskinen (1993). Plasmid pJP4 and r68.45 can be transferred between populations of bradyrhizobia in nonsterile soil. Applied and Environmental Microbiology, 59: 1762-1766.

19. Kurland, B.; Canback, B.; and Berg, O. (2003). Horizontal gene transfer.Acritical view.Proc. Natl. Acad. Sci. USA. 100: 9658-9662.

20. Linz, S. ; Radtke, A. ; and Haeseler, A. (2007).A likelihood framework to measure horizontal gene transfer. Mol. Biol. Evolu.24: 1312-1319.

21. Meyer, B.; and Kuever, J. (2007). Phylogeny of the alpha subunits of the dissimilatory adenosine -5-phosphosulfate reductase from sulfate reducing prokaryotes. Microbiology. 153 :2026-2044.

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

23. Payan, A.; J. Ebdon; H. Taylor; C. Gantzer; J. Ottoson; G.T. Papgeorgiou; A.R. Blanch; F. Lucena; J. Jofre and M. Muniesa (2005). Method for isolation of bacteroides bacteriophage host strain suitable for tracking sources of fecal pollution in water. Applied and Environmental Microbiology, 71: 5659-5662.

24. Perumbakkam Sudeep, Thomas F.; Hess and Ronald L. Crawford (2006). A bioremediation approach using natural transformation in pure-culture and mixed-population biofilms. Journal of Biodegradation. 17: 545- 557.

25. Rodolphe, B., Sung- Silk, Y., Frrederick, B., Henry, P.E. and Todd, R.K. (2002). Characterization of six *Leuconostoc Fallax* bacteriophages isolated from an industrial sauerkraut fermentation. Applied and Environmental Microbiology,68: 5452-5458.

26. Sharma, R.S.; A. Mohmmed and C.R. Babu (2002). Diversity among rhizobiophages from rhizobiophere of legumes inhabiting three ecogeographical regions of India. Soil Biology and Biochemistry, 34: 965-973.

27. Suchard, M.; R. Weiss; K. Dorman and S. Sivsheimer (2003). Inferring spatial phylogenetic variation along nucleotide sequences. Journal of the American statistical Association. 98: 427- 437.

28. Zaneveld Jesse R.; Diana R. Nemergut and Rob Knight (2008). Are all horizontal gene transfers created equal? Prospects for mechanism- based studies of HGT patterns. Microbiology. 154: 1- 15.

8/1/2010