J.Agric. Chem. and Biotechn., Mansoura Univ. Vol. 2 (3): 95 - 110, 2011

FITNESS OF GENETICALLY MODIFIED MICROORGANISMS *In situ* (RIVER WATER)

Hassan, Amina A.
Genetic Dept. , Fac. Agric., Zagazig Univ.

ABSTRACT

The present study aimed to determine the fitness of genetically modified microorganisms (GMMs) and their parents in aquatic environment (river water) through the genetic stability and transduction abilities. Two GMMs (RS1, RS2) *Pseudomonas aeruginosa* strains were used. The results show that the transferred genes were stable up to 15 days in RS1 and 30 days in RS2, but the cfu/ml was decreased. The survival of RS1 was decreased from $2.87x10^{11}$ at zero time to $8.9x10^{2}$ after 15 days, while the cfu/ml of RS2 was decreased from $9.39x10^{11}$ to $6.0x10^{1}$. The fitness of RS2 was higher than their parents.

The GMMs were used as a donor to study their transducing abilities *in situ* by transduction mechanism. GMMs were able to transfer their DNA to other bacterial strain. Transduction frequency was declined from $6.12x10^{-8}$ to $8.2x10^{-11}$ through 20 days for RS1 and from $4.4x10^{-8}$ to $1.6x10^{-10}$ for RS2. No transductants have been detected after 20 days.

The abiotic factors that may be effect on GMMs under environmental conditions were investigated under laboratory conditions. UV, pH, ions and temperature have been tested on the survival and transducing ability of GMMs. The remarker effect was observed with acidic pH, trivalent cation (Fe^{+++}) and 42°C. The survival and gene transfer were dramatically decreased.
Keywords: Gene transfer GMMs, *In situ*, survival, , transduction.

INTRODUCTION

An accidental or deliberate release of genetically engineered microorganisms into the environment can be a possible source of biological contamination of ground or surface waters. Although there seem to be many promising applications of GMMs in agriculture, industry, and medicine, their use has been limited so far by environmental concerns (Alvarez, *et al*., 1996). Fundamental concerns regarding GMMs in the environment include the ability of these organisms to survive, to compete with the indigenous microbiota, and the possible transfer of their manipulated DNA to other microorganisms (Demirtas, *et al.,* 2006). Predication of the fate of the GMMs and their engineered DNA in natural systems is a major component of a complete risk assessment process (Cuskey, 1990). The survival of GMMs in the environment depends on many factors, both biotic (such as population interactions) and abiotic (such as pH, salinity, temperature, illumination, humidity and the availability of nutrients) (Hong, *et al*., 1996, Sayler and Ripp, 2000, Kargatova, *et al*., 2001). In addition to the stable maintenance of engineered genes is required for their successful applications in the environment, it typically assumed that GMMs will exhibit a decreased level of fitness due to the extra energy demands imposed by introduced foreign

genetic elements and will therefore be unable to compete under real world conditions (Lenski, 1993 and Gidding, 1998).

The present study aimed to evaluate fitness of GMMs via genetic stability and transducing abilities, it compared with their parents and study of some factors that may effect on these mechanisms .

MATERIALS AND METHODS

This study was performed in Microbial Genetic Lab., Genetic Dept., Fac.of Agric., Zagazig Univ., Egypt.

Bacterial strains:

Bacterial strains used in this study as a model for GMMs are: RS1 (*Pseudomonas aeruginosa* lysogen with phage F116, containing streptomycin and chloramphenecol resistant markers), RS2 (*P.aeruginosa* lysogen with phage F116, containing streptomycin and ampcillin resistant markers). The parental strains (PAO1, PU21, MAM2) were obtained from M. Day, University of Wales, Cardiff, UK .

Growth media:

Nutrient agar (NA) and nutrient broth (NB) media were used. Soft agar (0.8% W/V agar) was prepared in distilled water and kept at 45°C on waterbath. Phosphate buffer (pb) was prepared from 1/15M potassium phosphate (KH_2PO_4) and 1/15M disodium phosphate (Na_2HPO_4. H_20). Streptomycin (12mg/ml), ampcillin (2mg/ml) and chloramphenicol (1mg/ml) were added as sterilized solution by filtration through 0.2 µm filter membrane (Whatman No.1) to the media after autoclaving.

***In situ* stability of GMMs:**

GMMs and their parents were grown independently in NB overnight. The cells were washed by centrifugation at 5000 rpm for 5 min., and resuspended in 10 ml fresh NB. One ml of each strain was layered by filtration onto separate nitrocellulose membrane filter (0.2 µm Whatman No.1) held in a swinnex filter holder. The membranes were held by clips and suspended by a nylon line from a tree branch overhanging the bank of Mowas River in Zagazig City. A weight was tied on the end of the nylon line to keep the filter membranes 20-30Cm below the surface of the water. After certain time intervals each membrane was removed, placed in 10 ml phosphate buffer, vortexed and counted by plating.

Gene transfer from GMMs:

GMMs strains were used as donors in transduction experiment *in situ* to study their ability to transducer genetic materials to another recipients. All strains were grown independently in NB overnight. The cells were washed by centrifugation at 5000 rpm for 5 min and resuspended in 10 ml fresh NB. One ml of donor and recipient cells was layered by filtration onto separate nitrocellulose membrane filter held in a swinnex filter holder. Membranes containing donor and recipient cells were tacked separately to the river for *in situ* mating experiment. Transfer from laboratory to the river site took a maximum of 15 min. The membranes were held face to face by clips and suspended by a nylon line from a tree branch overhanging the bank of the

river. A weight was tied on the end of the nylon line to keep the filter membranes 20-30 Cm below the surface of the water (Amin, 1988, and, Amina, 1995). Zero time (control) experiments were also performed. Filters were immediately removed from the water, placed in 10 ml pb. Held on ice and transported back to the laboratory for assaying. After certain time intervals, the filters were removed from the water, placed in 10 ml phosphate buffer, held on ice and transported back to the laboratory. The filters were then vortexed for 60 sec., viable counts of donor (GMMs), recipients, transductants and phage were recorded using the appropriate selective media.

Treatment of GMMs and their parents with some factors:-

UV treatment:

Ten ml of overnight strains were placed in petri dish and expoured to UV at different times (0,1,5,8,13,20,45,75,105 min.), survival was determined. Effect of UV on transduction ability of GMMs, 1.0 ml of treated GMMs and recipient was layered as previous. The two membranes were placed face to face on a NA plate for 24h at 30°C. After incubation time the membranes were vortexed for 60 sec. in 10 ml phosphate buffer. Counts for donor, recipient, transductants and phage were recorded.

pH treatment :

To study effect of different pH levels on survival of GMMs and their parents, the NB media with different pHs (2,5,7,10,12)were prepared and inoculated with GMMs and parents separately. The cultures were incubated at 30°C for 24h. Serial dilutions were prepared and counts were records. Effect of pH on transducing ability of GMMs was also studied. Flasks with different pH NB medium were prepared. The donor and recipient cells were layered onto filter membranes as previous. Two membranes were placed face to face and held in flasks, incubation at 30°C for 24h. After incubation time, the membranes placed in 10 ml phosphate buffer, vortexed, counts for donor, recipient, transductants and phage were performed.

Salts treatment:

Three different salts (mono, di, and trivalent) were choosed {NaCl, $CaC1_2$ (0,10,50,100,150,200 mM) , $FeCl_3$ (0,5,10,15,20,50 mM)}. Flasks with different concentrations of individual salts were prepared, inoculated with separate GMMs and parents. The flasks were incubated at 30°C for 24h. Serial dilutions were prepared, and counts were recorded. The effect of salts on transducing ability of GMMs was investigated.

Temperatures treatment:

NB flasks inoculated with GMMs and their parents were incubated at different temperatures (5,20,30,42°C)for 24h. Viable counts of cells were recorded. The effect of temperature on transducing ability of GMMs was investigated.

RESULTS AND DISCUSSION

Fitness and genetic stability of GMMs *in situ*:

The stability of the engineered genes was determined by plating the genetically modified strains on selective media. The results in (Table 1 and

Fig.1) appeared that the transferred genes were relatively stable up to 15 days for RS1.The colony forming units (cfu/ml) were declined from $2.87x10^{11}$ to $1.21x10^{4}$ through 10 days. The population of the introduced strain then remained stable for the next 5 days and rapid declined to zero after this time.

The other GMM strain (RS2) was survived up to 30 days but the cfu/ml was decreased from $9.39x10^{11}$ to $2.54x10^{5}$ after 5 days. The population remained relatively stable for the next 20 days. Comparing with parental strains, RS1 was similar to their parents (PU21A, MAM2A), RS2 has a good fitness than their parents (PAO1A, MAM2A). Altered organisms would have reduced fitness for survival and growth in the environment, due in part to the increased metabolic load imposed by maintenance and expression of the foreign genes(Sobecky, *et al*., 1992). Release of such organisms, it was argued, would be inherently safe since they would either die off quickly or gradually be eliminated by their naturally occurring analogs (Sobecky *et al*., 1992 and Ryder *et al*., 1994). Results from other laboratories demonstrated that post-release mutational changes can increase the fitness of genetically altered organisms to level equal to or higher than those of the wild – type organisms from which they were derived (Sobecky, *et al*., 1992).

Table 1: Fitness of GMMs and their parents at different times *in situ.*

Time(day) / Strains	Zero	1	5	10	15	20	25	30
RS 1	$2.87X10^{11}$	$5.8X10^{10}$	$2.16X10^{5}$	$3.19X10^{4}$	$1.21X10^{4}$	–	–	–
RS 2	$9.39X10^{11}$	$7.9X10^{10}$	$2.54X10^{5}$	$2.10X10^{4}$	$1.87X10^{4}$	$4.5X10^{3}$	$3.1X10^{3}$	$6X10^{1}$
PAO1A	$9.76X10^{11}$	$1.2X10^{10}$	$7.7X10^{5}$	$1.90X10^{4}$	$3.5X10^{4}$	$1.7X10^{3}$	—	—
PU21A	$3.17X10^{11}$	$1.99X10^{10}$	$2.31X10^{5}$	$1.69X10^{4}$	$2.3X10^{3}$	–	—	—
MAM2A	$7.28X10^{14}$	$3.9X10^{10}$	$2.81X10^{5}$	$9.68X10^{4}$	$3.7X10^{3}$	$1.6X10^{2}$	—	—

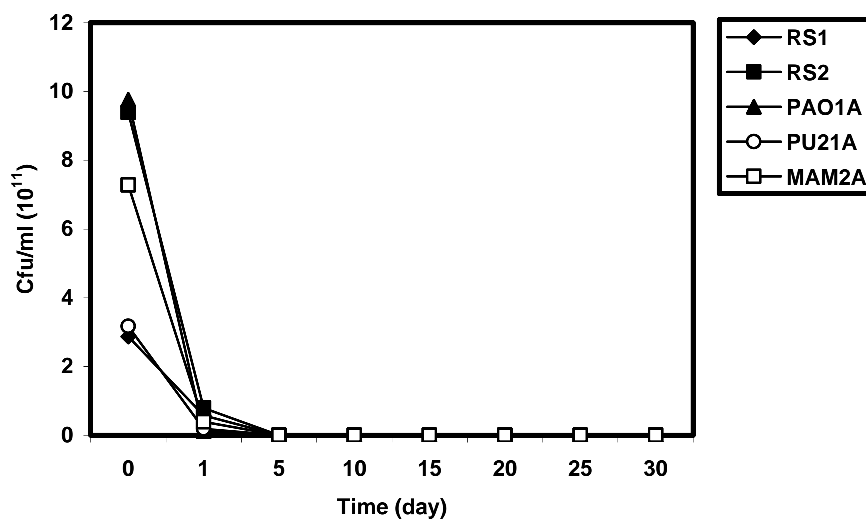

Fig. 1: Fitness of GMMs and their parents at different times *in situ.*

Alvarez *et al*. (1996) suggested that the presence of competing bacteria may limit the survival time of GMMs in the environment.

Similar results have been described previously, McClure *et al*., (1991) found that the level of the introduced strain declined rapidly from $1.8x10^{8}$ to $4.7x10^{5}$ after 6 days, the population of the introduced strain then remained stable for the next 10 days. Many engineered genotypes are unsuitable, such that their frequencies decline with time, instability may be caused by infidelity of replication or transmission of particular gene or it may be caused by a difference in the fitness genotypes(Lenski, 1991). Kargatova *et al*. (2001) noted a decrease in cfu/ml to 10^{2} with in a week. Alvarez *et al*., (1996) found differences in survival rates between GEMs and suggested that some GEMs are less resilient in the environment. Hong *et al*., (1996) found that number of 2.4-D- degrading bacteria were declined in natural river water. Fujita *et al*. (2003) found that the introduced GEMs declined rapidly.

Awong *et al*. (1990) noted that genetically engineered strains were better able to survive under given conditions than their parental strains, and suggested that the presence or addition of plasmids to a host bacteria may improve the fitness of the cell in certain environment. Recorbet *et al*. (1992) regarded that, *in situ* intraspecific competition studies can provide additional knowledge about the relative fitness of a modified bacteria as compared to a wild-type. VanElsas *et al*. (1994) noted that, the growth rates of both modified derivatives in different liquid media were similar to that of the parental strain, but altered fitness during intermittent growth in different liquid media in competition with the parental strain. In general, genetically engineered microorganisms will be poor competitors and therefore unable to persist in the wild due to energetic inefficiency, disruption of genomic coadaptation, or domestication. Many studies support the hypothesis that genetically modified microorganisms are less fit than their progenitors (Lenski, 1993 and Popova *et al*., 1997).

One should not try to predict survival times of a genetically engineered microorganism based on experiments conducted with another. Thus GEMs must be evaluated on an individual basis prior to their release into the environment (Alvarez *et al*., 1996).

Transducing ability of GMMs *In situ*:

GMMs were used as a donor *in situ* experiment to evaluate their ability to transducer acquired genetic material by transduction mechanism. The samples were tested at different time, counts of donor, recipients, transductants and phage were performed.

Results in (Table 2) show that, genetically engineered strains (RS1, RS2) are capable to transfer their DNA to other bacterial strains. Transduction frequency was declined from $6.12x10^{-8}$ to $8.2x10^{-11}$ through 20 days for RS1 and from $4.4x10^{-8}$ to $1.6x10^{-10}$ for RS2. No transductant cells were detected after this time. Comparing with results in (Table 3) (transducing ability of GMMs in Lab.), it can be noticed that, transduction frequency under Lab. conditions was higher than *in situ* in both genetically engineered strains. It ranged from $1.8x10^{-4}$ to $9.1x10^{-11}$ (RS1), $5.1x10^{-5}$ to $3.4x10^{-11}$ (RS2). Also, transducing ability was persistent up to 30 days in lab.

and at 20 days *in situ*. These results indicated that, acquired genes may be transferred from GMMs to other strains.

Table 2: Transducing ability of GMMs at different times *in situ*.

Strains / Time (day)	Zero	1	5	10	15	20	25	30
RS1(donor)(cfu/ml)	$1.28X10^{11}$	$6.18X10^{7}$	$2.16X10^{5}$	$1.22X10^{4}$	$8.9X10^{2}$	—	—	—
PU21A (recipient) (cfu/ml)	$3.17X10^{11}$	$2.77X10^{5}$	$2.43X10^{5}$	$2.12X10^{4}$	$1.62X10^{3}$	$8.2X10^{2}$	$2.0X10^{2}$	$8.0X10^{1}$
Transductants (cfu/ml)	—	$5.98X10^{4}$	$2.4X10^{3}$	$8.9X10^{2}$	$1.5X10^{2}$	$8.0X10^{1}$	—	—
Transduction frequency	-	$6.12X10^{-8}$	$2.45X10^{-9}$	$9.0X10^{-10}$	$1.5X10^{-10}$	$8.2X10^{-11}$	—	—
Phage(pfu/ml)	$9.97X10^{13}$	$2.4X10^{9}$	$2.61X10^{5}$	$7.8X10^{3}$	$9.1X10^{2}$	$5.6X10^{2}$	$3.3X10^{2}$	$1.22X10^{2}$
RS 2(dondr) (cfu/ml)	$9.23X10^{1}$	$8.12X10^{6}$	$2.52X10^{5}$	$2.10X10^{5}$	$1.2X10^{4}$	$4.5X10^{3}$	$1.0X10^{2}$	$6.0X10^{1}$
PAO1A (recipient) (cfu/ml)	$9.26X10^{11}$	$6.5X10^{4}$	$3.51X10^{4}$	$1.17X10^{4}$	$1.6X10^{3}$	$1.02X10^{3}$	$1.1X10^{3}$	$8.0X10^{1}$
Transductants(cfu/ml)	—	$1.4X10^{4}$	$7.0X10^{3}$	$7.0X10^{2}$	$9.8X10^{1}$	$5.0X10^{1}$	—	—
Transduction frequency	—	$4.4X10^{-8}$	$2.2X10^{-8}$	$2.2X10^{-9}$	$3.1X10^{-10}$	$1.6X10^{-10}$	—	—
Phage(pfu/ml)	$9.15X10^{11}$	$3.2X10^{8}$	$4.4X10^{3}$	$1.6X10^{3}$	$4.7X10^{2}$	$9.0X10^{1}$	$1.0X10^{1}$	—

PU 21A = $3.17 X 10^{11}$
PAO 1A = $9.76 X 10^{11}$

Previous studies shown that, genetically engineered microorganisms can transfer their novel genetic information to the indigenous microbial populations(Awong *et al*., 1990). The processes for genetic exchange and uptake of DNA within and between species are widespread in nature and have been documented(Colwell, 1986). New nucleotide sequences or genes, indeed, may occur, therefore any gene combination can potentially be found in any single organism and integration of introduced DNA can occur both by homologous and heterologous recombination (Colwell, 1986). Transformation, transfection, transduction, plasmid and conjugative transposon transfer during conjugation, and mobilization of non-conjugative plasmid are known to occur in natural environment.

Table 3: Transducing ability of GMMs at different times under laboratory conditions.

Strains / Time(day)	Zero	1	5	10	15	20	25	30
RS 1(donor) (cfu/ml)	$9.18X10^{11}$	$7.82X10^{9}$	$3.6X10^{7}$	$8.1X10^{6}$	$7.4 X10^{6}$	$8.2 X10^{5}$	$2.9 X10^{3}$	$9 X10^{2}$
PU 21A(recipient) (cfu/ml)	$3.7X10^{11}$	$9.87X10^{9}$	$5.17X10^{7}$	$2.9X10^{6}$	$1.3 X10^{6}$	$7.9 X10^{5}$	$5.5 X10^{3}$	$4.3 X10^{2}$
Transductants(cfu/ml)	-	$5.6X10^{7}$	$1.6X10^{7}$	$3.7X10^{5}$	$1.9 X10^{4}$	$1.6 X10^{3}$	$1.2 X10^{2}$	$2.9 X10^{1}$
Transduction Frequency	-	$1.8X10^{-4}$	$5.04X10^{-5}$	$1.2X10^{-6}$	$5.9 X10^{-8}$	$5.04 X10^{-9}$	$3.7 X10^{-10}$	$9.1 X10^{-11}$
Phage (pfu/ml)	$9.21X10^{13}$	$3.17X10^{10}$	$9.13X10^{9}$	$9.43X10^{7}$	$5.2 X10^{7}$	$9.1 X10^{6}$	$8.1 X10^{5}$	$7.2 X10^{5}$
RS 2(donor) (cfu/ml)	$9.81X10^{11}$	$9.51X10^{9}$	$4.59X10^{7}$	$9.8X10^{6}$	$8.1 X10^{6}$	$7.7 X10^{5}$	$3.7 X10^{3}$	$1.8 X10^{2}$
PA 01A (recipient) (cfu/ml)	$9.68X10^{11}$	$9.46X10^{9}$	$6.22X10^{7}$	$3.6X10^{6}$	$2.1 X10^{6}$	$3.2 X10^{5}$	$1.8 X10^{3}$	$1.2 X10^{2}$
Transductants(cfu/ml)	-	$4.99X10^{7}$	$1.16X10^{7}$	$3.9X10^{5}$	$1.3 X10^{4}$	$6.2 X10^{3}$	$1.5 X10^{2}$	$3.4 X10^{1}$
Transduction Frequency	-	$5.1X10^{-5}$	$1.2X10^{-5}$	$3.9X10^{-7}$	$1.33 X10^{-8}$	$6.3 X10^{-9}$	$1.5 X10^{-10}$	$3.4 X10^{-11}$
Phage(pfu/ml)	$8.36X10^{11}$	$1.9X10^{10}$	$8.32X10^{9}$	$9.1X10^{7}$	$3.9X10^{7}$	$9.5 X10^{6}$	$7.3 X10^{5}$	$9.2 X10^{5}$

PU21 A = $3.17 X 10^{11}$
PAO1 A = $9.76 X 10^{11}$

Control of the spread of the genes to resident organisms depends on many factors in a very complex environment, whether soil, water, or air (Colwell, 1986).

Consitent with these results, Lilley *et al*. (2003) reported that, gene transfer to *Pseudomonas* in the phyllosphere and rhizosphere was found only in the plasmid treatment bacteria.

In contrast, Kluepfel *et al*. (1991)were not able to detecte transfer of Tn7:: LacZY to microbial member of the rhizosphere. Prosser (1994) don't found evidence for transfer of the chromosomally encoded marker gene to the indigenous microflora, Alvarez *et al*. (1996) noted that no transfer of genetic information from GEMs.

Factors influncing GMMs:

It is important to know the effect of environmental factors on GMMs, including biotic (population interactions) and abiotic (UV, pH, salts, temperature) . In this study, abiotic factors were investigated under laboratory conditions. Effect of UV on survival of GMMs and their parents is presented in (Table 4 and Fig.2). The results showed that cfu/ml for all strains was decreased with exposure time.

Table 4: Effect of UV on the survival of GMMs and their parents.

Dose (min) / Strain	Zero	1	5	8	13	20	45	75	105
RS 1	$4.67X10^{11}$	$2.81X10^{11}$	$1.83X10^{11}$	$1.68X10^{11}$	$9.8X10^{10}$	$7.7X10^{10}$	$5.8X10^{10}$	$4.6X10^{10}$	$2.9X10^{10}$
RS 2	$6.36X10^{11}$	$5.48X10^{11}$	$4.32X10^{11}$	$2.96X10^{11}$	$2.65X10^{11}$	$1.98X10^{11}$	$1.02X10^{11}$	$9.8X10^{10}$	$4.3X10^{10}$
PAO 1A	$1.9X10^{12}$	$1.18X10^{11}$	$6.3X10^{10}$	$5.9X10^{10}$	$3.6X10^{10}$	$2.8X10^{10}$	$1.6X10^{10}$	$9.0X10^{9}$	$2.0X10^{9}$
PU 12A	$9.5X10^{12}$	$8.56X10^{11}$	$6.48X10^{11}$	$6.22X10^{11}$	$3.16X10^{11}$	$1.92X10^{11}$	$1.02X10^{11}$	$9.3X10^{10}$	$6.0X10^{9}$
MAM 2A	$1.32X10^{12}$	$1.32X10^{11}$	$1.31X10^{11}$	$1.26X10^{11}$	$1.21X10^{11}$	$1.14X10^{11}$	$1.09X10^{11}$	$8.15X10^{10}$	$4.0X10^{9}$

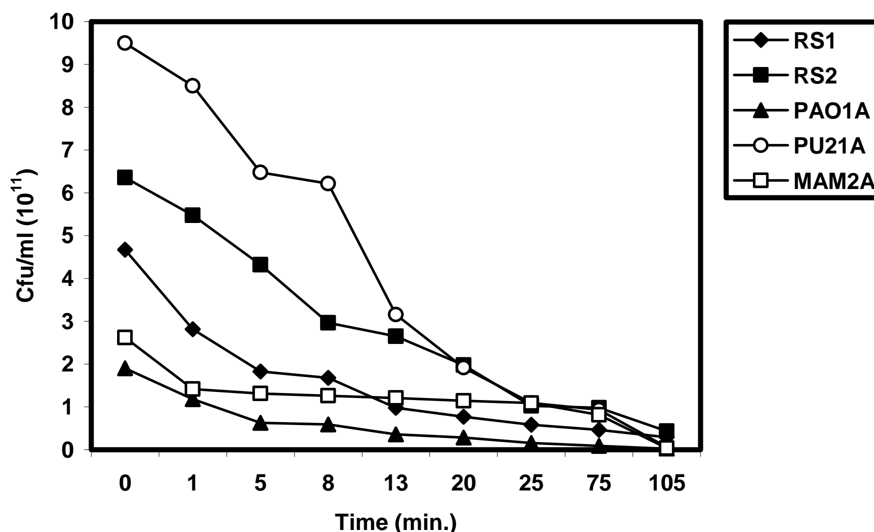

Fig. 2: Effect of UV on the survival of GMMs and their parents.

The GMMs that exposed to UV were used to assess their ability to transduce antibiotic resistant genes (Table 5). Number of transductants were decreased with time (with RS2) although increased of induced phage. This may due to effect of UV on number and quality of transducing particles. With RS2, number of transductants was increased than control and so transduction frequency.

Table 5: Effect of UV on transducing ability of GMMs.

Dose (min) / Stain	Zero	1	5	8	13	20	45	75	105
RS1(donor) (cfu/ml)	$9.21X10^{5}$	$8.6X10^{5}$	$7.8X10^{4}$	$5.3X10^{4}$	$7.0X10^{3}$	$5.0X10^{3}$	$4.1X10^{3}$	$3.3X10^{3}$	$2.8X10^{3}$
Recipient (cfu/ml)	$1.91X10^{6}$	$1.18X10^{6}$	$1.3X10^{6}$	$1.12X10^{6}$	$1.3X10^{6}$	$6.23X10^{5}$	$4.81X10^{5}$	$3.52X10^{5}$	$3.16X10^{5}$
Transductant (cfu/ml)	$1.36X10^{6}$	$9.78X10^{5}$	$4.16X10^{5}$	$2.56X10^{5}$	$1.59X10^{5}$	$1.12X10^{5}$	$4.1X10^{4}$	$3.2X10^{4}$	$2.1X10^{4}$
Transduction frequency	$1.7X10^{-6}$	$1.2X10^{-6}$	$5.2X10^{-7}$	$3.2X10^{-7}$	$2.0X10^{-7}$	$1.4X10^{-7}$	$5.1X10^{-8}$	$4.1X10^{-8}$	$2.6X10^{-8}$
Phage(pfu/ml)	$1.32X10^{10}$	$1.65X10^{10}$	$1.11X10^{10}$	$9.73X10^{10}$	$2.043X10^{10}$	$7.28X10^{9}$	$1.201X10^{8}$	$9.36X10^{7}$	$3.8X10^{6}$
RS2 (donor) (cfu/ml)	$1.65X10^{6}$	$1.93X10^{6}$	$1.12X10^{6}$	$9.31X10^{5}$	$1.39X10^{5}$	$1.14X10^{5}$	$1.08X10^{5}$	$9.45X10^{5}$	$7.12X10^{5}$
Recipient (cfu/ml)	$1.19X10^{6}$	$1.21X10^{6}$	$1.20X10^{6}$	$1.12X10^{6}$	$1.25X10^{5}$	$1.28X10^{5}$	$1.12X10^{5}$	$1.12X10^{5}$	$1.11X10^{5}$
Transductants (cfu/ml)	$1.65X10^{6}$	$2.14X10^{6}$	$2.17X10^{6}$	$1.26X10^{6}$	$2.11X10^{6}$	$2.29X10^{6}$	$2.34X10^{6}$	$2.61X10^{6}$	$2.69X10^{5}$
Transduction frequency	$2.0X10^{-6}$	$2.6X10^{-6}$	$2.7X10^{-6}$	$1.5X10^{-6}$	$2.6X10^{-6}$	$2.8X10^{-6}$	$2.9X10^{-6}$	$3.2X10^{-6}$	$3.3X10^{-5}$
Phage(pfu/ml)	$1.28X10^{10}$	$1.53X10^{10}$	$1.31X10^{10}$	$1.07X10^{10}$	$9.68X10^{9}$	$6.90X10^{9}$	$1.21X10^{8}$	$8.14X10^{7}$	$1.6X10^{6}$

PU 21A = $7.89X10^{11}$
PAO 1A = $8.14X10^{11}$

Effect of pH on survival and trnsducing ability was studied. The highest cfu/ml of all strains was at pH7, extreme pH values (2,12) were more effect on survival (Table 6 and Fig.3). The results of transduction in (Table 7) appeared that, pH7 was the better. No viable cells or phage counts have been observed in extreme acid (pH2). Transduction frequency was dropped to $0.6x10^{-4}$, $0.9x10^{-4}$ for two GMMs (RS1 , RS2) at alkaline pH (pH 12). These results indicated that pH more marked under acidic than under alkaline conditions.

Table 6: Effect of pH on the survival of GMMs and their parents.

pH / Strain	2	5	7	10	12
RS 1	$1.6X10^{4}$	$2.56X10^{11}$	$7.1X10^{12}$	$3.11X10^{11}$	$1.58X10^{8}$
RS 2	$8.0X10^{3}$	$3.7X10^{10}$	$6.9X10^{12}$	$2.7X10^{12}$	$1.14X10^{9}$
PAO1A	$9.0X10^{2}$	$1.4X10^{10}$	$1.6X10^{12}$	$1.7X10^{12}$	$5.0X10^{4}$
PU12A	$3.0X10^{2}$	$1.2X10^{10}$	$1.3X10^{12}$	$1.9X10^{12}$	$3.0X10^{4}$
MAM2A	$2.0X10^{2}$	$5.28X10^{11}$	$4.9X10^{12}$	$2.3X10^{12}$	$8.0X10^{4}$

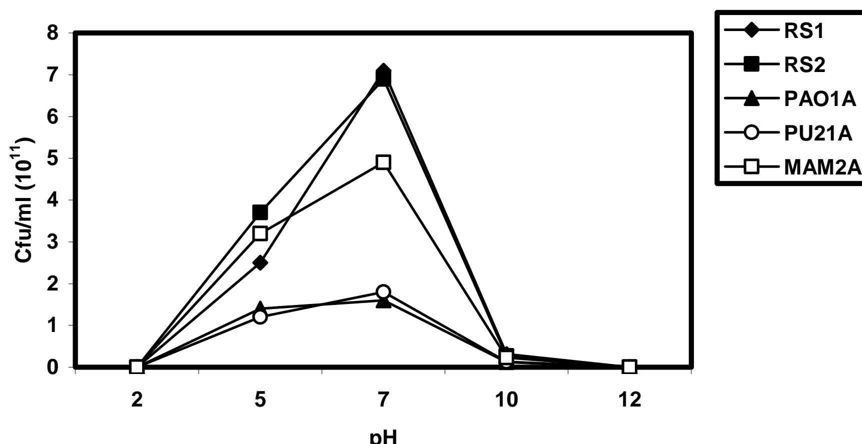

Fig. 3: Effect of pH on the survival of GMMs and their parents.

Table 7: Effect of pH on transducing ability of GMMs.

Strain \ pH	2	5	7	10	12
RS 1(cfu/ml)	—	$6.58X10^7$	$7.65X10^7$	$6.18X10^7$	$5.28X10^7$
Recipient(cfu/ml)	—	$9.46X10^7$	$1.07X10^8$	$9.39X10^7$	$9.11X10^7$
Transductants (cfu/ml)	—	$4.96X10^7$	$5.13X10^7$	$4.58X10^7$	$1.22X10^7$
Transduction frequency	—	$2.5X10^{-4}$	$2.6X10^{-4}$	$2.3X10^{-4}$	$0.6X10^{-4}$
Phage(pfu/ml)	—	$1.57X10^{10}$	$2.16X10^{10}$	$1.63X10^{10}$	$6.11X10^9$
RS 2(cfu/ml)	—	$7.21X10^7$	$9.83X10^7$	$8.43X10^7$	$6.17X10^7$
Recipient(cfu/ml)	—	$9.25X10^7$	$9.46X10^7$	$9.15X10^7$	$8.99X10^7$
Transductants (cfu/ml)	—	$3.89X10^7$	$4.99X10^7$	$4.13X10^7$	$1.41X10^7$
Transduction frequency	—	$2.4X10^{-4}$	$3.1X10^{-4}$	$2.5X10^{-4}$	$0.9X10^{-4}$
Phage(pfu/ml)	—	$1.25X10^{10}$	$1.94X10^{10}$	$121X10^{10}$	$4.33X10^9$

PU 21A = $1.97 X 10^{11}$ **PAO 1A = $1.63 X 10^{11}$**

The acidic pH may change the configuration of the cell surfaces which contain the site receptors for phage adsorption. There is no single optimum pH for gene transfer in general and optimum pH may depend on the transferred marker, characteristics of donor and recipient cells. Phage F116 is not able to form transductants at extremes pH even under laboratory conditions (Amin and Day 1988).

The influence of mono (Na^+) di (Ca^{++}). And tri (Fe^{+++}) ions on the survival and ability of GMMs to transduce their genetic material were studied. The cations that have been used in this study were chosen to chare one anion (Cl^-). So any observed effect will be mostly due to the action of the tested cation. The influence of Na^+ (Table 8, Fig.4 and Table 9), all examined concentrations (10-200mM) seem to have inhibition effect on survival and gene transfer, with the exception of concentrations (10,50,100) that increased transduction frequency with RS2 only. The influence of Ca^{++} (Table 10, Fig.5 and Table 11), no remarkable stimulation effect on survival. Transduction frequency was increased with RS2 only, it is ranged from