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THE FITNESS OF A GEM STRAIN OF *Pseudomonas aeruginosa* BACTERIA COMPARING WITH ITS PARENT STRAINS UNDER LABORATORY CONDITIONS

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ABSTRACT: The fitness of a genetically engineered microorganism strain that constructed as transconjugant by conjugation mechanism of horizontal gene transfer in *Pseudomonas aeruginosa* bacteria comparing with its parent strains has been assessed. Ecological fitness expresses interaction of an organism with its environment. This consider as a good indicator for the assessment of Genetically Engineered Microorganism (GEM). When released into the environment this assay was carried out under laboratory condition to investigate the effects of environmental condition on the fitness. These laboratory condition included the effect of different cations (Na^+, Ca^{+2}, and Fe^{+3}), different incubation temperatures, pH values and different ratios of the two parents. Increasing of mono, and di cations (Na^+, Ca^{+2}) concentrations revealed an increasing in the fitness of parents and transconjugant (GEM) strain up to the maximum concentration (105 mM) with Na^+ and (85 mM) with Ca^{+2} and then began to decrease. In the three strains temperature plays an important role in gene transfer rate and fitness, There was a direct relationship between the increasing in temperature until it reaches the optimum temperature and the rate of gene transfer. Also, the highest rate of gene transfer *via* conjugation appeared when the ratio between the donor and the recipient was 3:1. The best results for bacterial fitness for transconjugant strain and parents appeared at pH 9, which means increased fitness for both of them. These results show that the transconjugant strain can be have and survive well as its parent strain and the effects of some ecological factor are similar in the three strains.

Key words: *Pseudomonas aeruginosa*, conjugation, transconjugant, Ecological fitness, bacteria, genetically engineered microorganism (GEM).

INTRODUCTION

Horizontal gene transfer (HGT) is primarily responsible for the genetic diversity (evolution) in bacteria **(Wiedenbeck and Cohan, 2011)**. Plasmids play an important role in this process *via* conjugation **(Summers, 2009).** The most significant indication of the importance of plasmids in bacterial adaptation is their role in the spread of antibiotic resistance among strains **(Carattoli, 2013).** plasmids it play an important role in helping bacteria adapt to different conditions in bacterial environments **(Smillie *et al.*, 2010; Smalla *et al.*, 2015; San Millan *et al.*, 2016).**

Temperature is one of the most important factors affecting gene transfer rate and fitness, increased temperature increases fitness for parents and recombinant strain until the optimum temperature for bacteria is reached **(Headd and Bradford, 2018)**. The dependence of gene transfer on temperature is similar to the relationship between temperature and growth rate **(Johnson and Kroer, 2007).**

The value of PH affects the gene transfer rate in *Pseudomonas aeruginosa*, where gene transfer occurs at a pH degrees of 5-8 and the fitness between the parent and transconjugant GEM strains **(Amin and Day, 1988).**

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The compositions of bacterial genomes can be changed rapidly by some mechanisms such as horizontal gene transfer. This change plays a role to bacterial evolution. Horizontal gene transfer resulting in the incorporation of genetic elements transferred from another organism directly into the genome. These genetic elements whose function increase bacterial fitness. Fitness can be varied into ecological fitness, symbiosis fitness and pathogenicity fitness **(Hacker and Carniel, 2001).**

The ecological or environmental fitness describes the interaction of an organism with it's environment. The ecological fitness of an organism will differ according to the environment into which it is released. There is a lack of data about the ecological fitness of the genetically engineered microorganism (GEM) that can be released into the environment. The wider ecological can sequences of such release need to be determined. This is especially important if there is reason to believe that a GEM is ecologically more fit than the wild type organisms, or parent organisms from which it was derived **(Seidler, 1992).**

So, the aim of this study is to show the fitness between a genetically engineered microorganism (GEM) strain that constructed by conjugation mechanism of horizontal gene transfer and its parents under different abiotic and biotic stresses.

MATERIALS AND METHODS

All experiments was carried out at Microbial Genetics Research Laboratory, Faculty of Agriculture, Genetics Department, Zagazig University.

Used Strains

Pseudomonas aeruginosa bacteria strains PAO1, PU21 and MAM2 that used in this study were obtained from M. Day, university of wales, Cardiff, UK.

Nutrient Media

Different culture media were used in this study namely Luria broth (LB), nutrient broth (NB) and nutrient agar (NA). NB medium was prepared by mixing 10 g peptone, 5 g yeast extract and 5 NaCl in 1 L distilled water (dH2O). Medium pH was adjusted at the range of 7.0–7.2. For semi-solid nutrient agar (NA) medium, 1.5% agar was added. Then medium was autoclaved at 121ºC for 20 min.

Phosphate buffer was prepared from 1/15 M potassium phosphate (KH_2PO_4) and 1/15 M disodium phosphate ($Na_2 HPO_4. 2H_2O$).

The concentration of tetracycline was 2.0 µg/ml and the concentration of Chloramphenicol was 1200 µg/ml. Both antibiotics are dissolved in distilled water.

Conjugation Experiment

Pseudomonas aeruginosa PAO1 strain was used as the donor strain and *Pseudomonas aeruginosa* MAM2 was used as the recipient strain in this experiment. They were inoculated in 25 ml liquid media, incubated for 24 hours at 30°C with shaking at 160 rpm, and then by syringe 1 ml of donor strain was taken and passed through a filter membrane filtered onto a 0.22 µm pore size so that bacterial cells are passed through the holes. The same was done for recipient strain. The donor and the recipient strain filters were placed face to face on NA media. Then it was incubated at a four different types of treatments, various temperature, various time, modified media contain different concentrations of salt and different pH degrees. This was to estimate the fitness of these strains under different types of laboratory conditions **(Amin *et al*., 2008).**

1 ml of the donor and 1 ml of the recipient were placed on filter membranes, and then both filters were placed face to face. In these experiments, the ratio 1:1 per volume has been changed into 2:1 and 3:1 donor to recipient and 1:2, 1:3 donor to recipient.

Isolation of Resistant Bacterial Mutants

The mutants resistant to the tetracycline and chloramphenicol were obtained by culturing both of MAM2 and PAO1 bacteria overnight in broth culture at 30°C, then spreading 1 ml of fresh broth culture on NA media containing different concentration of both antibiotics, plates were incubated at 30°C for 5:7 days until appearance of single colonies in the case of antibiotics resistant mutants which tested and propagated again on a fresh media supplemented with the same previously mentioned antibiotics.

Bacterial strains of *Pseudomonas aeruginosa*

Strain	Phenotype/Genotype	Reference
PAO1 (donor).	Prototrophic	**Holloway and Morgan (1986)**
	Tetracycline resistant (Tet^R)	This study
	Chloramphenicol (Chl^S)	
MAM2 (recipient).	Auxotrophic, Met^-	**Amin *et al.* (1987)**
	Chloramphenicol (Chl^R)	This study
	Tetracycline resistant (Tet^S)	
Pu21	Auxotrophic, Val^-	**Amin and Day (1988)**
	Chloramphenicol (Chl^R)	This study
	Tetracycline resistant (Tet^S)	

Effect of Incubation Temperature

Both the donor and the recipient strains were prepared as previously mentioned. The donor and the recipient strains filters were placed face to face on NA media. The plates were incubated at different temperatures (4°C, 15°C, 25°C, 30°C, 35°C and 40°C). After incubation, the incubated filters were taken overnight and placed in a tube containing phosphate buffer pH 6.8 in a glass test tube.

Then a series of dilutions was made. And from each dilution 3 replication was done to calculate the CFU/ml for both donor, recipient and transconjugants. To know the extent of the effect of high or low temperature on the gene transfer rate.

Enumeration of Bacteria

CFU/ml was calculated for both donor and recipient at zero time and 24 h. CFU/ml was calculated after incubation in each experiment for donor, recipient and transconjugants. After incubation, the two incubated filters were placed together in 10 ml phosphate buffer and then a series of dilutions were made. Three replica for each of donor, recipient, and Transconjugant on the selected media for each of them 0.1 ml is taken from this donor, recipient, and Transconjugant for each plate. The CFU/ml and Conjugation frequency were calculated according to the following formulas:

CFU/ml = no. of colonies x dilution factor x 0.1

Conjugation frequency = transconjugant/ recipient at zero time

Statistical Analysis

The average and standard deviation were calculated using Excel 2010 program for all analyzed data.

RESULTS AND DISCUSSION

Isolation of Antibiotic Resistant Bacteria

Tetracycline and chloramphenicol

The three strains of *Pseudomonas aeruginosa* bacteria (PAO1, MAM2 and PU21) were tested on tetracycline where a series of concentrations were made, 20 µg/ml, 30 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml and 200 µg/ml, the three strains were resistant to this antibiotic to a concentration of 100µg/ml but at concentration 200 µg/ml it gave resistant result with PAO1 while it was negative with MAM2 and PU21. Depending on these results, PAO1 was used as a donor and MAM2 as a recipient. The data also showed in Table 1. As well, the three strains of bacteria (PAO1, MAM2 and PU21) were tested on chloramphenicol where a series of concentrations were made, 20 µg/ml, 30 µg/ml, 40 µg/ml, 60 µg/ml, 8 0µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml, 600 µg/ml, 700 µg/ml, 800 µg/ml, 1000 µg/ml and 1200 µg/ml. The three strains were resistant to this antibiotic to a concentration of 1000 µg/ml, but at concentration 1200 µg/ml it gave positive result with MAM2 and PU21 while it was negative with PAO1. The data also showed in Table 1 **(Dean *et al.*, 2003; Amina and Amin, 2010).**

Table 1. Effect of different concentrations of tetracycline and chloramphenicol on *Pseudomonas aeruginosa* strains PAO1, MAM2 and PU21

Antibiotics / Strains	Tetracycline				Chloramphenicol				
	20 µg	80 µg	100 µg	200 µg	20 µg	100 µg	600 µg	1000 µg	1200 µg
PAO1	+	+	+	+	+	+	+	+	–
PU21	+	+	+	–	+	+	+	+	+
MAM2	+	+	+	–	+	+	+	+	+

Effect of different concentrations of NaCl on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

It was noted that the increase in the concentration of NaCl leads to an increase in the conjugation rate as well as an increase in the fitness of both parents and the recombinant strain until 105 mM from NaCl where conjugation frequency reached to 6.51×10^{-6} then decreased after this concentration. This decrease may be due to the fact that the surface roughness is expected to change (morphological change) and this is due to the large ionic strength of the solution **(Kim *et al.*, 2009)**. This result may be due to extreme difficult for the pilus to extend freely in a 125 mM NaCl media. This is compatible with its low salinity solutions the pilus would be free to extend into media **(Headd and Bradford, 2018).** The data also showed in Table 2.

Effect of different concentrations of $CaCl_2$ on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

It was noted that the increase in the concentration of $CaCl_2$ leads to an increase in the conjugation rate as well as an increase in the fitness of both parents and the transconjugant strain until 85 mM from $CaCl_2$ with conjugation frequency reached to (3.58×10^{-6}) then decreased at 105 mM, 125 mM and at 200 mM was zero these results do agree with others **(Amin *et al.*, 2008)** The data also showed in Table 3.

Effect of different concentrations of $FeCl_3$ on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

It was noted that the highest conjugation rate and the best bacterial fitness were (1.16×10^{-6}) at 0 $FeCl_3$ and at 15 mM concentration the bacterial numbers was significantly decreased for both parents and transconjugant (0.158×10^{-6}) while it was zero at the following concentrations of $FeCl_3$. This may be due to the fatal effect of ferric cation on *Pseudomonas aeruginosa* cells, as it may lead to DNA damage, enzyme protein oxidation or membrane lipid oxidation.as the ability of Fe^{+3} to cause mutagenicity in bacteria has been extensively documented **(Kanematsu *et al.*, 1980; Arlauskas *et al.*, 1985; Armijo *et al.*, 2020)** The data also showed in Table 4.

Effect of temperature on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

It was observed that with increasing temperature the conjugation rate and fitness increased as the fitness was highest at 35°C (3.23×10^{-6}), there was no fitness at 4°C and it rose somewhat at 15°C, 25°C and 30°C then decreased at 40°C to the lowest level. Data represented in Table 5 show that. These results were in agreement with others **(Bale *et al.*, 1987; O'morchoe *et al.*, 1988; Johnsen and Kroer, 2007; Headd and Bradford, 2018).**

Effect of pH on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

It was noted that at pH 2 and pH 11 there was no fitness for both parents and transconjugant strain, While the fitness was the best possible at pH 9 with conjugation frequency reached to (16.7×10^{-6}). These results were according with previous results **(Fry and Day, 1990; Amin *et al.*, 2008).** The data also showed in Table 6.

Table 2. Effect of different concentrations of NaCl on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

NaCl concentration mM	Donor (CFU/ml) at 24 hours	Percent survival for donor	Recipient (CFU/ml) at 24 hours	Percent survival for recipient	No. of transconjugants cells ($x10^2$) per Plate			Mean of transconjugant CFU/ml ($x10^3$) ± SD	Percent survival for □	Conjugation Frequency ($x10^{-6}$) per recipient at zero time
					R1	R2	R3			
Zero NaCl	4.1×10^6	100	3.3×10^6	100	0.47	0.57	0.62	0.55 ± 0.08	100	0.883
5 mM	4.2×10^6	97.6	3.5×10^6	106	1.04	0.84	0.85	0.91 ± 0.11	166	1.44
15 mM	4.3×10^6	102.3	4.7×10^6	142.4	0.58	0.60	0.47	0.64 ± 0.09	116	1.02
55 mM	5.2×10^6	123.8	5.4×10^6	163.6	1.28	1.43	1.27	1.3 ± 0.09	236	2.06
85 mM	7.2×10^6	171.4	6.2×10^6	187.8	2.23	2.25	2.29	2.26 ± 0.03	410	3.49
105 mM	7.8×10^6	185.7	6.5×10^6	196.9	0.44	0.49	0.31	4.1 ± 0.09	745	6.51
125 mM	1.1×10^4	0.268	7.1×10^5	21.5	1.61	1.50	1.48	1.53 ± 0.06	278	2.43

Recipient at zero time = 6.3×10^8 cfu/ml Donor at zero time = 9.7×10^8 cfu/ml

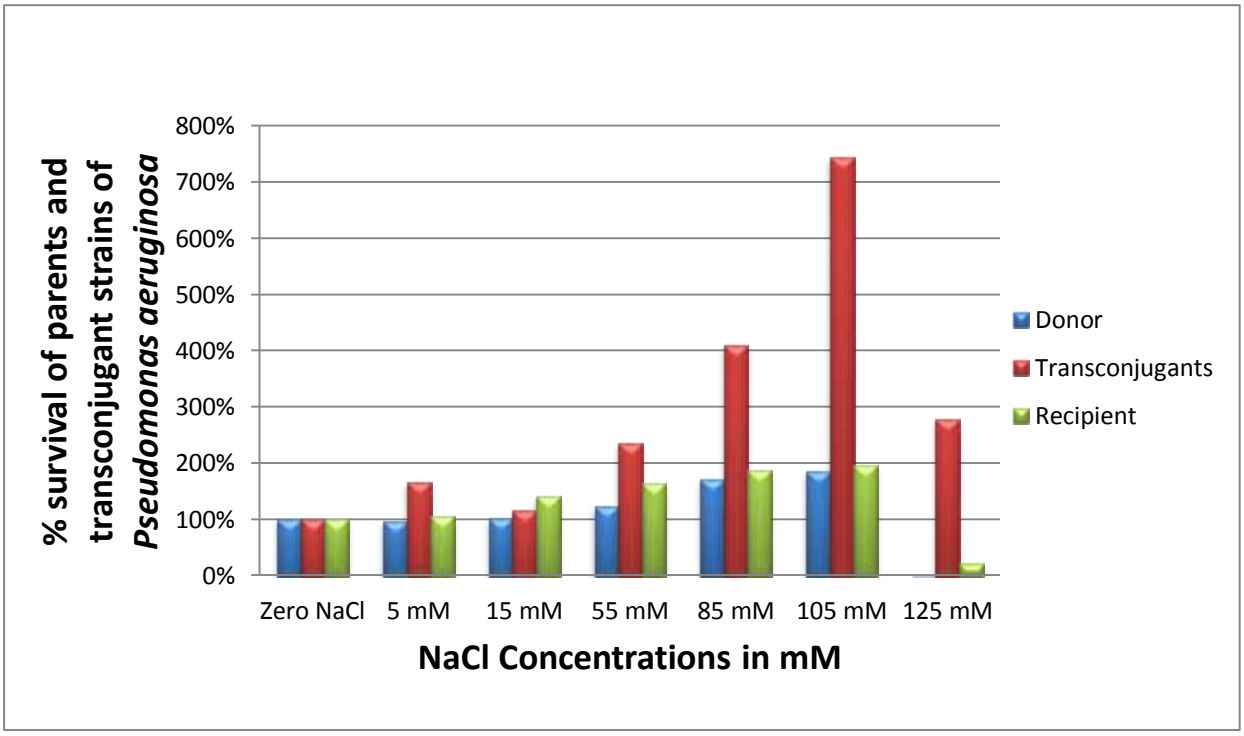

Fig. 1. Effect of different concentrations of NaCl on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Table 3. Effect of different concentrations of $CaCl_2$ on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

$CaCl_2$ concentration mM	Donor (CFU/ml) at 24 hours	Percent survival for donor	Recipient (CFU/ml) at 24 hours	Percent survival for recipient	No. of transconjugants cells ($x10^2$) per Plate			Mean of transconjugant CFU/ml ($x10^3$) ± SD	Percent survival for □	Conjugatio frequency ($x10^{-6}$) per recipient at zero time
					R1	R2	R3			
Zero NaCl	1.1×10^7	100	1.2×10^7	100	0.96	0.8	1	0.92 ± 0.5	100	0.766
5 mM	1.2×10^7	109	5.8×10^7	483	0.77	0.59	0.68	0.68 ± 0.09	73.9	0.567
15 mM	1.4×10^7	127	7.8×10^7	650	1.09	1.33	1.18	1.2 ± 0.12	130.4	1
55 mM	7.1×10^7	645	7.9×10^7	658	1.95	1.77	1.98	1.9 ± 0.11	206.5	1.58
85 mM	7.9×10^7	718	9.9×10^7	825	2.97	2.63	2.83	2.81 ± 0.17	305.4	2.34
105 mM	8.3×10^7	582	9.1×10^7	758	1.48	1.55	1.77	1.6 ± 0.15	173.9	1.3
125 mM	4.8×10^6	43.6	3.5×10^6	29.16	0.91	0.77	0.75	0.81 ± 0.09	88	0.675
200 mM	6.1×10^3	0.06	2.6×10^3	0.021	0	0	0	0	0	0

Recipient at zero time = 1.2×10^9 CFU/ml Donor at zero time = 9.2×10^9 CFU/ml

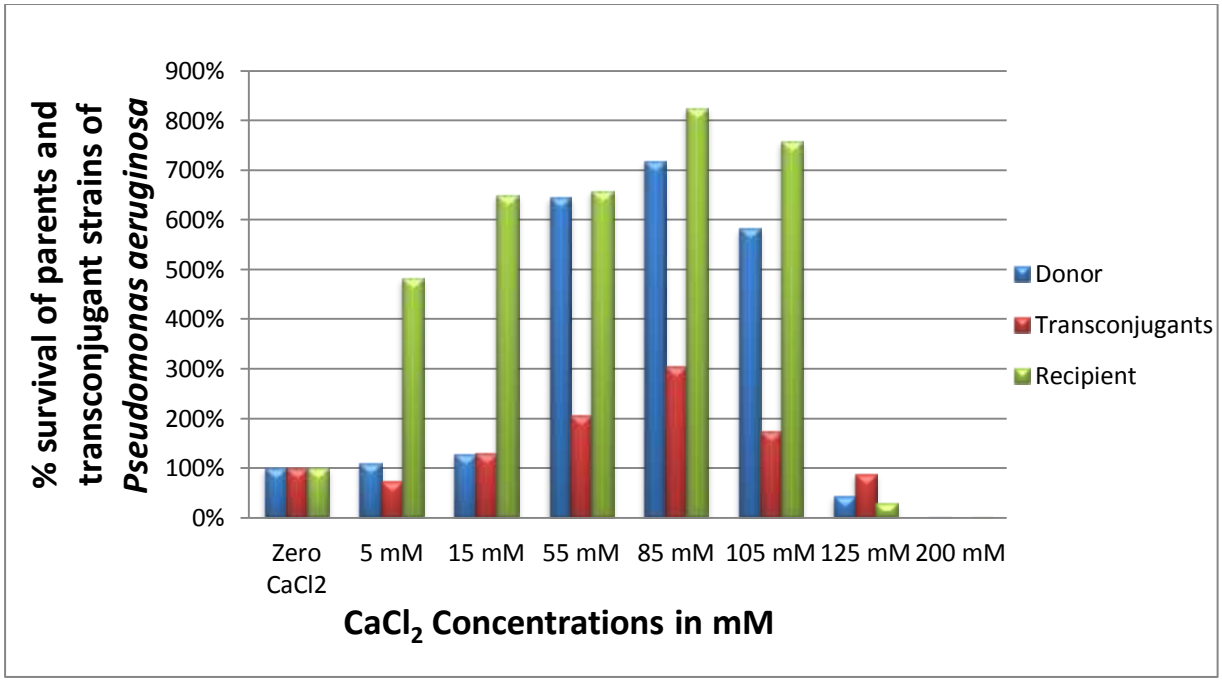

Fig. 2. Effect of different concentrations of $FeCl_3$ on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Table 4. Effect of different concentrations of $FeCl_3$ on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

$FeCl_3$ concentration mM	Donor (CFU/ml) at 24 hours	Percent survival for donor	Recipient (CFU/ml) at 24 hours	Percent survival for recipient	No. of transconjugants cells ($x10^2$) per Plate			Mean of transconjugant CFU/ml ($x10^3$) ± SD	Percent survival for □	Conjugation Frequency ($x10^{-6}$) per recipient at zero time
					R1	R2	R3			
Zero NaCl	7.3×10^7	100	9.2×10^7	100	1.35	1.46	1.41	1.4 ± 0.07	100	1.16
5 mM	5.97×10^6	8.18	8.3×10^5	0.9	0.81	0.76	0.71	0.76 ± 0.05	54.3	0.633
15 mM	6.41×10^4	0.09	9.2×10^3	0.0001	1.83	1.91	1.96	0.19 ± 0.07	13.57	0.158
55 mM	0	0	0	0	0	0	0	0	0	0
85 mM	0	0	0	0	0	0	0	0	0	0
105 mM	0	0	0	0	0	0	0	0	0	0
125 mM	0	0	0	0	0	0	0	0	0	0
200 mM	0	0	0	0	0	0	0	0	0	0

Recipient at zero time = 1.2×10^9 CFU/ml Donor at zero time = 9.2×10^8 CFU/ml

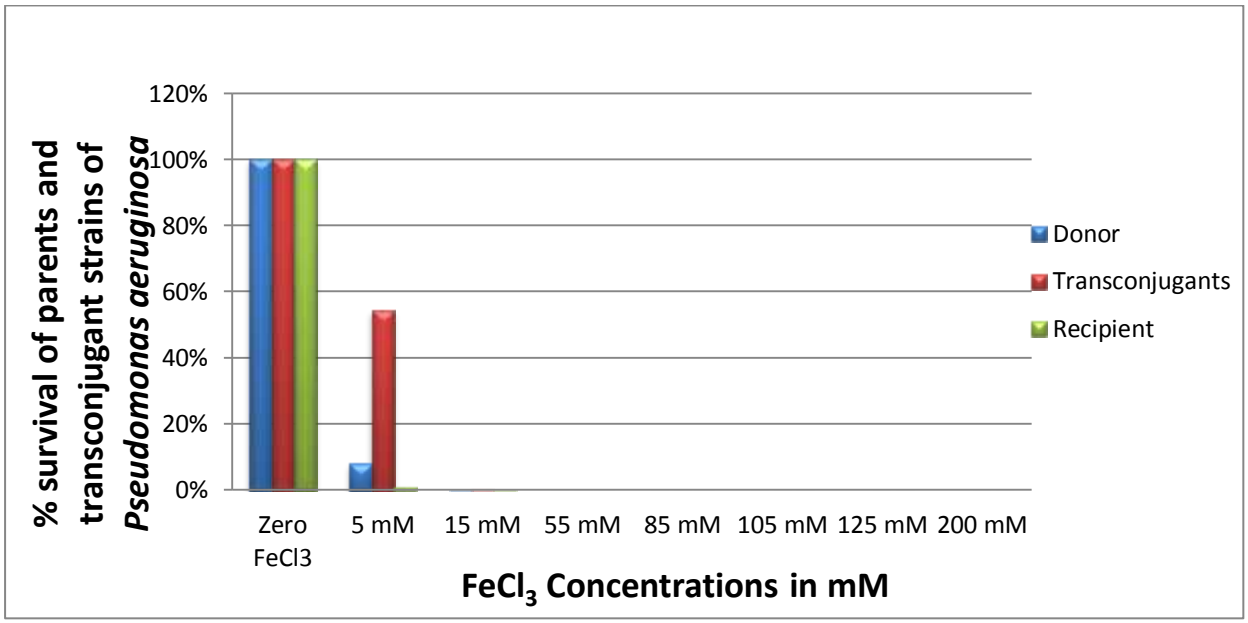

Fig. 3. Effect of different concentrations of $FeCl_3$ on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Table 5. Effect of temperature on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Temperature °C	Donor (CFU/ml at 2 hours	Percent survival for donor	Recipient (CFU/ml)at 24 hours	Percent survival for recipient	No. of transconjugants cells ($x10^2$) per Plate			Mean of transconjugant CFU/ml ($x10^3$) ± SD	Percent survival for □	Conjugation Frequency ($x10^{-6}$) per recipient at zero time
					R1	R2	R3			
4°C	7.2×10^6	12.2	1.1×10^6	1.33	0	0	0	0	0	0
15°C	8.2×10^6	13.9	2.5×10^7	30	1.31	1.36	1.47	1.38 ± 0.08	32.9	1.06
25°C	9.2×10^6	15.6	2.9×10^7	34.9	1.74	1.64	1.51	1.63 ± 0.11	38.8	1.25
30°C	1.4×10^7	23.7	4.1×10^7	49.4	2.5	2.62	2.35	2.49 ± 0.14	59.3	1.92
35°C	5.9×10^7	100	8.3×10^7	100	0.36	0.43	0.47	4.2 ± 0.06	100	3.23
40°C	1.9×10^4	0.03	7.2×10^4	0.09	1.08	0.97	1.07	1.04 ± 0.06	24.8	0.8

Recipient at zero time = 1.3×10^9 CFU/ml Donor at zero time = 9.1×10^8 CFU/ml

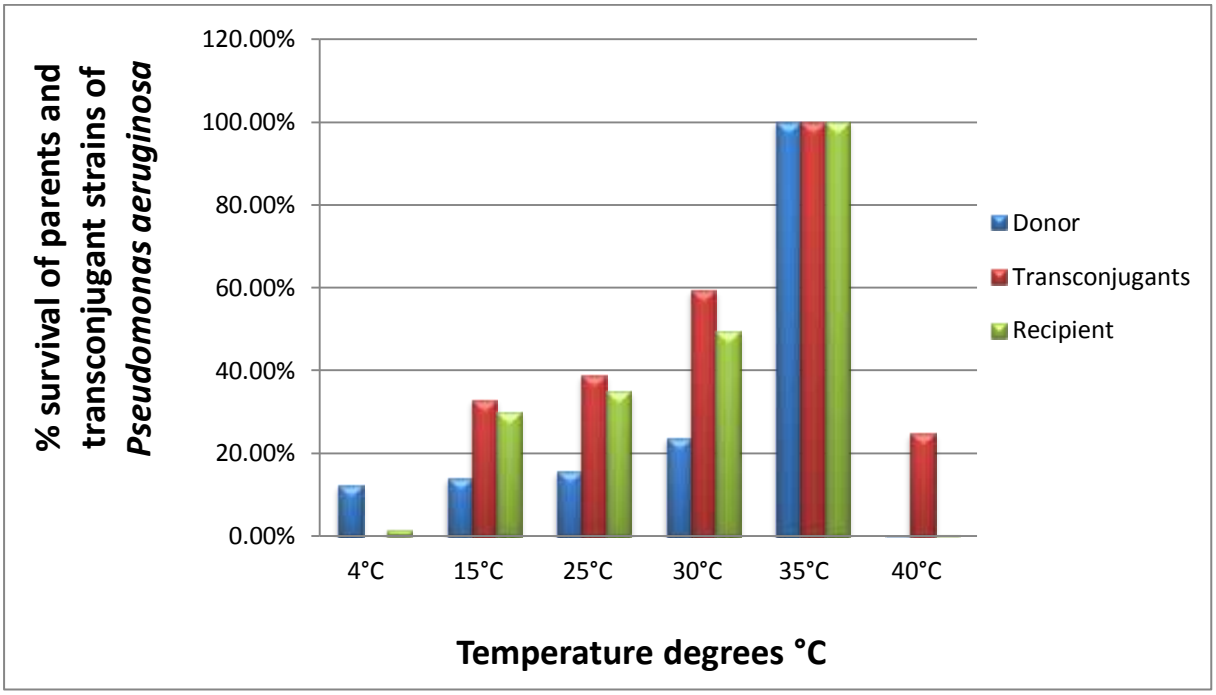

Fig. 4. Effect of temperature on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Table 6. Effect of pH on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

pH	Donor (CFU/ml) at 24 hours	Percent survival for donor	Recipient (CFU/ml at 24 hours	Percent surviva for recipient	No. of transconjugants cells ($x10^2$) per Plate			Mean of transconjugant CFU/ml ($x10^3$) ± SD	Pcent survival for □	Conjugation Frequency ($x10^{-6}$) per recipient at zero time
					R1	R2	R3			
PH 2	$1.4 X 10^3$	0.13	$1.7 X 10^5$	0.18	0	0	0	0	0	0
PH 4	$2.3 X 10^4$	2.09	$1.9 X 10^6$	1.96	0.41	0.43	0.39	0.41 ± 0.02	11.7	1.95
PH 6.6	$1.1 X 10^6$	100	$9.7X 10^7$	100	2.89	2.98	2.73	2.86 ± 0.13	100	13.6
PH 9	$1.76 X 10^4$	1.6	$3.5 X 10^4$	0.04	0.34	0.40	0.31	3.5 ± 0.05	81.7	16.7
PH 11	0	0	0	0	0	0	0	0	0	0

Recipient at zero time = $2.1 x 10^8$ CFU/ml Donor at zero time = $2.8 x 10^8$ CFU/ml

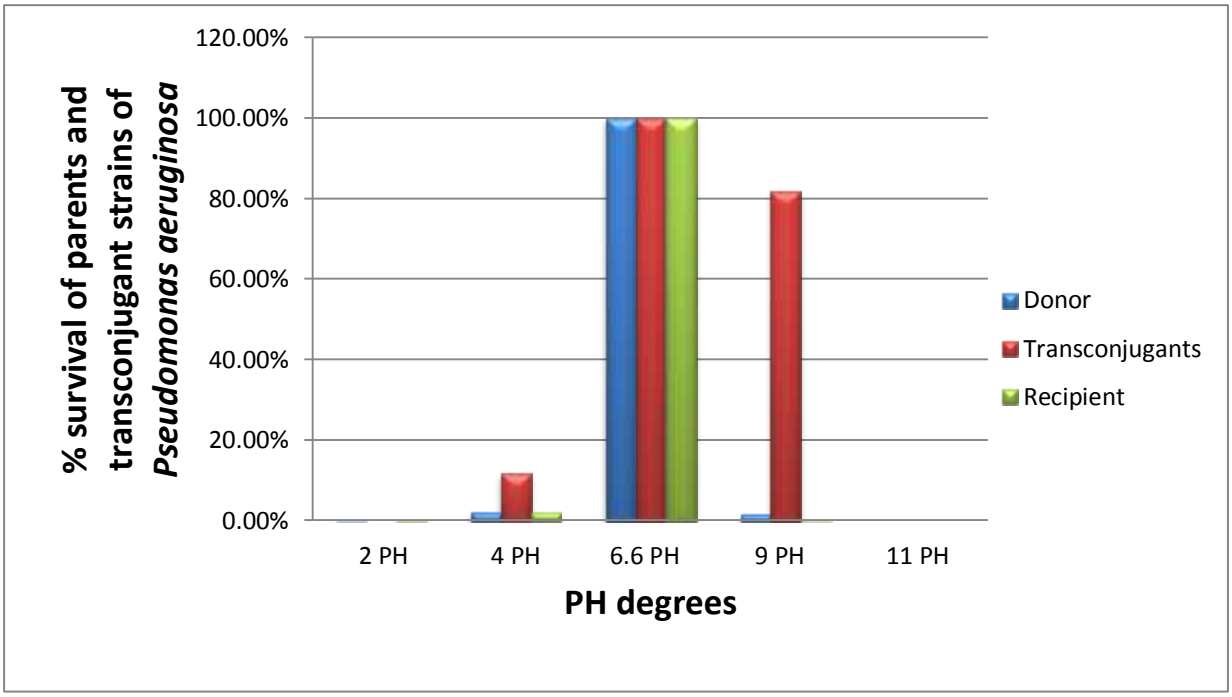

Fig. 5. Effect of pH on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Effect of ratio between donor and recipient on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

The highest number of the conjugation rate and fitness increased as the fitness when the ratio between donor and recipient was 3: 1 with conjugation frequency of (1.53×10^{-6}) per recipient at zero time. As for the other ratios, they gave fewer results. These results are according to **O'morchoe *et al.* (1988) and Amin *et al.* (2008)**. The data also showed in Table 7.

Table 7. Effect of ratio between donor and recipient on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

Ratio (♂ : ♀) ml/ml	Donor (CFU/ml) at 2 hours	Percent survival for donor	Recipient (CFU/ml) at 24 hours	Percent Survivalfor recipient	No. of transconjugants cells ($x10^2$) per Plate			Mean of transconjugant CFU/ml ($x10^3$) ± SD	Percent survival for □	Conjugation Frequency ($x10^{-6}$) per recipient at zero time
					R1	R2	R3			
1 : 1	7.9×10^6	100	9.5×10^7	100	0.72	0.61	0.74	0.69 ± 0.07	100	0.575
1 : 2	7.1×10^6	89.87	8.4×10^8	305	1.52	1.36	1.35	1.41 ± 0.10	204	1.18
1 : 3	7.4×10^7	93.67	2.4×10^8	378.9	0.92	0.82	0.71	0.85 ± 0.11	123	0.708
2 : 1	1.1×10^9	115	1.1×10^8	11.58	0.94	0.85	0.82	0.87 ± 0.06	126	0.725
3 : 1	2.11×10^9	177	1.1×10^8	13.68	1.94	1.69	1.86	1. 83 ± 0.13	265	1.53

Recipient at zero time = 2.1×10^8 CFU/ml Donor at zero time = 2.8×10^8 CFU/ml

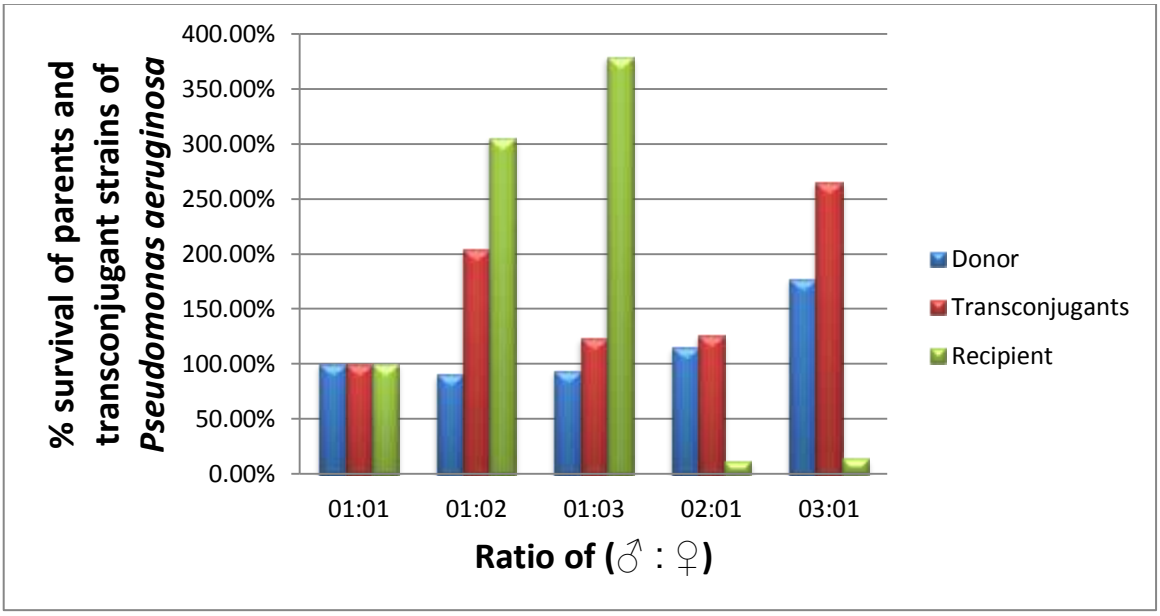

Fig. 6. Effect of ratio between donor and recipient on the fitness of parents and transconjugant strains of *Pseudomonas aeruginosa*

DISCUSSION

The progress of evolution is determined by an increase of the fitness of the organism. Fitness in this concept is considered to be a set of features that improve the survival, spread and or transmission of an organism within a specific ecological niche. The results of this study clearly show that the GEM strain (transconjugant) has a fitness nearly equal in survival when compared with its parental strains. More over the effects of tested laboratory conditions were similar in the three strains. These results are important in the field of microbial biotechnology. This field requires the release and dispersal of some genetically modified microorganisms (GMM) to the different ecosystems. The estimation of both, the efficacy and potential risk associated with the use of GMMs require an understanding of the fitness of microorganisms associated with different ecosystems.

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كفاءة سلالة مهندسة وراثيا من بكتريا *Pseudomonas aeruginosa* مقارنة بالسلالات الأبوية تحت الظروف المعملية

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توضح الدراسة الحالية تقييم ملاءمة سلالة من الكائنات الحية الدقيقة المهندسة وراثيًا والمحولة عن طريق ميكانيكية الأقتران البكتيري لنقل الجينات افقيا في بكتيريا *Pseudomonas aeruginosa* ومقارنتها بسلالات الأباء ، الكفاءة البيئية عبارة عن تفاعل الكائن الحي مع بيئتة ، وهذا مؤشر جيد لتقييم الكائنات الحية الدقيقة المهندسة وراثيًا (GEM) وتم إجراء الاختبارات تحت ظروف معملية لمعرفة تأثير الظروف البيئية المختلفة علي كفاءة البكتريا من حيث تاثير الكاتيونات المختلفة من Na^{+} و Ca^{+2} و Fe^{+3} ودرجات حرارة التحضين المختلفة وقيم الأس الهيدروجيني والنسب بين كلا من المعطي والمستقبل حيث كشفت زيادة تركيز كلا من الكاتيونات الاحادية والثنائية Na^{+} وCa^{+2} عن زيادة في كفاءة سلالات الاباء وكذلك كفاءة السلالة المتحولة الي اقصي تركيزعند 105 ملي مولر مع Na^{+} و 85 ملي مولر مع Ca^{+2} ، ثم بدأت في الأنخفاض عند التركيزات الأعلي، كما تلعب درجة الحرارة دورًا مهمًا في معدل نقل الجينات والكفاءة بين السلالات الثلاثة، حيث كانت هناك علاقة مباشرة بين زيادة درجة الحرارة حتى تصل إلى درجة الحرارة المثلى ومعدل نقل الجينات، كما ظهر أعلى معدل لنقل الجينات عبر الاقتران عندما كانت النسبة بين المعطي والمستقبل 1:3، وظهرت أفضل النتائج للكفاءة البكتيرية للسلالة المتحولة وسلالات الأباء عند الأس الهيدروجيني 9، مما يعني زيادة الكفاءة بينهما. تظهر هذه النتائج أن السلالة المتحولة يمكن أن تكون موجودة وتبقى على قيد الحياة وكذلك سلالات الأباء وأن تأثيرات بعض العوامل البيئية متشابهة في السلالات الثلاثة.

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