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EFFICACY AND MECHANISMS OF ACTION OF IONIZING RADIATIONS ON *PSEUDOMONAS AERUGINOSA* BACTERIOPHAGE F116.

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ABSTRACT: Influences of gamma rays and fast neutrons on efficacy and mechanisms of action on *Pseudomonas aeruginosa* bacteriophage F116 have been investigated. These mechanisms included, prophage induction, plaque forming ability, transducing particles and transduction efficiency. Survival percentages of 3 host strains, PAO1, MAM2 and PU17 have been seriously affected upon exposure to the ionizing radiation. Killing percentage ranged from 67.4 up to 78.9% when 5.6 x10^8 n/cm^2 of fast neutrons was used. Prophage F116 induction mechanism from the lysogenic strain PU17 (PU21 F 116) has also been influenced. At 3.0 KGy of gamma rays and 5.6 x10^8 n/cm^2 of neutrons resulting in 2-3 fold increase in prophage induction than control (zero- dose).

Whereas, the induced phage was doubling (15.54) in fast neutrons than gamma irradiation (7.43). Subsequently, transduction frequency of streptomycin resistance gene using these induced phase reached 25.3x10^{-5} and 6.1x10^{-5} upon using fast neutrons and gamma rays, respectively. This was correlated with number of transuding particles when calculated at these doses. Moreover, transduction frequency was also enhanced by treating the recipient host cells with different doses. In addition, a dramatically loss in the activation of phage F116 was observed upon treating the phage particles. This inactivation was occurred in plaque forming units and transducing abilities. The results of this study showed that fast neutrons were more effective on the previous phage F116 mechanisms than gamma rays. However, the effect was done depending on both.

INTRODUCTION

The integrity of DNA is essential for the well- being of a cell. Exposure of DNA to high energy radiation can undergo considerable alterations due to chemical reactions induced by the ionization of the molecules. Ionizing irradiation can cause mutations, reproductive cell death, and disappearance of some or all cell activities (Khare *et al.*, 1982; Isabele *et al.*, 1994). Two types of DNA damage are often distinguished, direct and indirect effect of irradiation (Jenny *et al.*, 1993). Four types of DNA damage can be generated, single- strand breaks, double- strand breaks, cross- links and nucleotide damage. (Kuipers and Lafleur, 1998). The hydroxyl radical OH., produced by the radiolysis of water is mainly responsible for this effect (Akinari *et al.*, 2002).

Although, it has been documented that fast neutrons are densely ionizing particles with a high relative biological effectiveness rather than to gamma rays, but, the molecular basis of their properties is not yet entirely understood. In spite of the fact that neutrons induce a different numbers of DNA frank strand breaks as compared to gamma photons, but, the attack occurs with almost the same probability at each nucleotide as reported for gamma rays. (Tae and Gyu, 1998). However, tow types of termini are produced by irradiation, the 3`-phosphate and the 3`-phosphoglycolate, but, upon neutrons, the 3`-phosphate end appeared with a higher yield than the 3`- phosphoglycolate- (Isabele *et al.*, 1994).

Therefore, the aim of this study is to compare the effect of gamma rays and fast neutrons on some mechanisms of the *P. aeruginosa* phage F116 (Pemberton, 1973). The phage has large size (145 n m) of double-stranded DNA make it a suitable model for this purpose. Beside, bacteriophages are suited for such investigations because of the properties of these bacterial viruses. a well- defined structure, non- pathogencity, low costs of propagation, availability of large amounts of phage particles and rapidity of assays.(Maillard *et al.*, 1998).

MATERIALS AND METHODS

1. Bacteriophage and Bacterial Strains:

In this study the generalized bacteriophage F116 (Holloway *et al.*, 1961) has been used. The phage particle has an isometric

head of 650 °A in diameters with tail to about 800 °A long. Phage DNA is double stranded molecule with a molecular weigh of $38x10^6$ Daltons (Miller *et al.*, 1974; and 1977).

Pseudomonas aeruginosa bacterial strains PAO1, PU21,PU17 and MAM2 that have been used in this study were obtained from M. Day, UWIST university, Wales, UK. PAO1 is prototrophic (Holloway and Morgan, 1986), whereas PU21 is auxotrophic and carrying streptomycin resistance gene, the strain PU17 is also auxotrophic and lysogenic (PU21 F116) (prepared in our lab.).

2. Growth Media:

Nutrient agar (NA) and nutrient broth (NB) media were prepared according to manufacture's instructions. The composition nutrient agar was(g/l): peptone(3); yeast extract(5); glucose(10); and agar (15-20). The composition of nutrient broth was(g/l): peptone (3); yeast extract (5); and glucose (10).Soft agar (0.8 % w/v agar) was prepared in distilled water and kept at 45°C on waterbath. Phosphate buffer was prepared from 1/15M potassium phosphate ($KH_2 PO_4$) and 1/15 M disodium phosphate ($Na_2 HPO_4$ $2H_2 O$). The streptomycin was added as sterilized solution by filtration through 0.2 µm filter membrane to the media after autoclaving at a concentration of 12 mg/ml.

3. Gamma Rays Irradiation:

The gamma chamber 400 A (Isotope Group, India) of the Radiation Research and Technology Nasr City, Cairo was used. The dose rate at time of irradiation was 0.0337 KGy/min. 10- ml of cell suspensions were irradiated using 60 Co gamma rays.

4. Fast Neutrons Irradiation:

10-ml of cell suspensions were irradiated with fission neutrons from Cf 252 pount (Radio Chemical Center, Amestedam, Engaland) in Fac. Sci. Biophys. Dept. Cairo University.

The streptomycin was added as sterilized solution by filtration through 0.2 µm filter membrane to the media after autoclaving at a concentration of 12 mg/ml.

5. Effect of Radiation on Prophage Induction from Lysogen:

The liquid culture of lysogenic strain (PU17) was exposed to 3 doses of fast neutron

($5.7x\ 10^6$), $6.3x\ 10^7$ and $5.6x\ 10^8$ n/cm^2) and gamma rays (1,2 and 3 KGy). After irradiation the survival of PU17 cells and phage F116 particles were assayed. The transduction frequency was also assayed.

5.1. Prophage Induction Assay:

After irradiation, a few drops of chloroform were added to cultures and centrifugated at 5000 rpm for 30 min. the supernatant was assayed by using the standard technique of phage titration (overlay method of Adams, 1959).

6. Transduction Frequency Assay:

Recipient cells (MAM2) were grown in NB overnight, then washed 2-3 times by phosphate buffer (pH 7.0) and resuspended. Viable count of the recipient strain was scored. Equal volumes (1ml) of phage lysate and recipient cell suspension were mixed and kept for 15-30 min at room temperature, to allow phage adsorption. Serial dilutions have been prepared and placed onto selective media. Number of transductants were recorded and transduction frequency per recipient was calculated.

7. Effect of Radiation on Phage Lysate:

The phage particles lysates were treated by the same previous doses. After treatment, the Pfu/ml and transduction frequency were calculated.

8. Effect of Radiation on Donor and Recipient:

The liquid cultures of donor (PU17) and recipient (MAM2) were exposed to radiation, the survival of donor, recipient. and transduction frequency were calculated.

9. Statistical Analysis :

Data have been statistically analyzed using standard deviation. Percentage of transducing particles have been calculated by dividing number of transductants on number of plaques and S percent by dividing Cfu/ml of each dose on Cfu/ml of zero dose as a percentage.

$$K\% = 100 - S\%$$

$$\%\ \text{of inactivation} = 100 - \frac{\text{Pfu or transductants}}{\text{Pfu or transductants of zero dose}}\ \%$$

RESULTS AND DISCUSSION

1. Survival Percentages of *Pseudomonas aeruginosa* Host Strains:

The effects of gamma irradiation and fast neutrons on the survival percentages of 3 host strains, PAO1, MAM2 and PU17 are shown in Tables (1and 2). S% reached 45.8, 32.5 and 65.7 when

the 3 host strains subjected to 3 KGy of gamma rays respectively. Upon comparing with neutrons, S % reached 32.6,29.7, and 21.1 for the tested host strains. It seems that most radiation cell lethality is the consequence for the cooperative effects of intracellular OH radicals on DNA which resulting in strand break formation, Klimczak *et al.*, (1993). Moreover, repeated DNA damage was brought about by OH radicals leading to lethal damage. However, the yields of strand breaks formed were proportionally to dose, and lethal damage decreased with increasing scavenging capacity. (Bertram and Hagen, 1992; Franchet *et al.*, 1993) Moreover, exposure the microorganisms to gamma radiation doses between 0 and 9.34 KGy resulted in the viability of some of them but non culturable and lost metabolic capacity and injured cell membrane (Pitonzo *et al.*, 1999).

2. Prophage Induction Mechanism Upon Exposure to Ionizing Radiation:

The lysogenic strain PU17 (PU21 F116) of *pseudomonas aeruginosa* was exposed to different doses of gamma rays and neutrons (Tables 3). Both ionizing radiations were able to induce phage F 116. Gamma rays of 1.0,2.0and 3.0 KGy resulted in 5.6,6.7 and 7.3 of induced phage. Whereas fast neutrons of $5.7x10^6$, $6.3x10^7$ and $5.6x10^8$ n/cm^2 resulted in 6.5, 9.4, and 15.5 of induced phage. So fast neutrons were able to double the number of induced phage when compared with gamma rays. However, the fold increase in induced phage was 3.1 and 2.1 for fast neutrons and gamma rays respectively. The high induced phage from lysogens observed in this study is a proof for the damage of DNA. Science, agents that damage DNA in bacteria are known to induce the SOS response (Radman, 1974). One of SOS functions, the induction of lysogenic phage has been suggested as a bioassay for DNA damage (Elespuru 1987) Moreover, single- stranded DNA which results from the processing of DNA lesions is an inducing signal for the SOS response leading to prohage induction mechanism (De Marini and Lawrence, 1992). This may explain the high yield induced phage that has been shown in this investigation.

3. Transducing Phage Particles:

The induced phage particles from the pervious experiments

were allowed to transduce the streptomycin resistance gene in order to calculate the percent of transducing particles. Transduction frequency increased from $2.04x10^{-5}$ up to $6.1x10^{-5}$ at 3.0 KGy of gamma, and from $3.4x10^{-5}$ up to $25.3x\ 10^{-5}$ at 5.6×10^{8} n/cm^2 of fast neutrons. So, fast neutrons were more effective in transuding ability than gamma irradiation. This remarkable increase in transduction frequency may due to an increasing in the number of transducing particles that formed among the induced phage. Data in Table (5) show that number of transducing particles has increased 3- fold than control in the case of fast neutrons. It seems that fast neutrons may able to induce an illegitimate recombination mechanism that was responsible for high percent of transducing particles. these results do agree with lambda transducing phage that were formed by radiation (Shanado *et al.*, 2001). They suggested many models for irradiation- induced illegitimate recombination and sequences at the recombination junctions were also determined. Breaks that induced at random sites were mainly responsible for the introduction of the site-specific or region- specific DNA strand breaks that leading to the recombination process.

4. Influence of Physiological State of Recipient Host Cells:

In order to demonstrate the dependence of transduction mechanism on the physiological state of the recipient host, MAM2 strain exposed to different doses of gamma and neutrons radiation before adding the phage lysate to it. Data are shown in Table (6). Transductants have been increased by increasing dose of radiation. Transduction frequency has increased twice than control in the case of gamma irradiation ($3.3x10^{-5}$ up to $7.2x10^{-5}$). Whereas fast neutrons have increased the transduction frequency up to 3-fold ($3.2\ x10^{-5}$ up to $9.7x10^{-5}$). This enhancement of transduction frequency upon treating the recipient cell by ionizing radiation may due to the induction of the repair enzymes in the host by the preexisting DNA damage that results by the action of irradiation. Dale (1994) found that, in the presence of damaged DNA, the expression of a number of genes involved in DNA repair is induced. These genes included the excision repair genes uvrA, uvrB, recA, and the gene involved in error- prone repair mechanism. Even, the activity of rec A arises after it recognizes certain types of DNA damage. Furthermore, the interplasmidic and intrachromosomal

Table (1): Survival percentages of some *Pseudomonas aeruginosa* bacterial strains upon exposure to gamma rays.

Dose (KGy)	Cfu/ml 10^{10}			S %			K %		
	PAO1	MAM2	PU17	PAO1	MAM2	PU17	PAO1	MAM2	PU17
0.0	3.6±0.1	2.4±0.3	1.4±0.02	100	100	100	0.00	0.00	0.00
1.0	3.1±0.06	1.9±0.03	1.14±0.03	86.11	79.16	81.43	13.89	20.84	18.57
2.0	2.4±0.07	1.4±0.04	1.11±0.01	66.66	58.33	79.29	33.34	41.67	20.71
3.0	1.65±0.003	0.78±0.002	0.92±0.001	45.83	32.50	65.71	54.17	67.50	34.29

Table (2): Survival percentages of some *Pseudomonas aeruginosa* bacterial strains upon exposure to fast neutrons radiation.

Dose (n/cm^2)	Cfu/ml 10^{10}			S %			K %		
	PAO1	MAM2	PU17	PAO1	MAM2	PU17	PAO1	MAM2	PU17
0.0	4.3±0.1	3.7±0.2	2.7±0.2	100	100	100	0.0	0.0	0.0
$5.7x10^6$	3.4±0.1	3.1±0.1	1.9±0.07	79.1	83.8	70.4	20.9	16.2	29.6
$6.3x10^7$	2.1±0.2	2.4±0.05	0.98±0.03	48.8	64.9	36.3	51.2	35.1	63.7
$5.6x10^8$	1.4±0.04	1.1±0.02	0.57±0.01	32.6	29.7	21.1	67.4	70.3	78.9

Table (3): Prophage F116 induction from the lysogenic strain PU17 (PU21 F116) upon exposure to ionizing radiations.

Gamma Rays				Fast Neutrons			
Dose (KGy)	Pfu/ml 10^5	Induced phage	Fold-increase	Dose (n/cm^2)	Pfu/ml 10^5	Induced phage	Fold-increase
0.0	6.79±0.1	0.00	0.00	0.0	7.43±0.2	0.00	0.0
1.0	12.39±0.2	5.60	1.83	5.7×10^6	13.94±0.1	6.51	1.9
2.0	13.47±0.07	6.68	1.98	6.3×10^7	16.35±0.3	9.42	2.3
3.0	14.13±0.02	7.34	2.08	5.6×10^8	22.97±0.4	15.54	3.1

Table (4): Ability of induced phage F116 in transducing streptomycin resistance gene upon exposure to ionizing radiations.

Gamma Rays				Fast Neutrons			
Dose (KGy)	No. Transductants 10^3/ml	Fold-increase	♂/♀ 10^{-5}	Dose (n/cm^2)	No. Transductants 10^3/ml	Fold-increase	♂/♀ 10^{-5}
0.0	9.54±0.11	0.0	2.04±0.03	0.0	6.35±0.04	0.0	2.37±0.25
1.0	17.99±0.21	1.9	3.84±0.07	5.7×10^6	17.29±0.15	2.7	6.45±0.07
2.0	19.73±0.24	2.1	4.20±0.09	6.3×10^7	36.94±0.27	5.8	13.78±0.2
3.0	28.42±0.41	3.0	6.10±0.21	5.6×10^8	67.85 ± 0.31	10.68	25.32±0.2

Table (5): Percentage of transducing F116 phage particles carrying streptomycin resistance gene among induced phage by radiation.

Gamma Rays		Fast Neutrons	
Dose (KGy)	% Transducing particles	Dose (n/cm^2)	% Transducing particles
0.0	1.41	0.0	1.05
1.0	1.45	5.7×10^6	1.24
2.0	1.46	6.3×10^7	2.19
3.0	2.01	5.6×10^8	2.95

Table (6): Effect of irradiated recipient bacterial cells in transducing streptomycin resistance gene.

Gamma Rays				Fast Neutrons			
Dose (KGy)	No. Transductants 10^4/ml	Fold-increase	♂/♀ 10^{-5}	Dose (n/cm^2)	No. Transductants 10^4/ml	Fold-increase	♂/♀ 10^{-5}
0.0	1.72±0.02	0.00	3.3±0.01	0.0	1.95±0.03	0.0	3.2±0.04
1.0	1.83±0.07	1.1	3.6±0.05	5.7×10^6	3.27±0.04	1.7	5.3±0.02
2.0	3.11±0.09	1.8	6.04±0.04	6.3×10^7	4.58±0.07	2.3	7.4±0.04
3.0	3.69±0.01	2.2	7.2±0.1	5.6×10^8	5.99±0.2	3.1	9.7±0.05

MAM2 bacterial strain has been used as recipient cells
Phage lysates have been prepared on the donor strain PU21

Table (7): Inactivation of phage F116.

Gamma Rays					Fast Neutrons				
Dose (KGy)	Pfu/ml 10^9	No. ♂ ♀ 10^3	%of inactivation		Dose (n/cm^2)	Pfu/ml 10^{10}	No. ♂ ♀ 10^4	%of inactivation	
			Pfu/ml	♂ ♀				Pfu/ml	♂ ♀
0.0	9.9±0.1	15.8±0.4	0.0	0.0	0.0	3.1±0.07	2.3±0.01	0.0	0.0
1.0	7.9±0.2	14.2±0.03	20.2	10.1	5.7×10^6	1.9±0.03	1.98±0.02	38.7	13.9
2.0	1.2±0.01	13.7±0.1	87.9	13.3	6.3×10^7	0.77±0.02	1.53±0.03	75.2	33.5
3.0	0.65±0.04	9.1±0.3	93.4	42.4	5.6×10^8	0.49±0.01	0.98±0.01	84.2	57.4

Each value is the mean of 3 replica ± SD

recombination occurred at high frequency following exposure to low dose of gamma, 17.5 KGy. Moreover, Birge (2000) observed that treatment that enhance recombination process e.g. introduction of nicks into the recipient DNA by means of irradiation improved transformation mechanism.

5. Phage F 116 Inactivation:

The ability of gamma and neutrons radiation to inactivate phage F 116 was determined (Table 7). Bacteriophage F 116 lost about 93%, and 84% of its ability to form plaques when treated with 3KGy of gamma and 5.6x 10^{-5} n/cm^2 of neutrons, respectively. Whereas the inactivation percent to transduce streptomycin resistance gene reached 42.4% and 57.4% at the same doses. This remarkable inactivation in phase F116 ability to form plaque or to transduce may be due to the radiation degradation of DNA in the phage particle so, the phage was not able to form the effective phage particle. A previous studies do support this suggestion. When coliphage T_7 was exposed to a mixture of fast neutrons and gamma rays, the plaque forming ability was affected, beside, strand, breaks and DNA – to- protein cross linkage were observed (Hawkins, 1979). Moreover, the plaque forming ability was influenced by treating phage particles with radiation (Hradecna and Kittler, 1990). However, phage adsorption, DNA injection and replication processes have been also influenced (Kuipers and Lafleur, 1998). Furthermore, the degradation effect of gamma rays on DNA has been also reported (Chen *et al*, 1995; Akinari *et al.*, 2002).

The results of this investigation clearly showed that the *Pseudomonas aeruginosa* bacteriophage F 116 is extremely useful for studying the mechanism of action of ionizing radiation and to understand the processes that leading to radiation degradation of DNA in cells.

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ميكانيكية عمل وقدرة الإشعاع المؤين على التأثير على فاج *Pseudomonas aeruginosa* Phage F116

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تم دراسة ميكانيكية عمل وفاعلية أشعة جاما والنيترونات المسرعة على بكتريوفاج F116 الخاص بـ *Pseudomonas aeruginosa*، وهذه الميكانيكيات تضمنت، حث البروفاج، القدرة على تكوين المناطق الرائقة Plaques، جزيئات الفاج الناقلة وكفاءة النقل.

وقد تأثرت النسبة المئوية لثلاث عوائل من بكتريا *P.aeruginosa* وهى PAO1, MAM2, PU17 تأثراً خطيراً بالتعرض لـ Fast neutrans حيث وصلت نسبة موت الخلايا من ٦٧,٤% إلى ٧٨,٩% عندما استخدمت الجرعة $5.6 \times 10^8 n/cm^2$ كما تأثرت أيضاً عملية حث البروفاج من السلالة الليسوجينية PU17، حيث لوحظ زيادة عملية الحث بمقدار ٢–٣ أضعاف عندما استخدمت الجرعات 3.0 KGY من أشعة جاما، $5.6 \times 10^8 n/cm^2$ من النيترونات المسرعة.

إلا أن الفاج المستحث قد تضاعف عند استخدام النيترونات المسرعة (١٥,٥٤) عنه فى حالة أشعة جاما (٧,٤٣). وبالتالى فقد وصل معدل نقل جين المقاومة للمضاد الحيوى استربتوميسين إلى $٢٥,٣ \times ١٠^{-٥}$ ، $٦,١ \times ١٠^{-٥}$ باستخدام النيترونات المسرعة وأشعة جاما على التوالى وهذا يتناسب مع عدد جزيئات الفاج الناقلة عندما تم حسابها على هذه الجرعات.

علاوة على ذلك فإن معدل النقل قد ارتفع بمعاملة خلايا السلالة المستقبلة بالجرعات المختلفة، ولكن لوحظ فقد مفاجئ فى نشاط جزيئات فاج F116 عند معاملتها بالإشعاع وقد ظهر هذا فى وحدات Plaques المتكونة والقدرة على النقل.

وقد أوضحت النتائج فى هذه الدراسة أن النيترونات المسرعة كانت أكثر تأثيراً على الميكانيكيات المختلفة لفاج F116 عن أشعة جاما.