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MUTAGENICITY STUDY OF INDUSTRIAL POLLUTED WASTE WATER IN ZAGAZIG CITY

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ABSTRACT

The mutagenicity study of industrial waste water of oil and soap factory of Zagazig City was carried out using two microbial assayes, prophage induction and transduction. *Pseudomonas aeruginosa* bacterial strain MAM2A1 (Lysogen with phage F116) was used to prophage induction assay. The results showed that all the volumes that used before treating of the waste water had a mutagenic activity. The fold increase than spontaneous release was 11.3 when added 20 ml of the waste water. However, after treating the high volumes (15 and 20 ml) had only a mutagenic activity. Phage induced by different industrial waste water volumes allowed to transduce streptomycin resistance gene. Number of transductants and subsequently transduction frequency were increased when waste water was used before treating. Transduction frequency reached $1.3X10^{-4}$, with 6 fold increase by adding 20 ml of the waste water. After treatment, the transduction frequency was increased in all volumes but did not reached the mutagenic activity. Effect of waste water on phage F116 lysate was tested. Then the phage was assayed in its ability to form plaques and transduce streptomycin resistance gene. The efficiency of phage to form plaques was dropped from $2.1x10^9$ to $1.4x10^7$ pfu / ml by using 0.5 ml of waste water. However by using 20 ml, the efficiency dramatically decreased to $1.9x10^5$ pfu/ ml.

The effect of industrial waste water on survival of two *Pseudomonas aeruginosa* bacterial strains (MAM2 A1 and PU 21A3) was assessed. The survival percentage of both strains was influenced. It was reached 1.3 in strain MAM2A1 and 0.14 in strain PU21 A3 when 20 ml of the waste water before treating was added. After treating it was ranged from 1.2 to 1.4 for both strains. It seemed that the treatment of these waste water did not enhance the survival of both bacterial strains that used in this study.

The results of this investigation clearly showed that the industrial waste water of oil and soap factory hade a strong mutagenic response in two short assayes, prophage induction and transduction.The waste water had a powerful killing effect on two *Pseudomonas aeruginosa* strains. The treatment of the wastes did not protect these strains in surviving. However, the efficiency of the phage has been seriously affected when the phage treated with waste water. Also treating the waste water before releasing into Mowas River had no remarkable effect in reducing the mutagenic activity of these wastes. Therefore, strict quality requirement should be set to protect public health.

Keywords: Waste water, mutagenicity, prophage induction, transduction, survival.

INTRODUCTION

Waste water are universal solvent and may contain a wide diversity of substances arising from industrial, agricultural and other sources and from supply systems (De Marini *et al.*, 1989 and Reutova *et al.*, 2005). Waste water containing diverse substances, can be modified by treatment. The genotoxicological safety of waste water represents an important issue for safeguarding of health and well-being of human. It should be assumed that there is a potential for all members of pollutions may present in the waste

water which might contain possible hazardous contaminants (Durgo *et al.,* 2005).Mutagenicity studies are rapid, relatively cheep and predictive of integral mutagenic activity and can evaluate the combined action of potentially hazardous compounds present in waste water (Lah *et al*., 2005).

The difficulties encountered in performing physico- chemical analyses (i.e detecting only known chemicals, classifying the chemicals present in a sample and not knowing what their biological effects are, not being able to detect very low quantities of particular chemical, etc.), long term mutagenicity assayes have encouraged the analysis of waste water using short term mutagenicity study.(Fernanda *et al*., 2004).

The Mowas River which is a branch of Nile River flows through Zagazig City in Sharkia Governorate , Egypt, is a source of drinking water to that City. It has over the years, become a dumping ground for wastes from sewage canals and industrial effluents especially from oil and soap industries. The effluents are a complex mixture of hazardous wastes such as mutagenic and carcinogenic heavy metals which may potentiate a major biological hazard so, the aim of this study was to perform a mutagenicity study of an industrial waste water of the oil and soap factory in Zagazig City which release to Mowas River. A battery of short-term model systems revealing different genetic endpoints was used in this study.

MATERIALS AND METHODS

1- Bacteriophage and bacterial strains:

The generalized transducing F116 phage and bacterial strains of *Pseudomonas aeruginosa* (PAOl, PU21 and MAM2) that used in this study were obtained from M. Day, University of Wales, Cardiff, UK. The strain MAM2 A1 is lysogen MAM2 with F116 phage and resistance to streptomycin. The strain PU21A3 is non lysogen PU21 and resistance to ampcillin.

2- Growth media:

The 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 was prepared from 1/15M potassium phosphate (KH_2PO_4) and 1/15M disodium phosphate ($Na_2HPO_4 . 2H_2O$). Streptomycin (12 mg/ml) and ampcillin (600 μg/ml) were added as sterilized solutions by filtration through 0.2 μm filter membrane to the media after autoclaving.

3- Prophage induction from lysogenic strain:

The overnight culture from lysogenic MAM2Al strain was prepared. Two ml were added to each individual volume(0.5, 2, 5,10,15 and 20 ml) of waste water and incubated at 30°C for overnight. A few drops of chloroform were added, centrifuged at 5000 rpm for 30 min and filtered. The supernatant was assayed, the plaque forming units (pfu/ml) were calculated for each waste water volume.

4- Phage titration:

Serial hundred- fold dilutions of phage were prepared in phosphate buffer (PH 7.0). Phage titer was determined by mixing equal volumes (0.1 ml) of a phage dilution with host cells (growing overnight in NB at 30°C), adding

soft agar, and pouring immediately onto an NA plate (Gulig *et al*., 2002) . Plates were incubated at 30°C for 24h., and pfu/ ml was recorded.

5-Transducing streptomycin resistance gene from MAM2A1 to PU21A3:

The induced phage particles from treated lysogenic strain were used to transduce streptomycin resistance gene. Recipient cells were grown in NB overnight. Viable count of the recipient strain was made. Equal volumes (0.5 ml) of phage lysate and recipient cell suspensions were mixed. The mixture was kept for 15 to 30 min at room temperature, to allow phage adsorption. Serial dilutions were prepared and placed onto selective media. Number of colonies (transductants) were recorded and transduction frequency was calculated.

6- Survival percentage of bacterial strains:

The overnight bacterial cells was prepared, two ml of each individual strain (MAM2A1 and PU21A3) were added to each individual volume from industrial waste water before and after treatment. After incubation at 30°C for overnight, serial dilutions were prepared ,0.1 ml of each dilution was spreaded onto NA plates. The plates were incubated at 30°C for 24h. The colonies were counted and the survival was calculated as colony forming units (Cfu /ml).

7- Treating industrial waste water of becteriophage F116:

The phage lysate was treated with the same previous volumes of waste water for 24h. The plaques were counted and pfu /ml was calculated. These phage lysates were used to transducing streptomycin resistance gene.

This investigation was carried out at the Microbial Genetic Lab., Genetic Dept., Fac., Agric., Zagazig Univ.

RESULTS AND DISCUSSION

1- Mutagenicity Study of Industrial Waste Water of Oil and Soap Factory of Zagazig City:

1-1- The mutagenic activity through prophage F116 induction assay:

Data in Table 1 showed the evaluation of mutagenic activity of the industrial waste water using prophage induction assay.

Table 1: Effect of industrial waste water (I.W) on prophage F116 induction from lysogenic Strain MAM2A1 of *P. aeruginosa.*

Vol of I.W (ml)	Before treatment			After treatment		
	(Pfu / ml)	F.I	M.I	(Pfu / ml)	F.I	M.I
0.5	$6.7x10^7$	3.2	+	$2.2x10^7$	1.1	-
2.0	$10.8x10^7$	5.1	+	$2.8x10^7$	1.3	-
5.0	$15.4x10^7$	7.3	+	$3.1x10^7$	1.5	-
10.0	$18.9x10^7$	9.0	+	$3.4x10^7$	1.6	-
15.0	$21.4x10^7$	10.2	+ +	$7.9x10^7$	3.8	+
20.0	$23.7x10^7$	11.3	+ +	$13.1x10^7$	6.2	+

The spontaneous release of prophage F116 from lysogen was $2.1x10^7$ pfu / ml.
F.I = Fold- increase than spontaneous release.
M.I: Mutagenic index according to Heinmnann 1971.
- = No mutagen. + = Moderate mutagen. ++ = High mutagen.

Before treating the waste water, all the volumes used had a mutagenic activity. The fold increase than spontaneous release was 11.3 when 20 ml of the waste was added. However, after treating the wastes, high volumes (15 and 20ml) had only a mutagenic activity, but, still the other volumes were able to induce the prophage whereas the fold increase raged from 1.1 up to 1.6 than the spontaneous release. Treating the waste water has no remarkable impact on inducing the prophage, since high volumes of treated industrial waste water (15 and 20 ml) result in mutagenic activity with 3 to 6 fold increase in prophage induction than those observed in the spontaneous release.

1-2. Mutagenic activity through transduction assay:

Phage induced by different industrial waste water volumes have been allowed to transduce streptomycin resistance gene. Before treating the waste, an increase in number of transductants and subsequently transduction frequency per recipient was correlated with increasing the volume of the waste water. Transduction frequency reached $1.3x10^{-4}$ with 6 fold increase by adding 20 ml of the waste(Table 2).

Table 2: Effect of industrial waste water (I.W) on transducing streptomycin resistance gene.

Vol of I.W (ml)	Before treatment				After treatment			
	Transductants10^4	Transduction frequency	F.I	M.I	Transductants10^4	Transduction frequency	F.I	M.I
0.5	12.5	$5.2x10^{-5}$	2.6	-	7.3	$3.04x10^{-5}$	1.5	-
2.0	14.7	$6.1x10^{-5}$	3.1	+	9.5	$3.9x10^{-5}$	2.0	-
5.0	16.2	$6.8x10^{-5}$	3.4	+	11.7	$4.9x10^{-5}$	2.4	-
10.0	17.4	$7.3x10^{-5}$	3.6	+	10.5	$4.4x10^{-5}$	2.2	-
15.0	27.3	$1.14x10^{-4}$	5.7	+	8.4	$3.5x10^{-5}$	1.8	-
20.0	31.2	$1.3x10^{-4}$	6.5	+	8.3	$3.5x10^{-5}$	1.8	-

- Cfu/ ml of recipient strain was $2.4x10^9$
- Number of transductants in control was $4.8x10^4$ cfu /ml
- Transduction frequency in control was $2.0x10^{-5}$

It seemed that the waste water increased the number of transducing particles resulting in increasing the number of transductants. All the volumes used showed a mutagenic activity except volumes 0.5ml.

After treatment, the transduction frequency has been increased in all volumes but did not reach the mutagenic activity. According to Heinmnann, 1971 3 fold increased than spontaneous or control experiment results in mutagenic response.

2-The Effect on Survival Percentage of Some *Pseudomonas aeruginosa* Strains:

Two ml of overnight bacterial cells have been added to different volumes of the industrial waste water in order to assess the survival percentage of two strains. Data in Tables 3 and 4. According to the safety rules of the oil and soap factory, the waste water should be treated before released to Mowas River and used for many purposes. The survival percentage of both strains have been, greatly influenced when mixed with

waste water. Before treating the waste water, S% reached 1.3 in strain MAM2A1 and 0.14 in strain PU21A3 when 20 ml of the waste water was added. However, after treating the waste water, the S% ranged from 1.2 to1.4 for both strains. It seemed that the treatment of these waste water did not enhance the survival of both bacterial strains.

Table 3: Survival percentage of *P. aeruginosa* strain MAM2A1 upon exposure to industrial waste water (I.W).

Volume of (I.W) ml	Before treatment		After treatment	
	Cfu / ml	S%	Cfu / ml	S%
0.5	$4.4x10^{13}$	52.4	$6.3x10^{13}$	75.0
2.0	$2.5x10^{13}$	29.8	$3.4x10^{13}$	40.5
5.0	$1.1x10^{13}$	13.1	$2.1x10^{13}$	25.0
10.0	$0.91x10^{13}$	10.8	$1.2x10^{13}$	14.3
15.0	$0.37x10^{13}$	4.4	$0.7x10^{13}$	8.3
20.0	$0.11x10^{13}$	1.3	$0.1x10^{13}$	1.2

Cfu / ml of strain MAM2A1 in control was $8.4x10^{13}$

Table 4: Survival percentage of *P. aeruginosa* strain PU21A3 upon exposure to industrial waste water (I.W).

Volume of (I.W) ml	Before treatment		After treatment	
	Cfu / ml	S%	Cfu /ml	S%
0.5	$1.4x10^{13}$	48.3	$2.1x10^{13}$	72.4
2.0	$0.9x10^{13}$	31.0	$1.9x10^{13}$	65.5
5.0	$0.42x10^{13}$	14.5	$0.8x10^{13}$	27.6
10.0	$0.13x10^{13}$	4.5	$0.3x10^{13}$	10.3
15.0	$0.01x10^{13}$	0.35	$0.1x10^{13}$	3.4
20.0	$0.004x10^{13}$	0.14	$0.04x10^{13}$	1.4

Cfu / ml of strain PU21A3 in control was $2.9x10^{13}$

3- Effect of waste water on phage F116:

Phage F116 lysate was treated with the different volumes of waste water, then the phage was assayed in its ability to form plaques and transduce streptomycin resistance gene (Table 5). The efficiency of phage F116 to form plaques has been dropped from $2.1x10^9$ up to $1.4x10^7$ pfu / ml by using 0.5 ml of industrial waste water. However by using 20 ml of the waste water the efficiency of the phage dramatically decreased up to $1.9x10^5$ pfu /ml. Number of transductants have been increased up to $9.6x10^4$ by using 5 ml of the waste water.

Table 5: Effect of industrial waste water(I:W) on phage F116.

Vol of I.W (ml)	Ability to forming plaques		Ability to transducer			
	Before treatment (Pfu / ml)	After treatment (Pfu / ml)	Before treatment		After treatment	
			Transductants	Transduction frequency	Transductants	Transduction frequency
0.5	$1.4x10^7$	$2.5x10^7$	6.9	$3.3x10^{-5}$	5.7	$2.7x10^{-5}$
2.0	$2.2x10^7$	$7.8x10^7$	8.2	$3.9x10^{-5}$	6.3	$3.0x10^{-5}$
5.0	$1.9x10^7$	$6.3x10^7$	9.6	$4.6x10^{-5}$	7.4	$3.5x10^{-5}$
10.0	$1.8x10^7$	$4.9x10^7$	8.9	$4.2x10^{-5}$	7.1	$3.4x10^{-5}$
15.0	$1.2x10^6$	$4.2x10^7$	8.2	$3.9x10^{-5}$	5.7	$2.7x10^{-5}$
20.0	$1.9x10^5$	$3.5x10^7$	7.2	$3.4x10^{-5}$	4.8	$2.3x10^{-5}$

- Pfu / ml of phage F116 in control was $2.1x10^9$
- Number of transductants in control was $4.7x10^4$
- Cfu / ml of recipient strain was $2.1x10^9$

The results of this investigation clearly showed that the industrial waste water of oil and soap factory hade a strong mutagenic response in two short assayes, prophage induction and transduction.

The waste water had a powerful killing effect on two *Pseudomonas aeruginosa* strains. The treatment of the wastes did not protect these strains in surviving. However, the efficiency of the phage has been seriously affected when the phage treated with waste water. Also treating the waste water before releasing into Mowas River had no remarkable effect in reducing the mutagenic activity of these wastes. Therefore, strict quality requirement should be set to protect public health.

Mutagenic pollution of natural environments seemed to be a general and serious problem that has been extensively investigated (Sanchez *et al.*, 1992, Malachova 1999 Czyz *et al.*,2000, Hu *et al*., 2003). However, many studies have been carried out to assess the mutagenic activity of some Rivers (Otsu *et al*., 1998 , Czyz *et al*., 2000 Tsukatani *et al*., 2003, Vijayushree *et al.,* 2005).

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دراسة القدرة الطفرية لمياه الصرف الصناعي الملوثة في مدينة الزقازيق

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تم دراسة القدرة الطفرية لمياه الصرف الصناعي لمصنع الزيوت والصابون بمدينة الزقازيق باستخدام اثنين من التقييم الميكروبي هما:

استحثاث البروفاج والنقل الجيني بالفاج. تم استخدام السلالة البكتيرية الليسوجينية MAM2A1 من بكتريا *Pseudomonas aeruginosa* لتقييم استحثاث البروفاج, وقد أوضحت النتائج أن كل الأحجام التي استخدمت من مياه الصرف الصناعي قبل معالجتها كان لها نشاط مطفر.

عند إضافة ٢٠ ملل من مياه الصرف الصناعي أدي ذلك إلي زيادة الفاج المستحث بمقدار ١١,٣ ضعف الفاج المنطلق تلقائياً. أما استخدام مياه الصرف الصناعي بعد المعالجة فإن الأحجام العالية (١٥, ٢٠ ملل) هي فقط التي كان لها نشاط مطفر.

الفاج المستحث عن طريق مياه الصرف الصناعي سمح له بنقل جين المقاومة للإستربتوميسين, وقد لوحظ زيادة في عدد Transductants وكذلك معدل Transduction في حالة الفاج المستحث بمياه الصرف الصناعي قبل المعالجة, حيث وصل معدل Transduction إلي $1.3x10^{-4}$ بزيادة ٦ أضعاف عند إضافة ٢٠ ملل من مياه الصرف الصناعي.

في حالة استخدام الفاج المستحث بمياه الصرف الصناعي بعد المعالجة فإن معدل Transduction قد ازداد ولكنه لم يصل إلي حد النشاط المطفر.

تم اختبار تأثير مياه الصرف الصناعي علي فاج F116 ثم تم تقييم قدرة الفاج المعامل (بمياه الصرف الصناعي) علي تكوين Plaques, وكذلك قدرته علي نقل جين المقاومة للإستربتوميسين.وجد أن فاعلية الفاج علي تكوين Plaques قد انخفضت من ٢,١× ١٠^٩ إلي ١,٤×١٠^٧ Pfu/ml باستخدام ٥, ملل من مياه الصرف الصناعي, ولكن باستخدام ٢٠ ملل انخفضت الفاعلية إلي ١,٩ × ١٠^٥ pfu/ml.

تم تقييم تأثير مياه الصرف الصناعي علي بقاء سلالتين من بكتريا *Pseudomonas aeruginosa* هي MAM2A1, PU21 A3.وجد أن النسبة المئوية لبقاء كلتا السلالتين قد تأثرت, حيث وصلت هذه النسبة إلي ١,٣% في السلالة MAM2A1, ١٤, % في السلالة PU21 A3 عند استخدام ٢٠ ملل من مياه الصرف الصناعي قبل المعالجة ولكنها تتراوح بين ١,٢ إلي ١,٤% لكلتا السلالتين في حالة استخدام مياه بعد المعالجة.وهذا يظهر أن معالجة هذه المياه لم يزيد بقاء كلتا السلالتين البكتيريتين التي استخدمت في هذه الدراسة.

أظهرت نتائج هذه الدراسة بوضوح أن مياه الصرف الصناعي لمصنع الزيوت والصابون لها تأثير مطفر قوي كما أن لها تأثير قاتل علي سلالتين من بكتريا *Pseudomonas aeruginosa* , كما أن فاعلية الفاج F116 قد تأثرت بمعاملته بهذه المياه ، و أن معالجة هذه المياه لم يقلل من التأثير المطفر لها.