J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 8(4): 123 - 130 , 2017

Genetical Studies on Bioremediation of Some Chemical Pollutants - Contaminated Water Byspirulina (*Arthrospirafusiformis*)

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ABSTRACT

Bioremediation of crystal violet and ammonia was investigated by Spirulina (*Arthrospirafusiformis*) in aquatic system.As well as, studying effect of crystal violet against genetic structure of Spirulina using biochemical and molecular genetic techniqes was the other aim of this study. Spirulina was capable of using crystal violet and ammonia as sole sources of carbon and nitrogen, respectively.Crystal violet and ammonia were completely remediated by Spirulinain liquid medium at pH 9 and 35 °C after 25 incubation days comparing with uninoculated medium (control). No toxicity of crystal violet was appeared after 25 days of incubation with *A. fusiformis*and*Bacillus subtilis*E5 as a microbial bioassay test. The dissipation of crystal violet and ammonia was coinciding with increasing *A. fusiformis* biomass. Estraseenzyme activity and RAPD analysis wereappliedin order to study thegenotoxic effect of crystal violet on genome structure of Spirulina. Enzyme activity of estrase was markedly differed in crystal violet treated algae in comparison with control group, indicating theinhibition of enzyme activity as bands number andhigh polymorphism percentage(87.5%) between control and treatment. Seven RAPD primers generated 44 DNA fragments, 37of them were polymorphic (84.09%).The results indicated that crystal violet induced genotoxic effect on the genomic structure of Spirulina.So, it is worth to conclude that*A. fusiformis* could be applied to remediate crystal violet and ammonia contaminated water.

Keywords: Crystal violet, decolorization, ammonia, *A. fusiformis*, estrase enzyme activity, RAPD analysis

INTRODUCTION

Microalgae mass culture seems to be a feasible way to remove from wastewaters inorganic nutrients, among which nitrogenous compounds, which are known as primary agents of pollution and eutrophication. Also, Microalgae is a good option for the bioremediation of dyes polluted wastewater. The one more advantage of using algae for bioremediation is that they do not require carbon for their growth, derives energy from sunlight and carbon from the air, and scavenges atmospheric nitrogen (Saha *et al*., 2010).

Crystal violet (C V) belongs totriphenylmethane dyes which are used in the clothing industry **(**Kim *et al*., 2005**).** It is used in the treatment of pinworms and added to feed to prevent fungal growth (Azmi *et al*., 1998). This dye or its degraded products are known as mutagenic orcarcinogenic agents. Therefore, the treatment of industrial effluents contaminated with dye becomes necessary prior totheir final discharge to the environment. Wastewater contaminated with dye could be treated with different physico-chemical methods. These methods are highly cost-effective and not environment friendly so thatbecome commercially unattractive (Nigam *et al*., 1996 and Azmi *et al*., 1998). Many applications of suspended microalgae on wastewater nutrient removal have been successfully applied (Wong *et al.,* 1995 and Lincoln *et al.,* 1996).

In previous studies, wastewaters from urban, industrial, or agricultural sources (Fallowfield and Garrett, 1985 and Noüe *et al*., 1992) were used for removing nitrogen and phosphorus by using *Chlorella pyrenoidosa* (Sánchez *et al*., 2001), *Spirulina maxima* (Kosaric *et al*., 1974) and *Spirulina* (*Arthrospira*) (Olguín *et al*., 2003).

The development of the RAPD (Random Amplified Polymorphic DNA) technique (Williams *et al*. 1990) has provided a good tool for research in genetic variability and genotoxicological assays to detect DNA damage and mutations. It consists of the PCR multiplication of small, inverted repeats scattered throughout the genome, using a single, short primer of arbitrary sequence. The ability to evaluate genomic variation without previous sequence information (Williams *et al*. 1990), low cost, and used of very small amount of template DNA, are all advantages of RAPD in genetic studies. This technique was utilized in cyanobacteria by Nishihara *et al*. (1997). Therefore, the aim of this study was to use Spirulina (*Arthrospirafusiformis*) in bioremediation of crystal violet and ammonia - contaminated water, as well as, studying the genotoxic effect ofcrystal violet on the genetic structure of Spirulina (*A.fusiformis*).

MATERIALS AND METHODS

Chemicals

Crystal violet ($C_{24}H_{28}N_3Cl$) was donated by Agric. Microbiol. branch, Agric. Botany Dep., Fac. of Agric., Kafrelsheikh Univ., Egypt.

Medium

Zarrouk's liquid medium was used in this study for growing Spirulinaas described by **(**Pandey *et al*. 2010**).**

Isolation and identification of microorganism

Spirulina (*A.fusiformis*) was isolated, identified and optimized in previous study (Belal *et al*., 2012). In addition, *Bacillus subtilis*E5 (Belal *et al*., 2013), as the representative gram positive bacterium was used as the test organism.

Decolorization of crystal violet by *Arthrospirafusiformis*

The homogenised culture of *A. fusiformis* (10^7cfu/ml) was used to inoculate 200 ml Zarrouk's medium containing crystal violet (50 mg/L) of the dye as a carbon source. The cultures were incubated under optimum conditions (35 ºC and pH9) for 25 days and illuminated with day - light fluorescent tubes having 5 Klux at the surface of the vessels. The test is based on the decrease of the optical density of the dye during the dyes decolorization. The decolorization percentage of the dye was determined photometrically using UV–visSpectrophotometer at OD_{590nm} for crystal violet (CV). This percentage was evaluated as described by Hauka *et al*. (2014). Control flasks with the equal volumes of Zarrouk's liquid medium and dye without any microbial inoculation were incubated in parallel at all intervals to assess abiotic loss. The remaining of crystal violet using UV–visSpectrophoto meter was determined. All the experiments were done in triplicates.

Analytical methods

Biomass concentration (gl^{-1}) was calculated by measuring dry weight. For measuring dry weight, homogenous suspensions ofspirulina sample were filtered through filter paper 8 mm pore size (Screen printing paper) and oven dried at 75 ^{o}C for 4 to 6 hrs. The dried filter paper containing spirulina biomass were cooled and weighed. The difference between initial and final weights wascalculated as the dry weight of spirulina biomass. The dry weights were expressed in terms of gl-1. Samples were taken in triplicates. Chlorophyll a content was calculated by the Mackinney method (Mackinney, 1941). Protein was estimated by the Lowry method (Lowry *et al*., 1951).

Genotoxic effect of crystal violet on genetic structure of *A. fusiformis*

Isozyme analysis

For extraction of isozyme, algae samplehomogenates weretaken from 0.5 g fresh algae for each sample (control and treatment), and crushed in liquid nitrogen in an ice chilled mortar and pestle with 1.0 ml of ice cold extraction buffer [0.2 M Tris-HCL buffer PH 8.5 containing 20 % sucrose, 5mM dithiotritol (DTT), 0.03% (v/v) mercaptoethanol, and 4% (w/v) polyvinyl-polypyrrolidone]. The extract was centrifuged twice at 12,500 rpm for 30 min. at 4ºC and the supernatant was separated. Polyacrylamide gel electrophoresis (PAGE) described by Laemmli (1970) was applied for qualitative analysis of enzymes. Estrase isozyme bands were detected on the gel by using α-naphthylacetate as substrate and subsequent color was developed with fast blue RR salts (Scandalios, 1969).The visual bands were recorded and their relative mobilities (Rf) were calculated using GelAnalyzer 3 program software.

Molecular studies using RAPD analysis

DNA extraction and amplification conditions

Spirulina (*Arthrospirafusiformis*) sampleswere analyzed using the PCR-RAPD method (Williams *et al*., 1990) using seven 10-mer arbitrary primers (Operon Technology),Table (1). Total DNA was extracted from 50-70 mg of algae biomass by using DNeasy Tissue kit Manufacturer's protocol (Qiagen, cat. No. 69504). The extracted DNA of each sample was estimated by comparison with known standard DNA ladder on 0.8 % agarose gel against 1 kb plus DNA Ladder (TIANGEN BIOTECH, Cat.no.MD113). DNA samples were diluted to a final concentration of 40 ng/µl before PCR amplification. The DNA was stored at −20°C until use.

Table 1. Primers name and sequences used in the present study.

Number	Primer name	Sequence (5'→3')
1	OPA-01	5'- CAGGCCCTTC -3'
2	OPA-02	5'- TGCCGAGCTG -3'
3	OPA-03	5'- AGTCAGCCAC -3'
4	OPA-04	5'- AATCGGGCTG -3'
5	OPA-08	5'- GTGACGTAGG-3'
6	OPB-01	5'- GTTTCGCTCC-3'
7	OPB-08	5'- GTCCACACGG-3'

PCR-RAPD analysis was carried out in a final volume of 20.0 μL containing 1 µl of 40 ng genomic DNA, 1 µl of 10 µM primer, 10 µl master mix [2x *Taq* PCR MasterMix (TIANGEN], cat. No. KT201), 8 µldd H_2O using the following amplification protocol: 94°C for 3 min, followed by 35 cycles consisting of denaturation step for 30 sec at 94°C, primer annealing step for 30-60 sec at 30°C - 34°C (annealing step was optimized for each primer), and extension step for 1 min at 72°C. The amplification was completed with a final extension period for 5 min. (72°C)and mixtures were stored at 4°C until use.

The PCR-RAPD amplified fragments were separated on 1.5%agarose gel by electrophoresis. The banding patterns were compared as presence (1) or absence (0), also molecular size of bands was calculating using GelAnalyzer 3 program software.

Genomic template stability (GTS) was determined according to Atienzar *et al*. (2002) as follow: GTS (%) = (1 – a/n) × 100 , where "a" refer to the number of RAPD polymorphic bands detected in each treatment and "n" refer to the number of total bands in the control (un treated treatment). Polymorphism observed in the RAPD profile included disappearance of a normal band and appearance of a new band in comparison with control profile.

Toxicity test

*Bacillus subtilis*E5 was used as test organism. The bioassay of the restcrystal violet toxicity was performed on the aqueous solutions after 25 days of incubation with the tested microbial isolate. *Bacillus subtilis* E5, as the representative gram positive bacterium was used as the test organism. Toxicity was evaluated by comparingthe inhibition zone in growth of *Bacillus subtilis*E5 with untreated treatment(control). Nutrient agar medium was poured into Petri dishes (9 cm in diameter, 15 ml/dish), after solidification, 100 µl from *Bacillus subtilis*E5 (107cfu/ ml) were transferred onto agar plates and spread evenly, wells (5 mm diameter) were punched in each plate. After that, 50 µl from the supernatant wereadded in punched holes (5 mm in diameter) in the nutrient agar medium, where the culture broth was obtained by culturing crystal violet decolurizing strain with crystal violet in Zarrouk's liquid medium after 25 days as mentioned above. Filtration wasapplied of the culture broth through a

sterile membrane filter (0.2 µm). Also, 50 µl from sterilized liquid medium were put in punched holes which were used as control treatments. Experiments were made in three replicates. Plates were incubated at 35°C for 7 days. Inhibition zone diameter (mm) surrounding each hole was recorded. The toxicity was determined as percentage of inhibition in the growth of the tested bacteria comparing to untreated treatment(Hauka *et al*. 2014).

Remediation of ammonia contaminated water by *A. fusiformis*

The homogenised culture of *A. fusiformis* (107cfu/ml) was then used to inoculate 200 ml Zarrouk's medium containing ammonia (20 mg/L) of the dye as a nitrogen source. The cultures were incubated under optimum conditions (35 ºC and pH9) for 25 days and illuminated with day - light fluorescent tubes having 5 Klux at the surface of the vessels. Control flasks with the same volumes of wastewater without any microbial inoculation were incubated in parallel at all intervals to assess abiotic loss. Thedecrease of ammonia concentration was assessment by spectrophotometer after incubation period. Remaining value of ammonia was calculatedas mentioned by APHA, (2005).

Statistical analysis

Data were calculated as mean ± standard deviation (SD) and analyzed using analysis of variance (ANOVA). Probability of 0.05 or above was considered significant. All calculations were determined as described by the statistical package of Costat Program (1986).

RESULTS AND DISCUSSION

Algae are photosynthetic, pigment-producing, protein-rich microorganisms especially play a vital role for treating wastewater for their unique ability to generate their own carbon source and oxygen, greater visibility that aids growth monitoring, and high commercial value.

The remediation potential of *A. fusiformis* was characterized via decolorization percentage determination of dyes in Zarrouk's liquid medium. The remediation potential was obtained for crystal violet with *A. fusiformis*at 35°C pH9 after 25 days. Results in Table (2) and Fig. (1) summarize the differences of decolorizationof C V by*A. fusiformis*in aquatic system. Dyes decolorization was increased by increasing incubation period with *A. fusiformis*in all cases.The application of C V decolorizing strain increased the number of cultivable C V - decolorizing cells in the aquatic system during the incubation period (7 days). Results were compared with the uninoculated (control), which appearedlowdecolorization of the dye due to a biotic stress.

Table 2. Bioremediation of crystal violet contaminated water by *A. fusiformis*.

Treatments		% Remaining C V and dry weight in g/200ml					
		C V					
		0 time	5days	10 days	15 days	20 days	25 days
Control (un-inoculated) (C V)	%Remaining	100±0	99±0.01	97±0.22	96±0.12	94±0.11	93±0.21
A. fusiformis + C V	%Remaining	100±0	74.3 ±0.2	52.2±0.1	24±0.3	6.1 ±0.2	0.5 ±0.01
	Dry weight in g/200ml	0.07±0.03	0.09±0.02	0.12±0.03	0.15±0.02	0.22±0.02	0.28±0.03

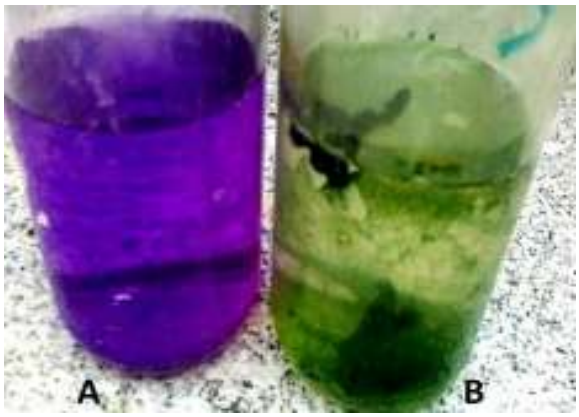

Fig. 1. Where A: control (crystal violet – uninoculated) and B: inoculated with *A. fusiformis*. Remove of C V by *A. fusiformis* in aquatic system.

Chengalroyen (2011) found that bacterial strain *Amycolatopsisorientalis* SY6 was able to decolorize amido black, janus green and several triphenylmethane dyes effectively. This suggests that the same enzyme/s might play a role in reduction of all these dyes. It was noticed that bacteria capable of degrading C V were also able to mineralize other triphenylmethane dyes such as malachite green, brilliant green and basic fuchsin. This allows for the speculation that enzymes responsible for triphenylmethane are more lenient in substrate interactions or the dye structural differences are minor andhence easier to accommodate. Additionally, research done on *P. pseudomallei* proved that decolorization of triphenylmethane dyes was not linked to the molecular weight or permeability of the compound through the membrane (Azmi *et al*., 1998).

Data in Table (3) showedthat the dry weight, chlorophyll-a content and protein content of *A. fusiformis* were higher on Zarrouk medium only thanZarrouk medium supplemented with crystal violet.

Table 3. Effect of crystal violet presence on dry weight, chlorophyll a and protein content of *A. fusiformis*

Treatments	Dry weight in g/200ml	Chlorophyll a content in mg/200ml	Protein content of dry weight
A. fusiformis + Crystal violet	0.25±0.02	5.2±0.21	42±0.34
A. fusiformis	0.49±0.02	13±0.23	62±0.23

Toxicity evaluation

Toxicity of the rest crystal violet that appeared in the aqueous solution after 25 days ofincubation do not affected by the simultaneous presence with the tested microbial strain. Toxicity was estimated using *Bacillus subtilis*E5 as a bioassay test. The supernatant of crystal violet after 25 days of incubation with *A. fusiformis* had no toxicity which could be detected against *B.subtilis*E5 as a test organism. Toxicity of the rest C V in the aqueous solution after seven daysof incubation was estimatedby*B. subtilis*E5 as a bioassay test.*A. fusiformis* exhibited the highest decolorizationofCV. The results also pointed out that the supernatant of C V after seven days of incubation with *A. fusiformis*had no toxicity against *B. subtilis* E5. Comparison between treatments with control (C V only) revealed 100% of inhibition against *B. subtilis*E5 under the same conditions which was recorded as inhibition zone absent in treatment and present with 5 ml diameter in control. Hauka *et al*. (2014) found that the aqueous solution spiked with C V was completely detoxified after seven days of treatment with *P. geniculata*strain AT 17. Also, Azmi *et al*. (1998) reported that these dyes inhibit cell growth by interfering with nucleic acid synthesis thus decreasing protein synthesis. Furthermore, Yatome *et al*. (1993) were the first to elucidate the degradation of C V by *Nocardia* spp.

Remove of ammonia in aquatic system by *A. fusiformis*

The results shown in Table (4) appeared that this isolate was tested to its growth ability and remediation of ammonia as a nitrogen source. *A. fusiformis*achieved completely removal of ammonia after 25 days. The disappearance of ammonia was coinciding with increasing in growth rate of *A. fusiformis*.

Table 4. The removal percentage of ammonia by *A. fusiformis* in aquatic system.

Treatments		% Remaining of ammonia anddry weight in g/200ml Ammonia					
		0 time	5 days	10 days	15 days	20 days	25 days
Control (un-inoculated with C V)	%Remaining	100±0	98±0.31	96±0.23	95±0.22	92±0.21	91±0.31
A. fusiformis + ammonia	%Remaining	100±0	69.3 ±0.11	45.2±0.2	19±0.23	4.3 ±0.2	0.1 ±0.01
	Dry weight in g/200ml	0.065±0.02	0.08±0.01	0.11±0.02	0.14±0.01	0.19±0.02	0.22±0.02

Spirulinawas employed for this study due to the capability in removing nutrients, non-toxic to its surroundings and high salinity tolerance. *Spirulina*has already shown no toxicities –neither acute nor chronic– hence, it is used as a safely human food. Therefore, it would appear that no problem is related to the present use of this cyanobacterium as a single cell protein source (Salazar *et al*., 1998). Chaowanapreecha *et al*.(2007) reported that Spirulinamats can be stored under 1000 lux light intensity, in 0.1x Zarrouk's medium concentration and for six weeks storage time according to ammonia removal efficiency of 85% and effluent ammonia concentration of 0.15 mg/l which still be accepted (compared with fresh Spirulina mat).

Different microalgae have been used with success for wastewater biotreatment (Craggs *et al*., 1995 andVoltolina *et al*., 2005). Although *Spirulinaplatensis* is the most widely studied photosynthetic microorganism, only a few attempts have been made until now to utilize it for removal of nutrients from wastewaters. Particularly, its peculiar advantages, among which its preference for strongly alkaline environment that prevents external contamination (Olguin *et al*., 1997), suggest its employment for environmental applications. *Chlorella vulgaris* has been reported to be able to readily uptake nitrogen from ammonium ion and ammonia through the cell membrane from the wastewater (Kim *et al*., 2010).

Genetic studies

In the assessment of Spirulina damage by crystal violet, the determination of enzyme activity such as estrase (EST) and DNA profile alteration by RAPD marker were used.

Estrase isozyme analysis

As shown in Fig. (2) and Table (5), activity of EST was markedly differed in crystal violet treated algae compared to control group, indicating inhibition of enzyme activity.Six loci of esterase activities in control (untreated Spirulina);EST-2, EST-4, EST-5, EST-6, EST-7 and EST-8 were appeared. It was evident from zymogram that, only one common locus out of the 8loci was observed in bothcrystal violet treatmentand the control, indicating that percentage of polymorphism was 87.5%. Meanwhile,the appearance of two new isozyme loci (EST-1 and EST-3) were shown in the crystal violet treated Spirulina. The activity of EST was decreased as appeared in bands number after administration of crystal violet. This incidence indicated that Spirulinawas damaged due to usingcrystal violet.

Kranthi *et al*.(2001)announced that increase of esterase bands in treated spirulina with crystal violet may be a result of its role in detoxification mechanisms for heavy metal or as a result of*denovo* protein synthesis under metal stress conditions. Also, the reduction in enzyme activity at the same treatment may be due to the interaction of metal with functional sulphydryl (SH)-groups of the enzyme (Prasad and Prasad, 1987).

Furthermore, when Spirulina was treated with crystal violet, new loci (EST-1 and EST-3)were appeared with stronger intensity in EST-3. Results showed that some enzyme bands were disappeared while others were detectedin crystal violettreated Spirulina. The disappearance of enzyme bands might be because crystal violet acts on the DNA level, in which transcriptional, posttranscriptional, translational, or other inhibition processes might be involved. These results agreed with that of Azmi *et al*. (1998), who reported that dyes suppress cell growth by interfering with nucleic acid synthesis then decreasing protein synthesis.

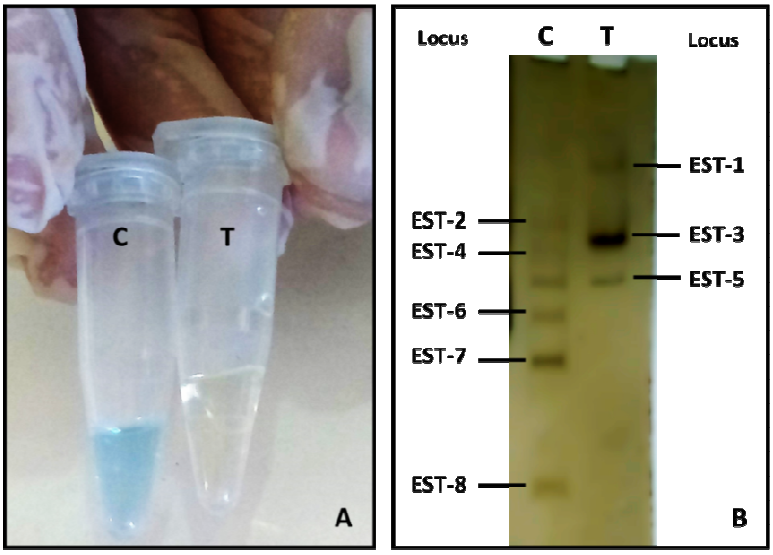

Fig. 2. Effect of crystal violet on estraseisozyme banding patteren of Spirulina (*A.fusiformis*).
(A): extracted total protein, (B): esterase isozyme banding profile, (C): control sample of Spirulina (untreated) and (T): Spirulina treated sample with crystal violet.

Table 5. Number of loci, relative mobilities (RF) and polymorphism percentage for esterase enzyme activity in Spirulina (*A.fusiformis*), control and treated sample with crystal violet.

Locus	RF	Control	Treatment
EST-1	0.174	-	+
EST-2	0.269	+	-
EST-3	0.296	-	+
EST-4	0.318	+	-
EST-5	0.362	+	+
EST-6	0.421	+	-
EST-7	0.491	+	-
EST-8	0.707	+	-
Total No. of loci		8	
Monomorphic loci		1	
Polymorphic loci		7	
Polymorphism %		87.5	

(+) present band (-)absent band

RAPD polymorphism between untreated and C V treated Spirulina

For visualization of algae DNA damage, sevenRAPD primers were applied to investigate the significant changes of genomic DNA isolated from algal cultures of Spirulina (*A.fusiformis*) resulted in specific banding profileswhich showed signs of polymorphism (Fig. 3). The seven primers generateda total of 44 scorable bands, varying from 2 to 9 per primer with an average of approximately 6.29(Table 6). Out of the generated bands, 37 were polymorphic withavalue of 84.09 % polymorphism. The highest percentage of polymorphism (100%) was generated by four primers (OPA-03, OPA-08, OPB-01and OPB-08) out of the seven primersused, while the lowest percentage of polymorphism (66.67%) was generated by OPA-01and OPA-02. Also, the results from RAPD analysis presented changes between control and treated crystal violet Spirulina.Control treatment appeared 30 bands resulted from seven primers,while the treated Spirulina with crystal violet showed only 21 variable bands, seven bands of them were monomorphic which are presented in control. Also, when treated Spirulinawas compared with control, 23 normal bands were disappeared and 14 new bands were detected.

Table 6. Primer name, polymorphism percentage and genome template stability percentage for the seven studied primer in control and crystal violettreated Spirulina.

Primer name	Size range (bp)	Total amplified fragment			Monomorphic bands	Polymorphic bands	P (%)	GTS (%)
		Control	Treatment	Total				
OPA-01	345-1698	5	3	6	2	4	66.67	20
OPA-02	214-1632	6	6	9	3	6	66.67	0.00
OPA-03	227-819	4	3	7	0	7	100	--
OPA-04	226-1039	5	4	7	2	5	71.43	0.00
OPA-08	385-1800	4	2	6	0	6	100	--
OPB-01	604-773	1	1	2	0	2	100	--
OPB-08	329-1415	5	2	7	0	7	100	--
Total		30	21	44	7	37	84.09	20
Average		4.29	3	6.29	1	5.29		2.86

P (%)= Percentage of polymorphism GTS(%) = Genomic template stability percentage.

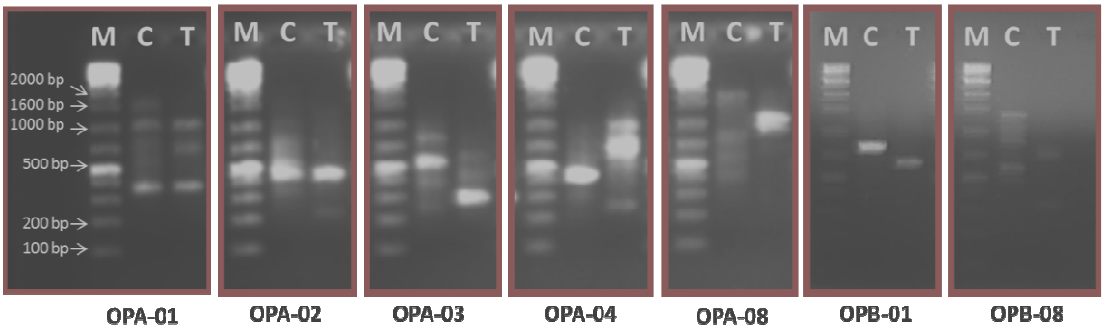

Fig. 3. DNA gel electrophoresis represents RAPD fingerprint for the seven arbitrary primers with isolated DNA from Spirulina (*A.fusiformis*).

Where, M refers to the 1 Kb plus DNA ladder, C refer to untreated Spirulina (A.fusiformis) and T refer to Spirulina (A.fusiformis)-treated with crystal violet.

Evaluation of genomic template stability % in RAPD patterns as decrease in GTS %, a qualitative measure reflecting the change in RAPD profile number sproduced by the crystal violet-treated Spirulina (*A. fusiformis*), in relation to profiles obtained from the control. According to these results, the RAPD profiles of treated and untreated groups showed differences in banding patterns. When the control and treatments were compared, these differences observed in all RAPD profiles are clearly exhibited by the appearance/ disappearance of some bands. Also, it was observed that the higher percentage (20%) of GTS was observed in OPA-01, while the lowest percentage (0.0 %) was recorded for OPA-02 and OPA-04 and the reset primers produced negative percentages of genome temple stability. The higher decrease of genome stability in Spirulina (*A.fusiformis*) was induced as the toxic effect of crystal violet.

The disappearance of normal RAPD product may be related to the events such as DNA damage (single and double strand breaks, mutated bases), point mutations and chromosomal rearrangements caused by genotoxins (Atienzar *et al.,* 1999; Atienzar *et al.,* 2002; Wolf *et al.,* 2004; Enan, 2006 and Liu *et al.,* 2009).

In conclusion, *A. fusiformis* has enormous potential to remediate chemicals contaminated water and resolve the problem of unnecessary dyes presented in the effluents of textile industries. *A. fusiformis* showed also detoxifying C V. This observation has proved that the cyanobacteria are adaptive in nature and can degrade contaminants. Application of traditional wastewater treatment is very expensive and continuous input of chemicals, which becomes uneconomical and causes further environmental damage. Also, *A. fusiformis* showed better efficiency in bioremediation of crystal violet and ammonia contaminated water. *A. fusiformis* have certainly proven to be leading species in basic nutrient treatment of wastewater, and the health, environmental and commercial benefits cannot simply be ignored. Furthermore these microalgae could be instrumental for the conversion of 'Waste to Wealth' in our environment.

Therefore, while the Spirulina (*A.fusiformis*) had the ability to remediate crystal violet and ammonia thatfound as a chemical pollutants, these chemicals were also able to caused different changes in the genetic constitution of this algae (*A.fusiformis*). The anti-effect was appeared on the biochemical and molecular levels by disappearance of some genetic loci and appearance of new genetic loci in algae treated these pollutants compared with untreated control. The appearance of new loci may be responsible or at least play an important role in the bioremediation activity of Spirulina (*A fusiformis*).

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دراسات وراثية علي المعالجة البيولوجية للمياه الملوثة ببعض الملوثات الكيميائية باستخدام طحلب سبيرولينا (*Arthrospirafusiformis*)

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أجريت هذه الدراسة بغرض المعالجة البيولوجية للمياه الملوثة بصبغه الكريستال البنفسجي والأمونيا باستخدام طحلب سبيرولينا (*Arthrospirafusiformis*) وأيضاً لدراسة التأثير العكسي لصبغة الكريستال البنفسجي علي التركيب الوراثي لطحلب سبيرولينا باستخدام تكنيكات الوراثة البيوكيميائية والجزيئية. أظهرت النتائج أن طحلب سبيرولينا كان له القدرة علي استخدام صبغة الكريستال البنفسجي والأمونيا كمصدر وحيد للكربون والنيتروجين علي التوالي، حيث تم التخلص نهائياً من صبغة الكريستال البنفسجي والأمونيا بواسطة الطحلب في بيئة سائلة بعد 25 يوم من التحضين علي درجه حرارة 35° م ودرجة حموضة الوسط PH=9 وذلك بالمقارنة بالبيئة الغير ملقحة بالطحلب (كنترول). لم تظهر أي سمية لصبغة الكريستال البنفسجي علي بكتريا *Bacillus subtilis* عند إستخدامها كاختبار تحليل بيولوجي بعد التحضين مع طحلب سبيرولينا لمدة 25 يوم كما أن تحطيم أو هدم صبغة الكريستال البنفسجي والأمونيا كان متلازماً مع زيادة كتلة طحلب سبيرولينا. تم ايضاً تحليل نشاط انزيم الأستيريز وبادئات الـ RAPD الجزيئية لدراسة تأثير السمية الوراثية لصبغة الكريستال البنفسجي علي تركيب الجينوم لطحلب سبيرولينا. أظهرت النتائج حدوث تغير شديد في نشاط إنزيم الأستيريز في عينات الطحلب المعاملة بصبغة الكريستال البنفسجي عند مقارنتها بالمجموعة غير المعاملة (كنترول) حيث أشارت النتائج إلي وجود تثبيط لنشاط هذا الانزيم والذي ظهر في عدد الحزم والنسبة المئوية المرتفعة لتعدد الأشكال المظهرية والتي وصلت إلي 87,5 % بين المجموعة المعاملة والغير معاملة. وتم أيضاً استخدام 7 بوادئ DNA عشوائيةRAPD والتي أنتجت 44 حزمة من الـ DNA كان منها 37 حزمة متعددة الشكل المظهري بنسبة 84,09 % مما يؤكد أيضا تأثير السمية الوراثية لصبغة الكريستال البنفسجي علي طحلب سبيرولينا. أثبتت هذه الدراسة أنه يمكن استخدام طحلب سبيرولينا (*Arthrospirafusiformis*)في معالجة المياة الملوثة بصبغة الكريستال البنفسجي والأمونيا علي الرغم من التأثير الوراثي الضارالذي تحدثه صبغة الكريستال البنفسجي علي الطحلب.