DNA Fingerprinting of Three Cyanobacterial Strains

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ABSTRACT

Cyanobacteria are photoautotrophic microorganisms and some can fix the atmospheric nitrogen, their use for biodegradation of surface water would circumvent the need to supply biodegradative heterotrophes with organic nutrients. Moreover, cyanobacteria have an interesting therapeutic and antimicrobial capabilities. Therefore , there is much attention is being paid on studying their genetic characteristic and gene manipulation techniques for cyanobacteria , to allow the development of clean bioprocesses based on their photosynthetic activities. In the present study, three cyanobacterial strains were classified into three different genera on morphological basis. Meanwhile, Biochemical and molecular genetic characterization of the studied strains were carried out via SDS-PAGE, and RAPD-PCR analysis in order to confirm the phylogenetic differences among the studied strains.

Keywords: Molecular genetic, Blue Green Algae, RAPD- PCR, SDS-PAGE, Photosynthetic, Phylogenetic, SDS-PAGE.

INTRODUCTION

Cyanobacteria have played a significant role in Earth history as primary producers and the ultimate source of atmospheric oxygen. To date, however, how and when the group diversified has remained unclear **(Tomitani,, A. *et al.* 2006)**. Filamentous cyanobacteria including nitrogen fixer strains that combine aerobic metabolism in the vegetative cells with anaerobic metabolism in their differenciated cells called heteurocystis, are widespread in many ecosystems including polluted once . Some of these filamentous cyanobacteria have a natural ability to degrade a highly chlorinated aliphatic pesticide, lindane (hexachlorocyclohexane), with the presence of evidence that this ability can be enhanced by genetic engineering ; and there are qualitative evidence that those two strains can be genetically engineered to degrade another chlorinated pollutant, 4-chlorobenzoate. **(Kuritz, Tanya and Walk, C.P., 1995).**

Phytoplankton and other aquatic vegetation can absorb and accumulate pesticides from the water bodies **(Khalil, Z., 1992)**.

Marine cyanobacterium Agmenellum quadriplicatum PR_6 metabolized phenanthrene to form trans-9, 10,- dihydrophenenthrene and 1- methoxyphenenthr. toxicity studies, using an algal lawn bioassay,

indicated that 9- phenenthrol and 9,10- phenenthrenequinone inhibit the growth of A. quadriplicatum PR-6.**(Narro, 1992)**

Algae are capable of carrying out biotransformation of aromatic pollutants, such as the hydroxylation of naphthalene and benzo {a} pyreneof there hydroxylated intermediates. These initial transformation may lead to making these pollutants more susceptible to attack by other microorganisms ofter rthe removal of a substituent group **(Semple *et al.*, 1999).**

Molecular phylogenies have been employed to resolve evolutionary relationships within the cyanobacteria (Henson, B.J. *et al.*, 2004),

Therefore, there is much attention is being paid on the development of bioreactor systems and gene manipulation techniques for cyanobacteria, to allow the development of clean bioprocesses based on their photosynthetic activities **(Kuritz, 1999).**

The aim of the present investigation is to optimize the conditions and establishing the techniques required for the biochemical and molecular genetic characterization of the three studied cyanobacterial strains .

MATERIAL AND METHODS:

Bacterial strains and culture conditions: The three studied cyanobacterial strains were grown in liquid modified SP medium as described by **Mohamed, 2000**. The cultures were tested for the presence of contaminating heteurotrophes microscopically and by plating on L agar and incubating for 1 week at 30°C. All cultures used were axenic. The strains were then classified on the morphological basis into four different genera (Table 1).

Molecular genetic characterization

B- Molecular genetic characterization:

RAPD Analysis:

Seven arbitrary 10 mer primers (Table:2)were used for PCR based on the protocol of **Mohamed, 2000.**

Reaction similar to that described by **(Mohamed, 2000)** was performed with some minor modifications. Reaction volume (25 ul) contained the following concentrations: 25 ng genomic DNA.0.2 uM primer,0.2 mM of each dNTP, (from Bohringer Manheim)1.5 units of taq DNA polymerase 2.5 ul 10x buffer Steril dd H2O up to 25 ul.

Reaction mixture was overlaid with a drops of light mineral oil.Amplification was performed in a thermal cycler (Perkin Elmer GeneAmp PCR System 2400) programmed for 1 cycle at 95°C for 5min., 4o cycles of 95°C for

1min., 37 °C for 1 min. and 72 °C for 2 min., final extention cycle at 72 °C for 15 min.

Agarose gel electrophoresis (0.7% in 1x TBE buffer) was used for separation of the generated PCR fragments.

Visualization and documentation were carried out by staining with ethidium bromide solution for 30 min. After that, gels were photographed under UV light.

Dendrogram construction

For constructing a combined dendrogram dealing with genetic relationships among the studied strains in the present work, the data generated from the protein banding pattern, and RAPD- PCR were introduced to SPSS package program according to binary values (1,0). The output results involved both different hirarchical pair- wise distance (UPGAMA) and constructed dendrogram.

RESULTS AND DISCUSSION

RAPD- PCR analysis:

As shown in Tables 10, and 11 a total of 118 DNA bands were detected by 7 random primers with at least 60% GC ratio (UBC3, and UBC 9), 70% GC ratio (UBC2, 6, 7), and 80% GC ratio in (UBC 4, and 5). These primers identifying and differentiated the three different samples of cyanobacteria based on DNA amplification using their template DNA.

In general, the sizes of the amplified DNA fragments generated by all primers are ranged from lower than 100 bp up to approximately 26573 bp. These primers did not produce any common bands, which means that all patterns were polymorphic, Moreover, the number of PCR products generated from every primer varied between 2 to 29 bands. Although informative profiles were generated in most cases, several samples produced profiles with only one to several bands. Arrangement of primers depending on the number of DNA fragments is UBC5 > UBC6>UBC3> UBC4> UBC9> UBC6> UBC7. Furthermore, Table 10, and 11 showed that the lowest number of bands was produced in strain Q2 (33 bands), while the highest number was 47 in strain Q1 was. Arrangement of strains depending on the number of fragments is Q1> Q3 > Q2. Moreover, six primers were reacted and produced PCR fragments with all strains (UBC 2, 3, 4, 5, 6, and 9). One primer (UBC 7) was reacted with one strain only.

Profiles of different primers:

Primer UBC 2:

Primer UBC 2 contains 70% G+C. In general, the MW of PCR products generated by this primer are ranged from about 522 - 2241 bp (Tables 3,10 and 11. The total band numbers produced by this primer was 6 bands. Also the number of total bands varied between the strains, Where the lowest number is one band in strains Q2. While, the highest number was 3 bands in strains Q1. There are 3 unique specific bands in strain Q1 with MW. Of about 2241, and 1638 and 522 bp. While, only one unique specific band was detected in strain Q3 with MW. of 712 bp. Thus, theses unique specific bands could be used to distinguish among all the studied strains. Moreover,a band with MW. Of 1318 bp was detected in the two strains Q2 and Q3. which may be, considered as specific markers for those group of strains.

Primer UBC 3:-

This primer contains 60% G+C. The PCR products of this primer ranged from 6 bands in the strain Q2 to 9 bands in strain Q3 with molecular sizes from 161 bp to 3922 bp (Tables 3, 10 and 11). Moreover, some strains had specific band(s) which could be used to distinguish among them. For instance, strain Q2 could be distinguished from the strain Q3 by the existence of two unique band that have a MW. of about 400, and 1247 bp.respectively. Moreover, strain Q1 had 6 specific bands of MW. of about 2373, 1990, 1500, 1300, 900, 876, and 800bp, respectively. Furthermore, strain Q3 has 5 specific band at the MW. Of about 2145, 733, 300, 250, and 161 bp respectively.

Primer UBC 4:

This primer has a 80% G+C. The PCR products of primer UBC 4 and analysis of these products are illustrated in Tables' 3, 10, and 11 respectively. This primer produced 6-8 bands among the studied strains with MW. Of about 247- 2072 bp. Some strains have unique bands and could be used to distinguish among them. For example, strain Q1 has 6 unique bands at MW. Of about 1690, 1400, 1300, 800, 600, and 400 bp, respectively. While, strain Q3 has two specific bandsat MW. Of about 267 and 247 bp, respectively.

Primer UBC 5:

This primer contains 80 % G+C. The PCR products of this primer ranged from 5-13 bands distributed in all strains with molecular sizes

ranged from 26573- 564 bp As shown in tables 3,10 and 11. Some strains have some specific bands. For example, strains Q1 and Q2 have 9 specific bands. Meanwhile, strains Q3 does not have any specific bands

Primer UBC 6:

As illustrated in tables 3,10, and 11, respectively. A total of 26 distinguishable bands with different molecular sizes were present in all strains. Also, the number of total bands varied between strains, where the lowest number was 7 bands in strain Q2 and the highest number was 11 in strain Q1. It is interesting to note that the two strains Q1 and Q2 have 3 specific band. Moreover, the strain Q3 has two specific bands.

Primer UBC7:

Only two specific bands with the strain Q3 of molecular sizes of 900 and 400 bp were produced by this primer (Tables 3,10 and 11). Moreover, no reactions were detected with strains Q1 and Q2 which means that this primer had no complementary sequences with those strains. Some strains had some unique bands and could be used to distinguish among them..

Primer UBC9:

Primer UBC9 has a 60% G+ C. The primer produced 3-7 bands distributed in all strains. The results of RAPD analysis using this primer are illustrated in Tables 3,10 and 11. All bands produced with the strain Q1 were spescific.While, all DNA bands produced with strains Q2 and Q3 were common bands. Therefore, this primer could be used to distinguish strain Q1 from the two other strains Q2 and Q3.

Dendrogram construction:

In the present investigation the results generated from the employed genetic criteria were pooled together for drawing the genetic relationships among the three examined cyanobacterial strains. The genetic distance was estimated for each pair-wise groups as an mathematical average of unweighed samples (UPGMA). The lowest distance was about 25 that between Q2 and Q3 strains. As shown in Fig (2) the phylogenetic tree showed that the three studied cyanobacterial were grouped into two groups at the distance about 25. In addition, the three strains Q2 and Q3, whose classified on morphological basis in one genus Anabeana and Oscilatoria, respectively, were grouped in one cluster. While, strain Q1 was grouped in a separate

cluster. Molecular genetic data have been used to draw phylogenetic relationships among and within cyanobacterial species. The present analysis is similar to that carried out by **Mohamed, 2000** who used RAPD-PCR to generate unique -DNA profiles for members of the cyanobacterial genera Anabaena, Lyngbya, spirulina and Oscillatoria. Analysis of DNA typing results obtained by the described method clearly distinguished between the two genera. The markers produced for each strain were also applied to a phylogenetic analysis to infer genetic relatedness in this group of prokaryotes.

Conclusion of the biochemical and molecular genetic. characterization:

As shown in Table 18, The best characterization criteria was primer UBC5 which produced 18 specific markers.. The highest number of specific marker bands were found in strain Q1 (32 bands). Meanwhile, the lowest number of specific marker bands were detected in strain Q3 (12 bands). The best characterization criteria for both strains is primerUBC5 which produces 9 marker specific bands. While, the best criteria for strain Q3 is primer UBC3 which produces 5 marker specific bands. Furthermore, data obtained from the molecular genetic characterization have been confirmed the preliminary morphological classification of the studied strains into three different genera.

Table (1): Cyanobacterial strains.

Genera	Code
Lyngbya	Q1
Anabaena	Q2
Oscillatoria	Q3

Table (2): Primer Sequences.

Primer	Sequences
UBC-2	CCTGGGCTTG
UBC-3	CCTGGGCTTA
UBC-4	CCTGGGCTGC
UBC-5	CCTGGGTTCC
UBC-6	CCTGGGCTTG
UBC-7	CCTGGGGGTA
UBC-9	CCTGCGCTTA

Table (3): Generated DNA fragments.

Primer	M.W	Q1	Q2	Q3
UBC2	2241	+	-	-
	1638	+	-	-
	1318	-	+	+
	712	-	-	+
	522	+	-	-
UBC3	3932	-	+	+
	2914	+	+	+
	2373	+	-	-
	2154	-	-	+
	1990	+	-	-
	1824	-	+	+
	1500	+	-	-
	1300	+	-	-
	1247	-	+	-
	900	+	-	-
	876	+	-	-
	800	+	-	-
	733	-	-	+
	466	-	+	+
	400	-	+	-
	300	-	-	+
	250	-	-	+
	161	-	-	+
UBC4	2072	+	+	-
	1690	+	-	-
	1400	+	-	-
	1300	+	-	-
	1243	-	+	+
	1159	-	+	+
	953	-	+	+
	800	+	-	-
	665	+	-	+
	600	+	-	-
	400	+	-	-
	371	-	+	+
	329	-	+	+
	267	-	-	+
	247	-	-	+

Primer	M.W	Q1	Q2	Q3
UBC5	26573	+	-	-
	21226	+	-	-
	19443	+	-	-
	17660	+	-	+
	8734	+	-	+
	4973	+	-	+
	4268	-	+	-
	3211	-	+	-
	314.8	-	+	-
	3106	+	-	-
	3000	-	+	+
	2684	+	-	-
	2373	+	-	-
	2027	-	+	-
	1904	+	-	+
	1744	+	-	-
	1584	-	+	-
	1375	-	+	-
	1296	+	-	-
	1030	-	+	-
	831	-	+	-
	669	+	-	-
	564	-	+	-
UBC6	1904	+	-	-
	1476	+	+	-
	1375	+	-	+
	947	-	-	+
	900	+	-	-
	854	+	+	+
	877	-	+	+
	614	+	-	-
	403	+	+	+
	205	+	+	-
	196	+	-	+
	125	+	+	-
	83	-	-	+
	22	+	+	-
UBC7	900	-	-	+
	400	-	-	+
UBC9	5451	-	+	+
	3305	+	-	-
	3080	-	+	+
	1508	+	-	-
	834	+	-	-
	364	+	-	-
	472	-	+	+
	101	+	-	-

Table (4): Number of bands of each isolate with seven different primers.

Isolate → / Primer ⇓	Q1	Q2	Q3	Total Amplified Bands	Total polymorphic bands
UBC2	3	1	2	6	6
UBC3	8	6	9	23	23
UBC4	8	6	8	22	22
UBC5	13	10	6	29	29
UBC6	11	7	8	26	26
UBC7	0	0	2	2	2
UBC9	5	3	3	11	11
Total	47	33	38	118	118

Table (5): Lowest and highest Number of bands produced by each primer/isolate.

Isolate	Lowest No. of bands	Highest No. of bands	No. of reacted primers	Total band No.
Q1	0	13	6	47
Q2	0	10	6	33
Q3	2	9	7	38

Table (6): Number of marker specific bands of strains with each primer.

strain⇒ primer ⇓	Q1	Q2	Q3	Total
UBC2	3	0	1	4
UBC3	6	2	5	13
UBC4	6	0	2	8
UBC5	9	9	0	18
UBC6	3	3	2	8
UBC7	0	0	2	2
UBC9	5	0	0	5
Total	32	14	12	58

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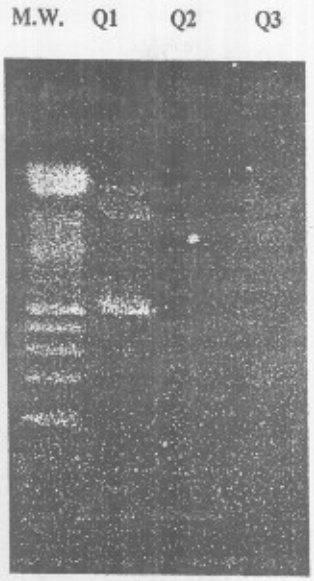

Fig (1): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC2

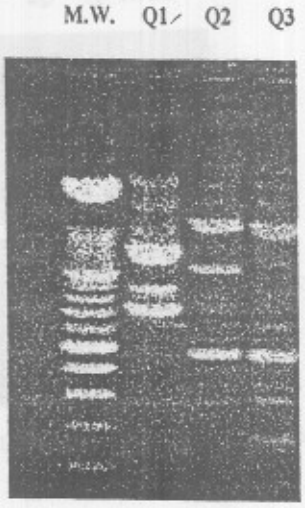

Fig (2): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC3

Fig (3): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC4.

Fig (4): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC5.

Fig (5): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC6.

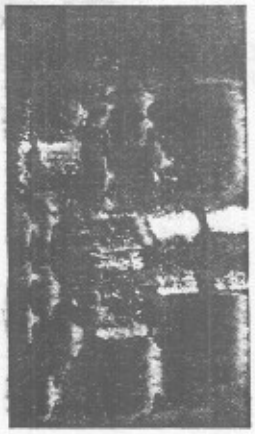

Fig (6): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC7.

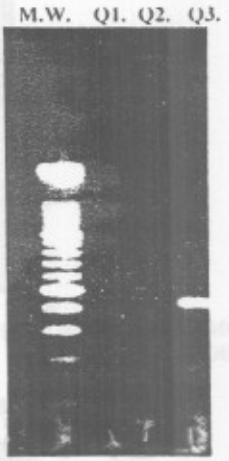

Fig (7): DNA polymorphism based on RAPD-PCR analysis of studied isolates with primer UBC9.

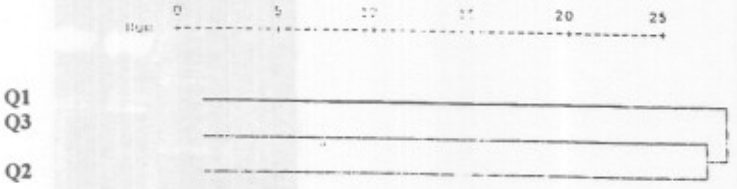

Fig (8): A phylogenetic tree construction of cyanobacterial strains based on RAPD-PCR analysis

الملخص العربى

البصمة الوراثية لثلاث عزلات من الطحالب الخضراء المزرقة

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المملكة العربية السعودية

تعتبر الطحالب الخضراء المزرقة من الكائنات الدقيقة ذاتية التغذية حيث أن لها القدرة علي القيام بعملية التمثيل الضوئي كما أن بعضها يستطيع تثبيت نيتروجين الهواء الجوي و قد تبين أن استخدامها في عملية التكسير الحيوي للملوثات الموجودة في المياه من الممكن أن يفي باحتياجاتها من المواد الغذائية العضوية . بالإضافة الي ذلك فإن الطحالب الخضراء المزرقة من الممكن أن تستخدم في مجال العلاج حيث أن لها بعض التطبيقات العلاجية كمضادات للبكتريا . لذلك فإنه يجب أن يكون هناك المزيد من البحوث ، لدراسة الصفات الوراثية و طرق تداول الجينات لهذه الطحالب و ذلك لتطوير طرق بيولوجية نظيفة و صديقة للبيئة اعتمادا علي قدراتها علي القيام بعملية التمثيل الضوئي . و في هذه الدراسة تم تصنيف ثلاثة عزلات من الطحالب الخضراء المزرقة اعتمادا علي صفاتها المورفولوجية . كما تم التأكد من هذا التصنيف اعتمادا علي التوصيف البيوكيماوي و الوراثي و ذلك باستخدام تقنيات التفريد الكهربي للبروتينات و تقنية الـ RAPD- PCR.