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PHYLOGENETIC ANALYSIS OF SOME INDIGENOUS CYANOBACTERIAL STRAINS BY RANDOM AMPLIFIED POLYMORPHIC DNA-PCR

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ABSTRACT

Molecular characterization for 11 cyanobacterial strains belonging to 3 genera namely *Nostoc* (4 strains of *N. muscorum* and 3 strains of *N. calcicola*), *Anabaena* (one strain of *A. oryzae* and one strain of *A. oscillarioides*) and *Calothrix* (2 strains of *C. clavata*) was done using the RAPD-PCR technique. Genomic DNA was extracted from these strains and amplified using the primers OPG-05, A- 01, OPV, OPT-07 and OPS-19 and distinct RAPD-PCR fingerprints were generated. Unique banding patterns were observed from all tested cyanobacterial strains and their molecular weights of each band were used to calculate the genetic distance among them, and the phylogenetic relations among these isolates was studied. RAPD fingerprinting results clearly showed the existence of wide range of genetic variation among the cyanobacterial strains. Data showed that phylogenetic relatedness between cyanobacterial strains was not highly influenced by with soil texture.

Keywords: RAPD-PCR, phylogenetic relatedness, cyanobacteria, *Nostoc, Anabaena, Calothrix.*

INTRODUCTION

Cyanobacteria are aquatic bacteria distributed world-wide and diverse assemblage of unicellular or multicellular photosynthetic prokaryotes that possess chlorophyll *a* and perform oxygenic photosynthesis. Cyanobacteria are responsible for a significant proportion of the biological fixation of nitrogen on Earth (Haselkorn and Buikema, 1992). These prokaryotes are valuable sources of various natural products of medicinal and industrial value (Cardozo *et al.*,

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2007). In addition, their inherent capacity to fix atmospheric nitrogen makes them ecologically important for rice-growing countries where they add to rice fields as natural biofertilizer (Vaishampayan *et al.*, 2001).

Molecular approaches to cyanobacterial identification are not influenced by ecological variables and in many instances do not require axenic or unicyanobacterial cultures for analysis. Indeed, due to the sensitivity afforded by DNA amplification technology, minute sample size, containing little cyanobacterial biomass, are required for the generation of genetic profiles (Neilan, 2002). Cyanobacteria have been analyzed using new molecular techniques such as DNA sequencing, random amplified polymorphic DNA (RAPD), and DNA polymorphisms (Neilan, 1995, Neilan *et al.*, 1997 and Otsuka *et al.*, 1999). For broad phylogenetic studies, sequence data from the 16S rRNA gene are most commonly employed due to its utility for distinguishing higher level taxonomic groups as well as traditional species (Neilan, 1995 and Neilan *et al.*, 1995).

Random Amplified Polymorphic DNA (RAPD) allows the detection of multi-locus genetic variation using short primers of arbitrary sequence (Welsh and McClelland 1990 and Williams *et al.*, 1990). This molecular technique is very easy to perform and requires no prior knowledge of the genomes under investigation (Weising *et al.*, 1995). This technique has been used for the analysis of diversity within germplasm populations (Virk *et al.*, 1995), phylogenetic relationships (Lunge *et al.*, 1994) and identification to the strain level (Welsh and McClelland 1990). Jeberlin Prabina *et al.*, (2003) stated that RAPD markers could be further used to identify and establish the genetic purity of the strains in the cyanobacterial inoculum. They found similarity of 60 – 90% within *Westiellopsis* cultures. *Nostoc* cultures shared 50 – 80% similarity with *Westiellopsis* cultures. *Anabaena* cultures were 60 - 70% similar to *Westiellopsis* cultures. The markers produced

for different cyanobacterial cultures were also applied to phylogenetic analysis to infer genetic relatedness in this group of prokaryotes. RAPD-PCR technology was used to evaluate the genetic diversity, and to provide rapid and reliable tool for differentiating strains of *Calothrix* isolated from different geographical locations (Shalini *et al.*, 2008). RAPD fingerprinting results clearly showed the genetic variation among the cyanobacterial isolates (Mahendra Perumal *et al.*, 2009).

This work was aimed to study the free-living cyanobacteria isolated from different rice fields in Sharkia governorate, Egypt and evaluate their diversity based on their widespread distribution and phylogenetic characterization based on RAPD-PCR technology.

MATERIALS AND METHODS

This study was carried out in the laboratory of Agric. Microbiology Dept. at the Faculty of Agriculture, Zagazig University. Molecular biological studies of cyanobacterial isolates were carried out at Genetic Engineering and Biotechnology Research Institute (GEBRI) Menofia University, Sadat city Egypt.

Cyanobacterial Culture and Culture Conditions.

In previous investigation (unpublished data), different cyanobacterial isolates (42 isolates) were performed from soils of different regions at Sharkia governorate included seven areas from the fertile soil namely Zagazig (4 isolates), Hihya (4 isolates), Mashtool El-Sook (2 isolates), Belbiese (3 isolates), Diarb Nagm (3 isolates), El-Hessenia (4 isolates), Abo-Hammad (2 isolates), and three areas of the newly reclaimed soils, which suffer from high salinity namely San El-Hagar (8 isolates), Shader Asaam (4 isolates) and Sahl Elteena (8 isolates). Physico-chemical analysis of fertile soil revealed that the pH ranged from 7.10 to 7.75, E.C from 2.57 to 6.4dsm^{-1}, organic matter from 1.08 to 1.95% and the soil texture from clay loam to clay. Meanwhile new

reclaimed soil revealed that the pH ranged from 7.95 to 8.52, E.C from 19.17 to 22.10 dsm^{-1}, organic matter from 0.46 to 1.01 % with a soil texture.

Among the 42 cyanobacterial isolates only 11 cultures were selected as effective biofertilizers for rice plants according to their capacities in N_2-fixation and phytohormonal production. After purification these isolates were subjected, in the laboratory, to morphological and physiological studies and hence their taxonomical patterns.

The study revealed that the selected isolates were belonging to 3 genera namely *Nostoc, Anabaena and Calothrix.* All isolates were cultivated on $BG^0$13 medium (Ferris and Hirsch 1991). All cultures were grown under continuous illumination with Philips Florescent white lamps, at a relatively low light intensity (400-500 lux) and incubated at 30°C for 3-4 weeks. Purification of these cyanobacterial isolates using antibiotics (nystatin and cycloheximide) were carried out according to Ferris and Hirsch (1991), and using UV radiation according to the methods described by Garcia-Pichel *et al.*, (1992). Identification of cyanobacterial isolates were described using the keys provided by Desikachary (1959), Rippka *et al.*, (1979), Komárek and Anagnostidis (1989), Rippka *et al.*, (2001) and Vashishta *et al.*, (2008).

Phylogenetic Analysis of Cyanobacterial Strains by Random Amplified Polymorphic DNA (RAPD-PCR)

DNA extraction and PCR template preparation

The selected eleven isolates were used to determine the extent of genetic diversity on the basis of DNA fingerprinting using the RAPD-PCR method. Cyanobacterial isolates were harvested immediately upon receipt, and total genomic DNA was extracted by a modified method for purification of DNA from gram-negative bacteria, including cyanobacteria according to Smoker and Barnum (1988). DNA concentration was quantified as µg/µl using the GeneQuant™ spectrophotometer, and DNA

purity was measured as optical density at 260/280 nm. For more accuracy, DNA quality and quantity of samples were assured through electrophoresis on agarose gel aside to serial dilution of λ DNA as a marker (25, 50, 75 and 100 ng) and comparing the intensity of samples with the λ DNA/ *Hind* III marker (Biron, cl857 Sam).

RAPD-PCR amplification condition and electrophoretic analysis

The amplification reaction was primarily based on the procedure suggested by Neilan, (1995). The reaction was carried out in 25 µl reaction volume containing in a final concentration 1X PCR buffer, 1.5 $MgCl_2$, 0.2 mM dNTPs, 2 pmol primer, 0.04 U *Taq* DNA polymerase and 0.5 µg template DNA. The PCR cycling condition involved initial denaturation at 94°C for 4 min followed by 35 cycles of amplification under the parameters, template denaturation at 94°C for 1 min, primer annealing at 32°C for 50 sec, and primer extension at 72°C for 2 min., final extension at 72°C for 5 min was given, followed by storage at 4°C. The random 10-mer oligonucleotide primers used are presented in Table 1. A total of 10 primers were initially chosen to generate RAPD patterns for 11 isolates of cyanobacteria. This number of primers was reduced to five, these being the primers which produced informative and reproducible genetic markers for the cyanobacterial isolates under investigation. Primers were obtained from commercial sources (Biron, cl857 Sam). PCR thermocycler machine from Biometra (T-Gradient Thermoblock) was used.

Agarose gel (1.5%) was used for resolving the PCR products and 1 kb DNA marker as a standard DNA was used in the present study. Agarose was stained with ethidium bromide solution (0.5 mg/ml) to visualize RAPD bands. The run was performed for 1 hour at 50 volt in SDE-PLAS submarine (10 cm x 10 cm). Bands were detected on UV-transilluminator, photographed by Gel Documentation System and were analyzed using the Phortix Program.

Calculation of Genetic Distances with RAPD Markers.

Each band visualized on a gel was considered a RAPD marker

and part of the total RAPD fingerprint generated from each selected cyanobacterial isolates. Bands bisected by similar perpendicular lines drawn across the gel were considered homologous characteristics. With this approach to RAPD marker identification, the comigration of bands between cyanobacterial accessions was used as an indication of genetic relatedness. The assumption that these bands contained homologous primer recognition sequences and identical intervening sequence lengths was made. Therefore, the presence or absence of a band at any position on the gel was used to construct a binary matrix for cyanobacterial RAPD markers from the described multiplex reaction. Genetic distances between strains were calculated by using the algorithm of Nei and Li (1979) as provided in the RAPDistance software package developed by Armstrong *et al.*, (1994). A pairwise comparison of genetic distances for all cyanobacterial patterns was used to create a dendrogram based on the neighbor-joining method and the program NJTREE (Saitou, and Nei, 1987), which was also supplied with the RAPDistance package. Another 17 metrics supplied by the RAPDistance package were applied to the data to support the tree inferred by the Nei and Li algorithm distances, including metrics previously used for RAPD marker analysis (Apostol, 1993).

Table 1. Decamer primers used for RAPD analysis of cyanobacterial strains

Primers	Sequence $5^- \longrightarrow 3^-$	C+G%
OPG-05	- CTG AGA CGG A -	60
A- 01	- CAG GCC CTT C-	70
OPV	- GCA CGG CGT T -	70
OPT-07	- GGC AGG CTG T -	70
OPS-19	- GAG TCG GCA G -	70

RESULTS AND DISCUSSION

The selected cyanobacterial strains obtained from our previous investigation (unpublished data) are shown in Table 2 indicating the morphological and physiological characters of the different cultures upon which they have been classified into 3 genera and 11cyanobacterial species.

Molecular Biological Studies

In the present investigation, RAPD- PCR was used to assess the phylogenetic relatedness amongst 11 selected isolates of cyanobacteria. A total of 10 primers were initially chosen to generate RAPD patterns for 11 isolates of cyanobacteria. This number of 10-mer oligonucleotides was reduced to five, these being the primers which produced informative and reproducible genetic markers for the cyanobacterial isolates under investigation. Primers which produced consistently even product intensities throughout a pattern were favored because of the high reproducibility of markers in these reactions. RAPD-PCR combined analysis with single primer was particularly useful for isolates in differentiation as it increased the number of informative genetic markers (Shalini *et al.*, 2008).

RAPD-PCR analysis

The electrophoretic patterns for 11 isolates of cyanobacteria derived from five single-primer reactions were analyzed to provide diagnostic fingerprints for each culture and to measure genetic distances between isolates based on RAPD marckers (Fig.2 A-E). Genetic diversity among isolates tested was determined with banding patterns from the RAPD reaction. Each band was considered a genetic marker for the isolate from which it was amplified. They showed that the DNA fingerprint consisted of band fragments ranged from 1300 bp to 50bp and were clearly distinguishable among all isolates tested (Table 3).

A total of 373 distinct polymorphic DNA fragments (bands), ranging from 50 to 1300bp were produced in PCR reaction with single primers (Table 3). The total number of fragments produced from single primer reaction ranged from 41 bands (A-01) to 110 bands (OPT-07).

Table 2. Morphological and physiological characteristics of cyanobacterial isolates

Characteristics \ Name of isolates	Sa83	Sh1	Z1	E3	D13	Se100	B4	Sa31	Se112	Se80	Se12
Morphological characteristics											
Color of culture	Brown	Olive green	Blue green	Brown	Brown	Brown	Brown	Blue green	Dark green	Blue green	Olive green
General shape	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous
Vegetative Cell: Shape	Barrel to ovoid	Disc-shape	Cylindrical	Barrel to Cylindrical	Barrel to Cylindrical	Barrel to Cylindrical	Barrel to Cylindrical	Cylindrical to ovoid	Isodiametric or Cylindrical	Barrel to Ovoid	Disc-shape
Vegetative Cell: Width (µm)	2.5-3.0	2.5-3.0	1.5-3.0	2.0-2.5	2.0-3.0	2.5-4.0	3.0-4.0	3.0-4.0	4.5-5.5	2-3.5.0	2.0-2.5
Vegetative Cell: Length (µm)	5.0-6.5	2.5-3.5	3.0-5.0	3.0-4.0	3.0-4.0	4.5-7.0	5.0-6.0	5.0-6.0	4.0-5.5	4.0-7.0	2.0-2.5
Heterocyst: Shape	Ovoid	Hemispherical	Spherical	Sub-spherical	Ovoid	Subspherical	Sub-spherical	Ovoid	Spherical	Ovoid	Hemispherical
Heterocyst: Width (µm)	3.0-4.0	5.0-6.0	2.5-3.5	3.0-4.0	3.0-4.0	3.0-4.0	3.0-5.0	4.5-5.5	6.0-7.0	3.0-3.5	5.0-7.0
Heterocyst: Length (µm)	5.0-6.5	6.0-7.0	3.0-4.0	4.0-5.0	4.0-6.0	5.0-6.5	4.0-5.0	5.0-6.0	6.0-8.5	3.5-4.5	6.0-8.0
Site of Heterocyst	Inter& Ter	Terminal	Inter.& Ter.	Inter.& Ter.	Inter.& Ter.	Inter& Ter	Inter.& Ter.	Inter& Ter	Inter& Ter	Inter& Ter	Terminal
Akinetes: Shape	Ovoid	Spherical	Cylindrical	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Cylindrical	Ovoid	Spherical
Akinetes: Width (µm)	5.0-6.0	4.5-6.0	5.0-6.0	4.0-5.0	4.0-5.0	5.0-7.0	5.0-7.0	5.0-7.0	8.0-11.0	4.0-6.0	4.0-6.0
Akinetes: Length (µm)	5.0-8.0	4.5-6.0	7.0-8.0	5.0-7.0	6.0-10.0	7.0-9.0	6.0-8.0	6.0-8.0	20.0-28.0	5.0-7.0	4.5-6.0
Baeocyste	-	-	-	-	-	-	-	-	-	-	-
Sheath	-	+	-	-	-	-	-	-	-	-	+
Physiological characteristics											
Nitrogenase activity µ mole C_2H_4/h/ml	9.316	11.350	16.049	21.056	10.468	15.010	3.489	13.210	4.664	10.450	8.384
Total protein (mg/g)	297.3	323.1	270.3	249.2	321.3	307.2	261.0	321.3	259.5	302.1	245.1
IAA (µg/ml)	7.30	3.25	9.31	9.50	6.60	3.20	5.95	3.25	3.50	12.00	3.45
Pigments (% Chlo. A/ Phycoc.)	32.28	44.46	18.49	31.52	16.65	21.63	34.00	25.26	50.30	14.90	55.23

According to these data the cyanobacterial isolates were classified (Desikachary, 1959 and Rippka *et al.*, 2001) as follows:

Sa83	*Nostoc calcicola*	**Sh1**	*Calothrix clavata*	**Z1**	*Anabaena oryzae*
E3	*Nostoc muscorum*	**D13**	*Nostoc muscorum*	**Se100**	*Nostoc muscorum*
B4	*Nostoc muscorum*	**Sa31**	*Nostoc calcicola*	**Se112**	*Anabaena oscillarioides*
Se80	*Nostoc calcicola*	**Se12**	*Calothrix clavata*		

Table 3. Number of amplicons produced by each primer was used for RAPD reaction for cyanobacterial strains

cyanobacterial strains / Primers	*N. calcicola* (Sa 83)	*Calothrix clavata* (Sh1)	*Anabaena oryzae* (Z 1)	*N. muscorum* (E 3)	*N. muscorum* (D13)	*N. muscorum* (Se 100)	*N. muscorum* (B 4)	*N. calcicola* (Sa 31)	*Anabaena oscillarioides* (Se112)	*N. calcicola* (Se 80)	*N. calcicola* (Se 80)	Total bands
OPG-05	5.00	4.00	6.00	7.00	4.00	8.00	3.00	8.00	9.00	5.00	5.00	65.0
A- 01	2.00	0.00	1.00	7.00	3.00	9.00	2.00	6.00	4.00	6.00	6.00	41.0
OPV	8.00	8.00	12.0	9.00	8.00	11.0	11.00	12.00	10.00	10.00	10.00	102.0
OPT-07	11.0	8.00	13.0	10.0	7.00	10.0	9.00	11.00	15.00	11.00	11.00	110.0
OPS-19	4.00	5.00	5.00	5.00	6.00	5.00	4.00	7.00	5.00	5.00	5.00	55.0

The primers OPO-05, A- 01, OPV, OPT-07 and OPS-19 generated a total of 65, 41, 102, 110 and 55 RAPD markers, respectively (Fig.1 A-E). The results indicated that the primers chosen were informative to differentiate isolates of cyanobacteria.

Measurement of genetic relatedness

The data produced, comprising 373 band fingerprints from 11 isolates of cyanobacteria, were used to calculate genetic distances (Table 4) and a phylogenetic tree was constructed (Fig. 2). The tree illustrates the similarity of RAPD patterns seen on the gels (Fig. 1 A-E). Numerical analysis of PCR profiles clustered by NJTREE program enabled the plotting of dendograms constructed from amplicons generated by single primer (Fig. 2).

The dendogram of genetic distances among isolates based on band polymorphisms generated by RAPD-PCR after using all primers is shown in Figure 3. This dendogram clustered the 11 isolates into four main clusters, where *N.calcicola* isolates Sa83, Sa31 and Se80 (group A) constituted one cluster correlated with *N. muscorum* isolates E 3, Se100, B4 and D13 formed the second cluster. The third cluster formed from *A.oscillarioides* Se 112 only. However, the isolates *C. clavata* Sh1, *C. clavata* Se12 and *A. oryzae* Z1 formed the fourth cluster.

The data computed exhibited great heterogeneity amongst the cyanobacterial strains with similarity percent ranging from

47% to 68%. Four strains from the same species namely *Nostoc muscorum* E3, *N. muscorum* Se100, *N. muscorum* B4 and *N. muscorum* D13 formed a single group, although these isolates originated from different soil textures. The maximum similarity of only 68% was detected only between *Nostoc muscorum* E3 and *N. muscorum* Se100 srains, which were isolated from El-Hessenia (fertile soil) and Sahl Elteena (new reclaimed soils), respectively. The first two strains namely *Nostoc muscorum* E3 and *N. muscorum* Se100 in turn shared a similarity coefficient of 64% with *N. muscorum* B4 which was isolated also from different soil textures. The later strain B4 was originated from fertile soil in Belbeise having closely related soil texture of El-Hessenia (E3).

On the other hand, isolate *Nostoc calcicola* Sa83 which isolated from San El-hagar showed similarity coefficient of 63% and 62% with *N. calcicola* Sa 31 and *N. calcicola* Se 80 indicating high genetic resemblance even though these belongs to the same sub-cluster and originated from the same soil texture (Fig. 2). The isolates *Calothrix clavata* Shl, *C.clavata* Sel2 and *Anabaena. oryzae* Zl formed a single sub-cluster, indicating a certain degree of genetic similarity. Data show that phylogenetic relatedness between cyanobacterial strains was not highly identical with soil texture. These results are inagreement with Shalini *et al.,* (2008) they reported that phylogenetic relatedness between *Calothrix* strains was unrelated with different geographical sites.

The cluster analyses based upon RAPD-PCR have been undertaken in similar studies (Jeberlin Prabina *et al.,* 2003). Neilan (1995) demonsterated that RAPD PCR was also used to generate unique and identifying DNA profiles for members of the cyanobacterial genera *Anabaena* and *Microcystis*, which were responsible for the production of nuisance blooms in various freshwater systems, including recreational and drinking water supplies. In studies of single primer PCR reactions, Shalini *et al.,* (2008) found a maximum closeness of only 60% was exhibited by strains (Ca28 and Ca29), which were isolated from same geographical location.

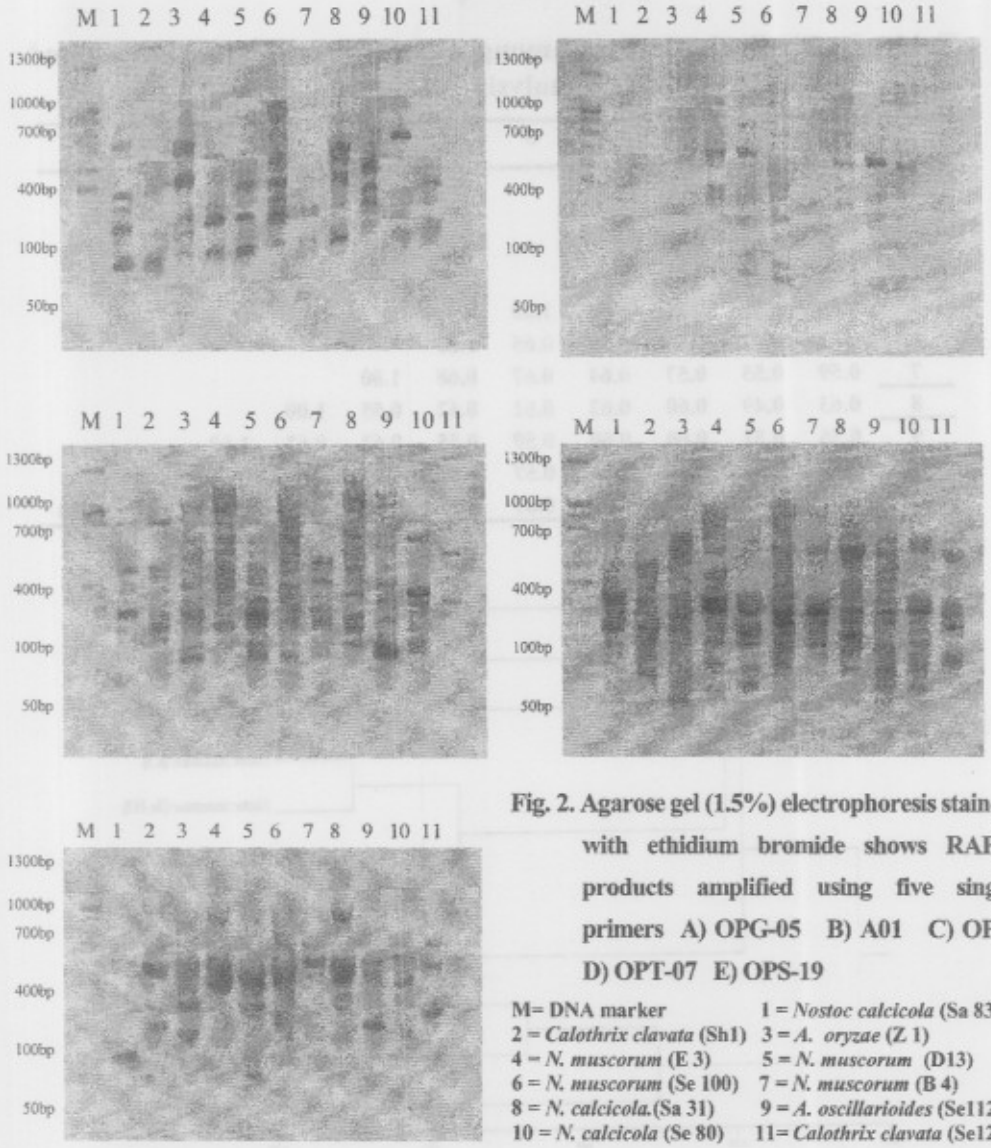

Fig. 2. Agarose gel (1.5%) electrophoresis stained with ethidium bromide shows RAPD products amplified using five single primers A) OPG-05 B) A01 C) OPV D) OPT-07 E) OPS-19

M= DNA marker
1 = *Nostoc calcicola* (Sa 83)
2 = *Calothrix clavata* (Sh1)
3 = *A. oryzae* (Z 1)
4 = *N. muscorum* (E 3)
5 = *N. muscorum* (D13)
6 = *N. muscorum* (Se 100)
7 = *N. muscorum* (B 4)
8 = *N. calcicola.*(Sa 31)
9 = *A. oscillarioides* (Se112)
10 = *N. calcicola* (Se 80)
11= *Calothrix clavata* (Se12)

Table 4. Similarity indices among 11 cyanobacterial isolates based on RAPD – PCR analysis

	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.59	1.00									
3	0.56	0.62	1.00								
4	0.54	0.49	0.55	1.00							
5	0.55	0.52	0.61	0.65	1.00						
6	0.54	0.47	0.60	0.68	0.65	1.00					
7	0.59	0.55	0.57	0.64	0.67	0.68	1.00				
8	0.63	0.49	0.60	0.62	0.61	0.62	0.55	1.00			
9	0.56	0.51	0.60	0.60	0.59	0.55	0.62	0.62	1.00		
10	0.62	0.56	0.52	0.56	0.57	0.65	0.67	0.63	0.56	1.00	
11	0.63	0.64	0.60	0.62	0.61	0.55	0.60	0.51	0.55	0.52	1.00

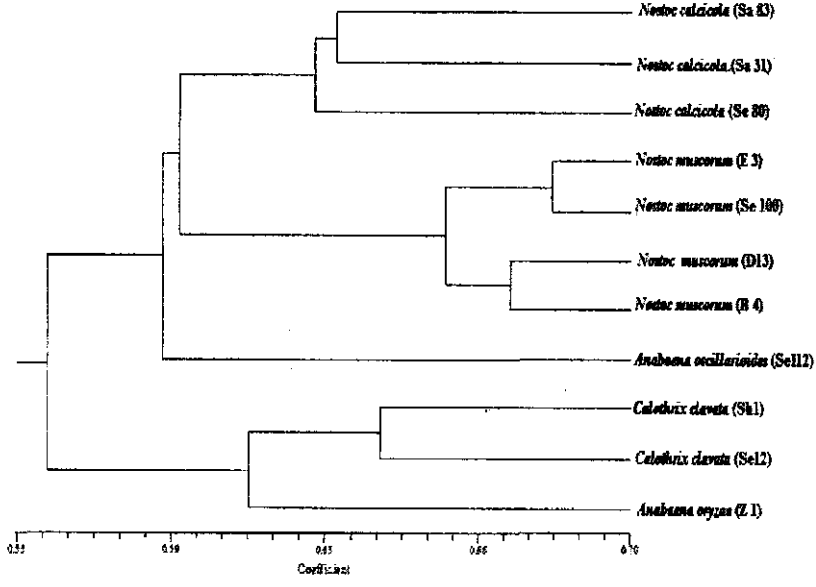

Fig. 2. Dendrogram for the genetic distances relationships among 11 cyanobacterial strains based on similarity indices data of RAPD analysis

In this regard, different authors reported also the usefulness of RAPD-PCR technique for the identification of cyanobacteria (Jeberlin Prabina *et al.*, 2003 and Mahendra Perumal *et al.*, 2009).

The present investigation has clearly shown the existence of diversity among the cyanobacterial isolates analyzed in terms of various morphological and physiological attributes. Molecular polymorphisms further aided in effective differentiation of the selected isolates, and besides revealing their genetic relationships. Molecular profiling could provide useful taxonomic criteria for distinguishing among the isolates belonging to the cyanobacterial group, when analyzed under a uniform set of conditions.

The data indicated that RAPD fingerprinting can be used successfully to differentiate closely related cyanobacterial strains. Mahendra Perumal *et al.*, (2009) concluded also that RAPD is well suited for fast and accurate strain differentiation and is an alternative and complementary approach to the traditional methods for studying cyanobacterial taxonomy.

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تحليل القرابة الوراثية لبعض سلالات السيانوبكتيريا المحلية بواسطة RAPD-PCR

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تم عمل توصيف جزيئي لـ١١ سلالة من السيانوبكتيريا ينتمون إلى ثلاث أجناس وهم جنس الـ *Nostoc* ويضم ٤ سلالات من النوع *N. muscorum* و ٣ سلالات من النوع *N. calcicola* و جنس الـ *Anabaena* ويضم سلالة واحدة من النوع *A. oryzae* وسلالة واحدة من النوع *A. oscillarioides* و جنس *Calothrix* ويضم سلالاتين من النوع *C. clavata* بإستخدام تقنية RAPD-PCR . وقد تم استخلاص DNA الجينومى من هذه السلالات و إكثاره بأستخدام RAPD-PCR فى وجود ٥ بادئات وهم OPG-05, A- 01, OPV, OPT-07, OPS-19 لعمل بصمة وراثية لهذه السلالات. من خلال نطاقات الحزم المفردة الناتجة لكل سلالات السيانوبكتيريا المختبرة والوزن الجزئيى لكل حزمة. تم حساب المسافة الجينية وكذلك علاقات القرابة الوراثية بين هذه السلالات. وأظهرت النتائج المعتمدة على البصمة الوراثية بواسطة تقنية RAPD-PCR بوضوح وجود مدى واسع من الاختلافات الوراثية بين سلالات السيانوبكتيريا المختبرة. أيضا أظهرت البيانات أن القرابة الوراثية بين سلالات السيانوبكتيريا غير مرتبطة بنوع التربة المعزولة منها.