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Short Communication

Biochemical and Molecular Genetic Studies on Some Cyanobacterial Isolates

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Abstract. In the present study, the isolation and purification of a set of Cyanobacteria strains belonging to genus *Oscillatoria* was undertaken, followed by the analyses of phylogenetic relationships using different biochemical and molecular genetic techniques (SDS-PAGE and RAPD-PCR). A total of 45 protein bands were observed within the studied *Oscillatoria* isolates by SDS-PAGE (only three unique bands, eight monomorphic bands and 37 polymorphic bands). On the other hand, extracted DNA from isolates was used to identify the molecular fingerprints. A sum of 94 polymorphic bands was generated by these primers in the *Ocsillatoria* genotypes under study. A total of 20 unique bands were identified out of the polymorphic ones. These unique bands were used to discriminate among the studied *Ocsillatoria* isolates. Most isolates of *Ocsillatoria* genotypes were discriminated by one or more unique bands. Numerical taxonomic using 45 protein attributes of 19 isolates and RAPD markers on five isolates. Two methods - Clustering (UPGMA) and Principal Component Analysis (PCA) were used for these analyses. The similarities and clusters produced between the studied isolates were discussed.

Keywords: cyanobacteria strains, protein banding pattern (SDS-PAGE), RAPD-PCR, numerical analysis, phylogenetic relationships, *Oscillatoria*

Cyanobacteria (blue-green algae) produce a large number and variety of bioactive allelochemical substances, with a diverse range of biological activities and chemical structures. Such chemicals are likely to be involved in regulating natural populations and are potentially useful as biochemical tools and as biological control agents (Wu *et al*., 2005; Biondi *et al*., 2004; Hirahashi *et al*., 2001). Microbial biotechnology, including DNA manipulation, facilitates the construction of new bacterial genomes that possess different potentialities.

Cyanobacteria were subjected to molecular genetic studies by Kumari and Parvathi *et al*. (2009); Havemann and Foster (2008); Guan *et al*. (2007); Gugger *et al*. (2002); Iteman *et al*. (2002); Wilson *et al*. (1999).

The use of phenotypic and genotypic characteristics in cyanobacteria taxonomy was pioneered by Rippka *et al*. (1979). Ezhilarasi and Anand (2009) used the sequences of 16S rRNA gene in a phylogenetic analysis of *Anabaena* spp.

Axenic cultures of cyanobacteria isolates were prepared and purified by different methods, then molecular genetic fingerprint were determined by using electrophoresis of proteins banding patterns. Molecular genetic fingerprint to biological active isolates were determined by using RAPD-PCR. Numerical taxonomic study was carried out by using 45 protein attributes on 19 isolates and RAPD markers on five isolates. Two methods used for numerical analysis; Clustering (UPGMA) and Principal Component Analysis (PCA). NTSYSpc version 2.02i (Rohlf, 1998) was used for these analyses.

Sample preparation. In the present study, biological material was nineteen isolates of *Oscillatoria* species. These isolates were collected from different locations in the eastern part of Qaroun Lake, Egypt. Isolation of cyanobacteria samples was carried out using modified procedure of Brand (1991) and purification was done according to Stein (1979). The extracted samples were centrifuged for 15 min at 4000 rpm at room temperature. The supernatants were taken and filtered through sterile cellulose nitrate membrane filters (0.45µm) for obtaining sterile aqueous cyanobacteria extracts. Whole cell cyanobacteria protein extraction was carried out according to Livne *et al*. (1992) with a lot of modification.

When preparation of samples were ready for electrophoresis, the frozen cyanobacteria extracts re-suspended

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in sample buffer and total protein concentration was determined by Bradford method (Bradford, 1976).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity one software ver. 4.0.3.

Molecular studies. DNA was extracted according to Neilan (1995), with some modification. Also, PCR-RAPD reactions applied were based on the protocol of Neilan (1995). PCR-RAPD reactions were conducted using 10 arbitrary 10-mer primers (Operon Technologies, Inc). The successful primers during PCR- are listed in Table 1. Bands were detected on UV-transilluminator and photographed by Gel documentation system (Biometra Bio Doc Analyze - 2000).

Numerical analysis. Among nineteen cyanobacteria isolates based on SDS-PAGE and RAPD-PCR markers polymorphism, similarity matrix was developed by SPSS computer package system ver.16. Numerical taxonomic study was carried out by using 45 protein attributes on 19 isolates and RAPD markers on five isolates. Two methods used for numerical analysis; Clustering (UPGMA) and Principal Component Analysis (PCA). NTSYSpc version 2.02i (Rohlf, 1998) was used for these analyses.

Biochemical analysis. The fingerprint of nineteen isolates of cyanobacteria is shown in Fig. 1.

RAPD-PCR amplification analysis. The randomly amplified polymorphic DNA (RAPD) technique, in conjunction with PCR, has been employed to identify many organisms up to strain level of classification (Williams *et al*., 1990). The resulting bands may then be used for distinguishing subspecies (Gow and Gadd, 1995).

Table 1. List of RAPD primers and their nucleotide sequence

Primer code	Sequence
C-02	5′- GTG AGG CGT C -3′
L-13	5′- ACC GCC TGC T -3′
Z-09	5′- CAC CCC AGT C -3′
E-06	5′- AAG ACC CCT C -3′
F-04	5′- GGT GAT CAG G -3′
G-05	5′- CTG AGA CGG A -3′
M-20	5′- AGG TCT TGG G -3′
O-04	5′- AAG TCC GCT C -3′

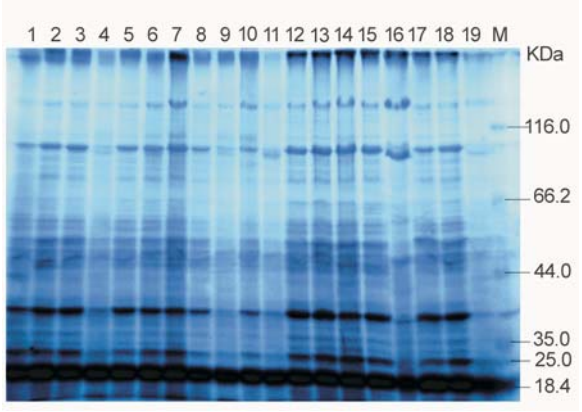

Fig. 1. SDS-PAGE of total soluble protein banding pattern of the ninteen cyanobacterial isolates.

In the present study, the using of RAPD-PCR technology in the detection of genetic heterogeneity among only five sample of the nineteen axenic cultures of cyanobacterial isolates have been discussed. Table 2 shows the comparison among the total number of amplified

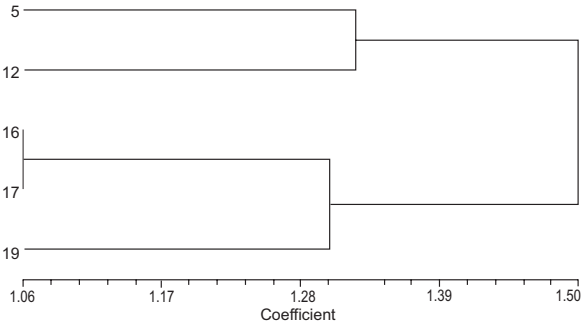

Fig. 2. Dendrogram using average linkage (between groups) of ninteen cyanobacterial isolates based on RAPD-PCR analysis.

Table 2. Number of bands for each isolate with different primers

Primers	Isolates					Total bands
	5	12	16	17	19	
L13	11	12	13	14	12	62
F4	6	6	6	12	8	38
O4	6	9	6	7	5	33
M20	9	9	10	10	10	48
C2	10	12	13	9	12	56
Z9	4	4	9	9	7	33
E6	5	6	2	2	11	26
G5	2	6	6	6	6	26
Total bands	53	64	65	69	71	322

DNA fragments for five selected cyanobacterial isolates that generated by the previously used primers in RAPD-PCR. Figure 2 illustrates the resulted dendrogram among five cyanobacteria isolates from RAPD-PCR analysis.

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