**Identification of some cyanobacterial strainsby molecular methods**

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**Abstract:** Cyanobacteria are reported to be aprolificsource of bioactive compounds and enzymes. Different protocols were tested for the extraction of DNA of cyanobacterial strains. 23S rRNA gene molecular analysis and phylogenetic reconstruction the reaction yielded single PCR amplicon for each cyanobacterial strain tested.

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**Key words:** Cyanobacteria, 23SrRNA gene,amplicon,phylogenetic analysis.

**1. Introduction**

Cyanobacteria are prokaryotes that lack plastids but possess thylakoids and perform oxygenic photosynthesis. All cyanobacteria contain chlorophyll a as the main photosynthetic pigment in addition to other accessory pigments such as carotenoids and phycobiliproteins (Douglas, 1994). Due to the simple morphological features of cyanobacteria and the lack of diagnostic phenotypic characters, some errors were made in the identification of cyanobacterial strains.This necessitated to the adoption of molecular methods in addition to traditional morphological in a polyphasic identification system (El Semary, 2005). This approach is more reliable as the identification is not based on phenotypic aspects only but it provides an insight into some portions of the genome that are discriminatory between taxa. This would also allow the deduction of phylogenetic relationships of cyanobacteria (El Semary and Abd El Nabi, 2010; El Semary, 2011). At the molecular level, the rRNA genes are the most employed genetic markers due to their conserved and variable regions that are taxonomically discriminatoryin addition to their homologous function and ubiquity (Nübel*et al*., 1997; Salomon *et al*., 2003; Sherwood and Presting, 2007). The 16S rRNA gene was largely employed as a genetic marker (Nübel*et al*., 1997; Salomon *et al*., 2003). However, the low discriminatory power of16S rRNA at the species level (Fox *et al*., 1992) due to thelow evolutionary rate variation in its conserved regionsmakes it less useful for phylogenetic studies of closely-related organisms. As a result, researchers have used other genetic markers that are more discriminatory such as the 16S-23S internal transcribed spacer region (ITS) (Iteman*et al*., 2000; El Semary, 2005) and the intergenic spacer region (IGS) of the phycocyanin genetic locus (Neilan*et al*., 1995; El Semary, 2005).

**2. Materials and Methods**

**Cyanobacterial strains and growth medium**

**Cyanobacterial cultures**

Three strains from cyanobacterial strains from two different localities (Wadi El NatrounandWadi ElRayan) were initially isolated and kept at Helwan culture collection pending further analysis. The cyanobacterial strains were further purified by streaking on agar plate of solidified BG11 medium(Allen and Stanier 1968; Watanabe *et al*. 2000 modified)(2 % agar, w: v). Microscopic examination was performed periodically to observe the cyanobacterial strains. The pure culture was then moved to 10 ml vials containing 6 ml liquid BG11 medium and incubated until visible growth was obtained then transferred to several 500 ml flasks.

**DNA extraction methods &PCR**

There were several methods used to extract total genomic DNA from cyanobacterial strain. One of this method used was: One ml of culture was pelleted by centrifugation at 6000 rpm for 20 min at room temp then the medium was decanted. The pellet was re-suspended in 500 µl of (50 mMtris-HCL, pH 8 -5 mM EDTA – 50 mMNACl). Lysozyme was added (1mg/ml). The solution was incubated at 37 °C for 2 hr with occasional mixing. After the addition of 10 µl of proteinasek (10 mg/ml) and 20 µl of (w/v) sodium dodecyl sulfate (SDS). The mixture was incubated at 55°C for 1hr or until the solution was clear. The solution was chilled on ice & extracted with an equal volume of (phenol – chloroform – isoamyl alcohol) (25: 24: 1).The previous organic extraction was repeated until obtaining a clear upper layer after centrifugation at 6000 rpm for ten minutes.The supernatant was added to one tenth of its volume of 3M sodium acetate. Total genomic was precipitated by incubation with an equal volume of isopropanol at -20°C for one hour followed by centrifugation at 4.500 x g for 10 min at room temperature.The pellet was washed with 70% ethanol, centrifuged then the ethanol was decanted and the pellet was left to dry in the air.The pellet was dissolved in 25 µl TE buffer (10 mMtris -1mM EDTA) & kept at 4°C overnight. The DNA was detected by running in gel electrophoresis using 0.8% agarose gel then visualized under UV-transilluminator.

**Polymerase chain reaction**

The extracted DNA was used in PCR to amplify partial 23S rDNA. The characterization of the partial ssurDNA locus was performed using the highly-specific universal algal forward 23S rDNA primer and reverse 23S rDNA primer primers (Sherwood and Presting, 2007). The reaction mixture contained the following: 12.5µl of master mix (One PCR™, Genedrix), 1µl of DNA template, 1µl of 23S forward primer (100 µM) and 1µl 23S reverse primer (100 µM) and 9.5 µl double distilled water.The amplification protocol was as follows:

Initial cycle: 94ºC for 5 min,Amplification cycles (35 cycles): 94 ºC for 1 min; 55 ºC for 1 min, 72 ºC for 1.5 min, Last cycle: 72 ºC for 10 min.

The PCR products were purified and sequencing was performed using sequencing service (Macrogen, South Korea).

**Phylogenetic analysis**

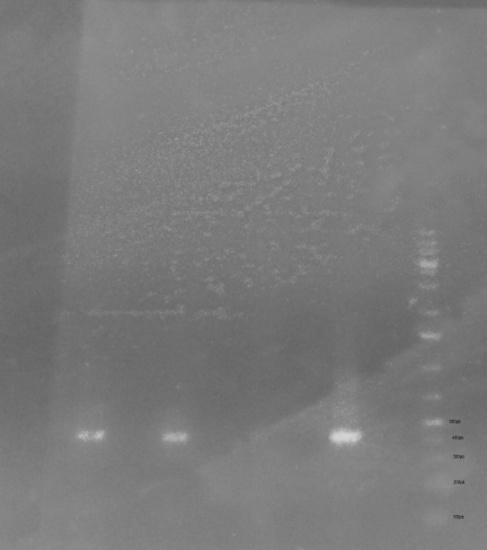
Cyanobacterial gene sequences were imported from GenBank and aligned. The alignment was performed using Mega 4 phylogenetic package. The bootstrap consensus tree was inferred from 500 replicates (Felsenstein, 1985).

**3. Result**

**Partial 23S rRNA gene and phylogenetic analyses**

The amplification reaction using partial 23S rRNA gene specific primers yielded single PCR amplicon for each cyanobacterium tested (Figure 1)**.** The sequence retrieved was compared to other sequences deposited at GenBank using nucleotide BLAST search. **Phylogenetic analysis**

The sequencing was resulted used for making the evolution history using the Maximum Parsimony method (Eck and Dayhoff, 1966). The phylogenetic tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches(Felsenstein, 1985). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar, 2003, pg. 128) with search level 3 (Felsenstein, 1985; Nei and Kumar, 2003) in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There were a total of 82 positions in the final dataset, out of which 5 were parsimony informative. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al*., 2007)

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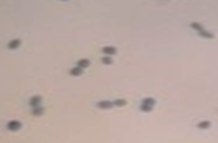
Lane1 lane2 lane3 L

**Figure (1):**Gel electrophoresis image of ssu23S rDNA amplification from 3 different species.

Bands appeared at about 460bp (Lane 1-3).L is 100 bp DNA marker.

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Figure(2). Evolutionary relationships of 8 taxa. The accession numbers are written to the right hand side of each taxon. Our isolate was arbitrarily designated as *Cyanothece* sp. Helwan 1

  
**Figure(3):***Cyanothece*-like strain under light microscope (magnification 100x).

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***Figure*(4). Evolutionary relationships of 10 taxa**

The evolutionary history was inferred using the Maximum Parsimony method (Eck and Dayhoff, 1966). The consensus tree inferred from 22 most parsimonious trees is shown. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. The consistency index is 0.847826 (0.833333), the retention index is 0.851064 (0.851064), and the composite index is 0.721554 (0.709220) for all sites and parsimony-informative sites (in parentheses). The percentage of parsimonious trees in which the associated taxa clustered together are shown next to the branches. The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Felsenstein, 1985 pg.

128) with search level 2 (Eck and Dayhoff, 1966; Felsenstein, 1985) in which the initial trees were obtained with the random addition of sequences (10 replicates). The codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There were a total of 89 positions in the final dataset, out of which 33 were parsimony informative. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al*., 2007).

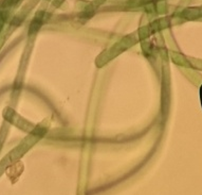


Figure (5): *Spirulina* sp. under light microscope (magnification 100 x).

**4. Discussion**

Because of failing in classification of Cyanobacteria by morphological method the molecular techniques were used. 23SrRNA and 16SrRNA genes are more reliable for identification of cyanobacterial strains because of conserved nature and lower evolutionary rate variation.

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