Molecular Cloning of 16 Sr RNA Gene Isolated from Two Cyanobacterial Strains

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ABSTRACT

Cyanobacteria are promising microorganisms due to it's ability to perform oxygenic photosynthesis as many of these Cyanobacteria are able to fix N2 aerobically in addition to it's medical, pharmaceutical and environmental potentialities. Moreover, Cyanobacteria represents one of the major bacterial phyla, being a large, diverse and widespread group inhabiting most of the Earth's environments. Therefore, there is a much attention is being paid to characterize and explore it's capabilities. the comparative analysis of *16S rRNA* gene sequences provides a new means to investigate the discrepancy between strain collections and natural communities. In the present study, the 16SrRNA gene of two cyanobacterial strains belonging to the genus Synechoccus was isolated via Polymerase Chain Reaction (PCR) in order to identify the variation between those two strains and to establish the protocols for further molecular genetic characterization of cyanobacterial strains. Because the generated PCR products have the same molecular weight (700 bp), the generated PCR products have been cloned successfully by the use of the commercial vector pCR2.1 for further characterization via sequencing analysis approaches.

Keywords: Molecular cloning, Cyanobacteria, *16SrRNA* gene, PCR,

INTRODUCTION

Cyanobacteria represents one of the major bacterial phyla, being a large, diverse and widespread group inhabiting most of the Earth's environments. They play an important role as primary producers **(Chisholm *et al.* 1988)**, but are also capable of extensive growth, resulting in bloom events that can cause a significant threat to human and animal health **(Carmichael, *et al.* 1992)**. Inadequate culture conditions leading to the loss of various morphological characteristics **(Castenholz, *et al.* 1989)**, researchers' inability to grow certain organisms in the laboratory **(Feris, M.J, *et al.* 1996)**, and misidentifications of strains in culture collections **(Wilmotte, A. 1994)** make it difficult in many cases to apply taxonomic assignments based on cultures to field populations. Both classification systems for the cyanobacteria based on bacteriological approach

(Castenholz, *et al.* 1989) as well as the traditional botanical approach **Geitler, L. 1932)** rely primarily on morphological characteristics of cells and colonies and do not necessarily lead to the identification of phylogenetically coherent taxa **(Castenholz, 1992)**. At all taxonomic levels above species, the sequence analysis of genes encoding small-subunit ribosomal RNA (*16S rRNA*) is currently the most promising approach for the phylogenetic classification of cyanobacteria **(Wilmotte, A. 1994)**. Furthermore, the comparative analysis of *16S rRNA* gene sequences provides a new means to investigate the discrepancy between strain collections and natural communities **(Feris, M.J, *et al.*1996)**. For instance, the genus Synechococcus should be revised extensively, taking into consideration that the phylogenetic diversity seen among this group as it stands, as inferred by 16S rDNA sequences, may be almost as wide as it is for all cyanobacteria (Robertson, et al. 2001). Also, Phylogenetic analyses based on *16S rRNA* gene sequences of sponge associated cyanobacteria showed them to be polyphyletic, implying that they derived from multiple independent symbiotic events **(Steindler, *et al.* 2005)**.

Sequences of *16S rRNA* genes are independent from cultivation or growth conditions and can be retrieved by PCR from small amounts of DNA extracted from laboratory cultures or natural environments **(Giovannoni, 1991).** Several different approaches to extend the analysis of *16S rRNA* gene from cyanobacteria beyond axenic cultures have been described. These approaches include antibiotic treatments to suppress heterotrophic bacteria in nonaxenic cultures **(Wilmotte,, *et al.* 1992)**, physical cleaning of cyanobacteria by micromanipulation **(Garcia-Pichel, F, *et al.* 1996)** and molecular cloning followed by screening for plasmid inserts of interest **(Nelissen, B., *et al,* 1996).** In a previous study, PCR products containing a single homogeneous population of DNA molecules are recognized as single bands after DGGE and can be directly sequenced, yielding information about approximately 700 nucleotides of the *16S rRNA* genes **(Nübel. U. *et al.* 1996).**

In the present study, *16SrRNA* gene was used to characterize two cyanobacterial strains on the molecular genetic level via PCR. Moreover, PCR product was cloned successfully in a commercial vector and the successful clones have been identified by the use of restriction enzyme analysis.

MATERIALS AND METHODS

- Cyanobacterial strains and culture conditions:-

- Cyanobacterial strains:

Two unicellular cyanobacterial strains (designated Q1 and Q3) were collected from an Egyptian brakish water. Culturing, isolation and purification processes were carried out as described by **(Mohamed, 2000)**.

Based on morphological basis, a preliminery classification of these strains Showed that both of them could be classified belonging to genus Synechoccus.

- Molecular Genetic characterization:

- DNA Extraction

- Rapid phenol Extraction method :

Extraction of total nucleic acid was carried out via phenol extraction method. This method includes lysing the washed cyanobacterial cell (1ml cyanobacterial culture) by the use of lysozyme and SDS, incubate for 1 h. , deproteinization by a mixture of phenol : chloroform : Isoamyl (25 :24 : 1) , precipitate the nucleic acid by equal volume of cold isopropanol and finally resuspend the dried pellet in d.H2O.

- Isolation of 16 *SrRNA* gene :

Primer sequences used to isolate the *16SrRNA* gene fragment were : **Forward:** CGGACGGGT GAGTAACGC GTG A **Reverse:** GAC TAC TGG GGT ATC TAA TCC CAT T. The PCR master mix. Contained 0.01nM primer 50 nMdNTPs, 1U Taq DNA pol., 3µl od 10x buffer, mixed with 1µl of DNA template , the sterile dH_2O was added to na final volume of 30 µl the thermal cycler (Perkin Elmer) was programed to 94 °C for 4 min., 94 °C for 1 min., 55 °C for 1 min., 72 °C for 1.5 min, the nomber of cycles was 35 cycle and the post PCR reaction time was 72°C(5 min.)

- Cloning of the *16SrRNA* gene :

The PCR product was eluted from the agarose gel (1%) by the use of commercial elution kit (Qiagene, USA) and the PCR product for each strain was cloned into the commercial vector (Invitrogen TOPO TA clonning kit which contains the commercial vector PCR 2.1 TOPO vector) as described by the manufacturer.

- Restriction enzyme analysis of successful clones:

In order to confirm that the *16SrRNA* gene fragment has been

integrated successfully in the right insertion site. Mixture of 1 µl *Rsa* (Promega) 1.5 µl 10x buffer, 13 µl plasmid DNA and 1-10/sample of Bovin Serum albumine(BSA) was incubated at 37 °C for 1 h. and 10 µl from each sample was loaded in 0.7% agarose gel electrophoresis, stained with ethidium bromide and visualized on the UV- Transelluminator.

RESULTS AND DISCUSSION

Isolation of the *16SrRNA* gene:

16S rRNA genes encode the small subunit ribosomal RNA. Analysis of the *16S rRNA* gene sequence is currently one of the most convenient approaches for phylogenetic characterization of Cyanobacteria. Because the purification of cyanobacteria can be a difficult and time-consuming procedure, and often they are cultivated more easily when accompanied by heterotrophic bacteria **(Castenholz, *et al.* 1989)**. The PCR protocol reported here has been used to amplify cyanobacterial 16S rRNA gene fragments exclusively and thereby retrieve them from impure culatures. Since the generated PCR product has the expected size documented by **Ulrich, N. *et al.*, 1996** (700 bp), we would suggest that this fragment representing the target *16SrRNA* gene fragment of cyanobacteria. However, the results of this experiment (Fig:1) showed that there was no variation, based on this molecular genetic criterion between the tested strains. Therefore, the PCR product of those two strains (700 bp) has been cloned and the restriction endonucleases pattern of the commercial vector have been compared to the corresponding standard pattern (Fig:2&3). The expected restriction enzyme patterns (that carries the 700bp fragment of the *16SrRNA* gene) of the cloned vector have been selected and stored at -80°C for further analysis via DNA sequencing analysis approaches.

Cloning of the 16SrRNA gene fragment:

Studies based on 16SrRNA gene sequencing analysis have been revolutionarise the phylogenetic analysis of many cyanobacterial genera **(Robertson, R.B. *et al.* 2001)** and provides researchers with a powerful tool for the detection of cyanobacterial strains in non-axenic cultures **(Steindler, L. *et al.* 2005).** As shown in Figures 2 and 3 the presence of the 943 bp DNA fragment (indicated by the black arrow) represents the target *16SrRNA* gene fragment (which has a 700 bp fragment length) inserted between the *Rsal* recognition sites (which has a length of 243 bp fragment DNA) compared to the restriction enzyme pattern of the restriction enzyme Rsal with the standard pCR2.1 vector used in this study as described by the manufacturer. These results confirms the successful cloning of the

16SrRNA gene fragments of both cyanobacterial strains Q1 and Q3. Moreover, These results are in accordance with the results obtained by **Nübel, U. *et al.* 1996** who described the application of 16SrDNA finger printing for the detection of cyanobacterial strains from nonaxenic cultures without employing and traditional purification techniques. .

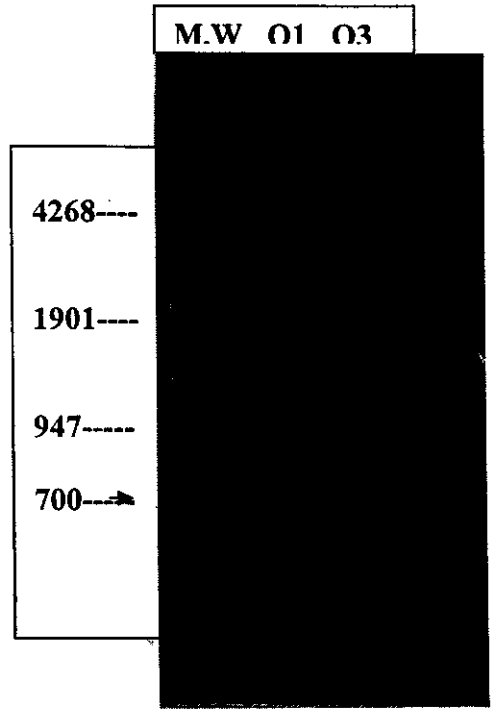

Fig (1): Isolation of *16 SrRNA* gene fragment of the two strains Q1, and Q3.

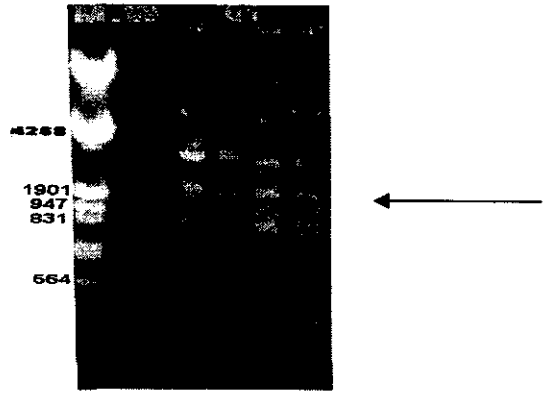

Fig (2): *Rsa*I pattern of the topo10 commercial vector harboring the 16 SrRNA gene fragment strains from the strain Q1.

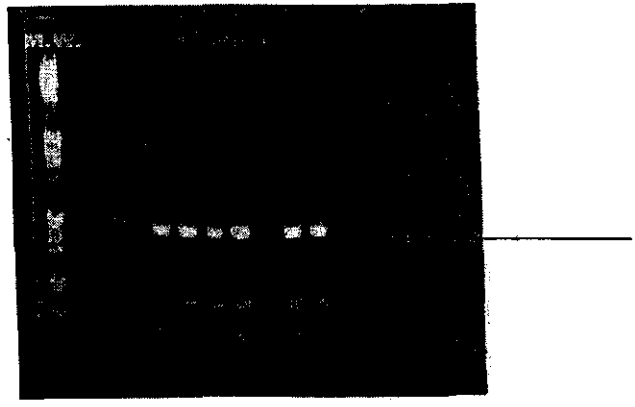

Fig (3): *Rsa*I pattern of the topo10 commercial vector harboring the 16 SrRNA gene fragment strains from the strain Q3.

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الملخص العربي

النسخ الجزيئى لجين 16 Sr RNA المعزول من سلالات سيانو بكتيريا

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تعتبر الطحالب الخضراء المزرقة من الكائنات الدقيقة الواعدة حيث تتميزبقدرتها علـى القيـام بعملية البناء الضوئى وكذلك تثبيت نيتروجين الهواء الجوى. بالإضافة إلى ذلك فإن الطحالب الخـضراء المزرقة تمثل واحدة من أكبر شعب الكائنات الحية و هو ماجعلها من أوسع الكائنات إنتشارا علـى وجـه الأرض. بالأضافة إلى أهميتها من الناحية الطبية و البيئية وهو ما جعل من الأهمية بمكان إجراء العديـد من الأبحاث لتعريفها وتوصيفها من الناحية الوراثية. و من افضل طرق التوصيف الـوراثى للطحالـب الخضراء المزرقة إستخدام تقنية التفاعل المتسلسل بإسـتخدام إنـزيم البلمـرة(PCR) لعـزل الجـين 16SrRNA gene وهو ما تم فى هذا البحث حيث تم عزل قطعة من الجين *16SrRNA* بإسـتخدام تقنية ال PCR وذلك لتعريف وتمييـز سـلالتين مـن الطحالـب الخـضراء المزرقـة مـن الجـنس Synechoccus حيث أظهرت النتائج أن ناتج عزل القطعة الجينية فى كلا العزلتين لهما نفس الـوزن الجزيئى (700 bp). ولذلك فقد تم كلونة ناتج ال PCR بواسطة الناقل البلازميدى pCR2.1 . كما تم التأكد من نجاح عملية الكلونة بإستخدام إنزيم القطع المحدد *Rsal*.