ISOLATION, MASS CULTIVATION AND DNA SEQUENCING OF *Arthrospira platensis*

K. Thamilarasi, M. Jayanthi, I. Ramya, M. Sriramani and R. Amutha*,

*Department Of Microbiology, Vivekanandha College of Arts and Sciences for Women, Tiruchencode, Namakkal, Tamilnadu, India.*

**Abstract**

*Spirulina* is a photosynthetic, filamentous, multicellular and blue green microalgae. It is commonly used as a natural colorant in food and cosmetic industries as a natural blue dye. *Arthrospira platensis* (presently known as *Spirulina platensis*) is species of Cyanobacteria used as health foods, animal feed, food additives and fine chemicals. People associate certain colors and can influence the perceived flavor in any food which it also causes disease due to artificial additives. Coloring of fermented milk products, ice creams, soft drinks, alcoholic drinks, sweet cake decoration, milk shakes, etc. Some naturally occurring pigment such as, blue pigment phycocyanin is found in Cyanobacteria species (*Spirulina platensis*) are used for food coloring which does not cause disease to human. Among different phycobiliproteins, phycocyanin (blue pigment) is greater importance because of its various biological and pharmacological properties. It has antioxidant property antimutagenic, antiviral, anticancer, anti-allergic, immune enhancing, hepato-protective, blood vessel relaxing, neuroprotective, antitumor, radical scavenging and anti inflammatory properties. Alzheimer’s and Parkinson’s can also be treated with phycocyanin. In this present study, the *Spirulina* cultivation was carried out in a Zarrouk’s medium. Then, we developed and tested a set of primers for the specific amplification of 16S gene segments from Cyanobacteria by PCR. The PCR amplification profile was checked on Agarose gel and the product size was compared with a size ladder of 100 bp. The PCR products were purified and sequenced. The 16S gene has 1100 bp in length and has conserve primer PCR. NCBI to obtain the closest hit of the query sequence. Phylogenetic analysis the sequences were aligned using Clustal W and the genetic distances were computed according to the Kimura 2-Parameter (K2P) model. DNA barcoding the genus of the sample is *Arthrospira* (*Spirulina*). The primer used for the analysis showed similarity to *platensis* species. Thus, we can conclude that the studied organism might be *Spirulina platensis*. The sequence was deposited in the Gene bank database under the Accession No KX423618 and KX423619.

**Article History**

Received : 25.02.2016  
Revised : 15.03.2016  
Accepted : 25.03.2016

1. **Introduction**

*Spirulina* are unicellular and filamentous blue-green microalgae that has gained considerable popularity in the health food industry and increasingly as a vitamin and protein supplement to aquaculture diets. It has long been used as a dietary supplement by people with living close to the alkaline lakes and bonds where it is naturally found. *Arthrospira* (*Spirulina*) is an economically most important filamentous Cyanobacterium.

**Key words:** *Spirulina platensis*, Phycocynin, 16S gene, PCR and Nucleotide Blast.
Microalgae can produce numerous products by photosynthesis during their growth. *Spirulina platensis* is one of the blue-green algae. The *Spirulina platensis* is larger than other species of microalgae which is easily digested and absorbed in the human body because its cell membrane does not include cellulose. *Spirulina platensis* consists a relatively low nucleic acid level which composed of 55% – 70% protein, 6% – 9% fat, and 15% – 20% carbohydrate and is rich in vitamins, minerals, fibers and pigments (Kay, 1991).

Environmental stresses affect growth and pigment accumulation of microalgae, including nutrients availability, high pH, light, salinity and temperature. The culture conditions can influence the growth of *Spirulina platensis* causing changes in its composition and proportion of phycobiliproteins. Phenolic compound increases by altering the culture conditions and antioxidant potential of *S. platensis* exploit as a nutritional supplement (Colla, 2007).

Phycocyanin are used in Food and beverage industry. Colouring of fermented milk products, ice creams, chewing gum, soft drinks, alcoholic drinks, deserts, sweet cake decoration, milk shakes etc. The C-phycocyanin is a major light-harvesting Phycobiliprotein in *S. platensis* and is produced in substantial amounts in the cell at levels up to 20% of the total protein. C-phycocyanin of *Spirulina*, a natural blue protein has potential application in food and cosmetic coloring. Its antioxidant, an anti-inflammatory, radical scavenging and hepatoprotective property gives a spectrum of pharmaceutical applications (Zahra Shoji et al., 2015).

*Spirulina* showed preventive effect against the skeletal damage more exercise induced oxidative stress. In addition, *Spirulina* has been shown to have protective effect against oxidative stress induced by lead acetate in the liver and kidney of rats. Feeding of *Spirulina platensis* reduces hepatotoxicity induced by cadmium and the effect is suggested to be mediated antioxidant properties. *Spirulina* is also known to have protective effects against nephrotoxicity due to oxidative damage induced by Gentamicin (Simsek et al., 2008).

The phylogenetic analysis using 16S sequences have shown that many unicellular and filamentous heterocysteous Cyanobacteria genera are probably polyphyletic and cannot be grouped as natural taxa, whereas heterocysteous strain form amnonophyletic group (Giovannoni et al., 1988). Sequences of 16S genes are independent from growth conditions and retrieved by PCR from small amounts of DNA extracted from laboratory cultures are natural environments. The combination of this procedure with denaturing gradient electrophores, a technique for the sequence dependent separation of DNA molecules, proved useful to visualize the diversity of cyanobacterial 16S genes in *Spirulina* samples, to detect the uniqueness of isolated strains and to PCR products derived from cultures to populations in the field. Accordingly, the aim of this study is to the cultivated strains of *Arthrospira* using Sanger’s sequence, including a compared of the phylogeny of *Arthrospira* strains based on the 16S gene. To define and delimit the genus *Arthrospira*, this includes other Cyanobacteria. Finally, this study attempts to identify specific strains of *Arthrospira platensis* and develop tools for species and strain identification.

2. Materials and Methods

**Algal Source**

The blue green algae were obtained from the water sample collected from the AWE CARE Research Laboratory, Erode.

**Isolation and Mass Cultivation of Spirulina on Zarrouk’s medium**

The water sample was inoculated into the Zarrouk’s medium for isolation of *Spirulina* and incubated in a growth chamber with light flux of 16:8 h. The purity of the culture was ensured by repeated inoculation, and identification was accomplished by determining cellular morphology using light microscope. The cultivation was carried out in a modified Zarrouk’s medium. The Zarrouk’s medium was prepared in sterilized
distilled water and the initial pH was adjusted to 9.0. The medium was inoculated with 10 % of mother culture of *Spirulina*. The medium was maintained under laboratory conditions and provided with light source and the medium was continuously aerated. Harvesting was carried out by the filtration process using cheese filter. After filtration, *Spirulina* was reduced in fine powder.

**Isolation of Genomic DNA**

Zarrouk’s medium was used for maintenance and propagation of *Spirulina* culture. The semisolid medium was used for maintenance of stock culture from the bulk culture grown for 7 days at 30 ºC, the genomic DNA was isolated following the cTAB method described by Sambrook and Russel (2001).

**PCR amplification**

Polymerase chain reaction (PCR) of the isolated sample was carried out in a 20 μl reaction mix containing 1X Taq buffer, Taq DNA polymerase and genomic DNA. The PCR was performed using Agilent sure cycler 8800.

**Agarose gel electrophoresis**

Prepare Agarose solution in the buffer provided (0.5x TEB) boil the Agarose until it is completely dissolved. When the gel temperature was around 40 ºC, 2 μl Ethidium bromide was added and mixed properly. The Agarose mixture was poured into the tray and solidify. Then, the gel was kept in the tray containing 0.5x TEB buffer with the wells in the cathode (Negative) side. The buffer level in the tank should be maintained above the gel tray. Connect the cords between the electrophoresis tank and the power pack before loading the samples. To prepare samples for electrophoresis, 5 μl of gel loading dye was added in to the sample and mix well. A volume of 20 μl of the sample was loaded and 3 μl of DNA marker in to the well. After loading, switch on the power pack and adjust the voltage to 50V or 100V. The electrophoresis was continued until the dye each the gel and observe the bands under UV transilluminator.

**Sequence Information**

The PCR products were purified and sequencing to Bangalore laboratory in Qtlomics traits to genes.

**NCBI: Nucleotide Blast (BLAST N) output**

The sequences obtained were subjected to BLAST using the BLAST N tool of NCBI to obtain the closest hit of the query sequence by Bangalore laboratory in QTlomics traits to genes.

**Phylogenetic Analysis (Altschul et al., 1997)**

The 16S gene sequences obtained from the *Arthrospira* strains were initially sequences available in the National Center for Biotechnology Information database using BLAST network services to determine their approximate phylogenetic affiliations. The sequences were aligned using Clustal W and the genetic distances were computed according to the Kimura 2-Parameter (K2P) model. The similarity values between the sequences were calculated from distance matrices by reversing the Jukes-Cantor distance formula. Phylogenetic trees were then inferred by neighbor joining (NJ) using the Kimura two-parameter model. The resulting NJ tree was evaluated by bootstrap analyses based. Finally, an overview of the phylogenetic position of *Arthrospira* in Cyanobacteria was created by the 16S gene sequences to corresponding cyanobacterial sequences available in databases and the sequences obtained in this study for *Spirulina platensis* evaluated with 1,000 bootstrap replicates.

**3. Results and Discussion**

The isolated *Spirulina* samples were mass cultivated in larger amount of Zarrouk’s medium. *Spirulina* is highly nutritive and shows great diversity and higher concentration of nutrients compared to other food sources. *Spirulina* is harvested by surface of the initially filter *Spirulina*. The *Spirulina* collected and reduced in fine powder was Stored in sterile container. The results was showed that Figure - 1.
Isolation of genomic DNA

From the isolated genomic DNA of *Spirulina*, when tested for purity, a ration of approximately 1.8 was observed in OD 260/280 indicating that the preparation was almost free of proteins.

PCR amplification

Polymerase chain reaction (PCR) of the given sample with the DNA barcode gene was carried out in a 20 μl reaction mix containing 1X Taq buffer, Taq DNA polymerase and genomic DNA. The PCR was performed using Agilent sure cycler 8800. PCR amplification products visualize the genetic diversity of Cyanobacteria and plastids as reflected in 16S gene sequences. Similarities andsuccessions in space and time of the composition of oxygenic phototrophic microbial communities can be observed by PCR products. The PCR amplification profile was checked on Agarose gel and the product size was compared with a size ladder of 100 bp. The PCR reactions gave products of identical lengths for all the Arthrospira strain was 1100 bp. The results were shown in the Figure - 2.

Phylogenetic Analysis

The sequences were aligned using Clustal W and the genetic distances were computed according to the Kimura 2-Parameter (K2P) model. Phylogenetic tree was constructed using Neighbor-joining method (NJ) on MEGA 6 software and the reliability of the tree was evaluated with 1,000 bootstrap replicates. Phylogenetic analyses DNA sequences obtained in this study were aligned and compared with sequence data for other Cyanobacteria available in the NCBI database using the CLUSTAL alignment algorithm contained (DNASTAR Inc., USA). Phylogenetic analyses of and Cyanobacteria one of the most informative genes is the16S gene. The 16S gene has a conserved function and is universally present in Cyanobacteria. The phylogenetic investigations using 16S sequences have shown that many unicellular and filamentous non-heterocystous Cyanobacteria.

4. Conclusion

The results of the present research showed that the DNA barcoding in the given isolated sample was *Arthrospira (Spirulina)*. Based on the NCBI BLAST hit result, the species of the sample was *platensis*. Thus, we can conclude that the sample might be *Arthrospira platensis* (*Spirulina platensis*). So, this product can be targeted for further compound identification studies.
Sequence Information

(A) 16S Gene Forward Sequence

> Powdered sample_16S_F

TCCCGAATGCGATCCACCATGACATGCGAGGCGCTTCTCGGACTAGTGGCGGACGGGTGAGTAACACGTGAGAATCTGGCTCCCGGTCGGGGACAACAGAGGGAAACTTCTGCTAATCCCGGATGAGCCGAAAGGTAAAAGATTTATCGCCGGGAGATGAGCTCGCGTCTGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCAGTATCTGGTCTGACAGGATGATCAGCCACACTGGGACTGAG

ACACGGCCCATACTCCTACGGGAGGCAGCAGTGGGGGAATT

TTCCGCAATGGGCGCAAGCCTGACGGAGCAAGACCGCGTGGGGGAGGAAGGCTCTTGGGTTGTAAACCCCTTTTCTCAAGGAAGAACACATTGACGGTACTTGAGGAATAAGCCTCGGCTAACTCCGTGCCACCAGCCGCGGTAATACGGGTGAAATGCGTAGATATCGGGAAAAACACCGGTGGCGAAAACGCTCTGCTGGGCCGTAACTGACACTGAGGGACGAAAGCTAGGGGAGCGAATGGGATTAGATACCCCAGTAGTCCTATCCGTAAACGATGGAAACTAGGTGTAGCCTGTATCGACCCGGGCTGTGCCAAAGCTAACGCGTTAAGTTTCCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCTATGCAACGCGACTACAGAGAGTAGCATGGAGACTGTCGTGCAGCTCCGTGTCTCGAAATGCTGGAGTTAGTACCACGCACTGAACGGCAACCCCTCGTCCATAGTGCCCAATCATTCATGTTGGACACTCTGCGGTGTAACAGAAACCGGACAGGGTAGACACATACCGTCTGCTT

Contig:

> Powdered sample_16S

GGTAATACGAGGGAGGCGAGACCTTACCTAGGCTAACGGGCTTGAACAGGCGATCAGGCTCCCTCTTCATCCGGCAATGGTTGGCATAAGTTTACGTGGTGTTCATATCTCGTCGCACTCAGCCCTCTCTCAGTTCAGATTGCAGGCTGCAAATCTCGTGCATGAAGGAGGA

©2015 Published by JPS Scientific Publications Ltd. All rights reserved
NCBI: Nucleotide Blast (BLASTn) output:

The sequences obtained were subjected to BLAST using the BLAST N tool of NCBI to obtain the closest hit of the query sequence.

(A) 16S

Figure - 3: Showing NCBI-blast hit search result for the given sample with 16S gene specific primer. The best 10 blast hit sequences were taken to construct a phylogenetic tree
Acknowledgement

The authors are thankful to Prof. Dr. M. Karunanithi, Chairman and Secretary, Vivekanandha Educational Institutions, and Dr. B. T. Suresh Kumar, Principal, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode, Namakkal District, Tamil nadu for providing all the facilities for our research work.

5. Reference


