BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net **Hypothesis**
Volume 10(7)

*A*nalysis of proteins involved in the production of MAA's in two Cyanobacteria *Synechocystis* PCC 6803 and *Anabaena cylindrica*

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Received May 19, 2014; Accepted May 26, 2014; Published June 22, 2014

Abstract:
Mycosporine- like amino acids (MAAs) are small (<400Da), colourless, water soluble compounds composed of cyclohexenone or cyclohexinimine chromophere conjugated with the nitrogen substituent of amino acid or its amino alcohol. These compounds are known for their UV- absorbing role in various organisms and seem to have evolutionary significance. The biosynthesis of MAAs is presumed to occur via the first part of shikimate pathway. In the present work two cyanobacteria *Synechocystis* PCC 6803 and *Anabaena cylindrica* were tested for their ability to synthesize MAAs and protein involved in the production of MAAs. It was found that protein sequence 3-phosphoshikimate 1-carboxyvinyltransferase is involved in producing mycosporine glycine in *Synechocystis* PCC 6803 and 3-dehydroquinate synthase is involved for producing shinorine in *Anabaena cylindrica*. Phylogenetic and bioinformatic analysis of Mycosporine like amino acid producing protein sequence of both cyanobacterial species *Synechocystis* PCC 6803 and *Anabaena cylindrica* provide a useful framework to understand the relationship of the different forms and how they have evolved from a common ancestor. These products seem to be conserved but the residues are prone to variation which might be due the fact that different cyanobacteria show different physiological process in response of Ultraviolet stress.

Keywords: Cyanobacteria, Mycosporine- like amino acids (MAAs), Mycosporine glycine, Shinorine, Ultraviolet.

Background:

Cyanobacterial species are the aerobic, photosynthetic, gram negative, nitrogen fixing bacteria varying in size and shapes (single cell, filamentous cell, colonial cell form) evolved 3.5 billion years ago in Precambrian period, cosmopolitan in distribution. The role of cyanobacteria in nitrogen fixation and in the maintenance of the fertility of rice is well documented **[1].** Cyanobacteria presumably faced high fluxes of ultraviolet radiations (UVR), which must have acted as an evolutionary pressure leading to the selection for effective protecting mechanisms. They make a good contribution to phytoplankton primary productivity of oceanic regions **[2].**

Due to UV exposure (UV-B, UV-A or both) cyanobacterial species experience reduction in growth, photosynthetic pigments, proteins and increase proline and lipid peroxidation activities. Apart from the deleterious effects, cyanobacteria have ability to withstand under UV-B stress condition by counteracting the damaging effects like repair of UV-induced damage of DNA by photo reactivation and excision repair, accumulation of carotenoids and detoxifying enzymes or radical quenchers and antioxidants for harmful radicals or oxygen species **[3, 4, 5].** Also involve the production of UV-B protecting compounds such as mycosporine like amino acids (MAAs) which enhance the life of cyanobacteria to some extent **[6].**

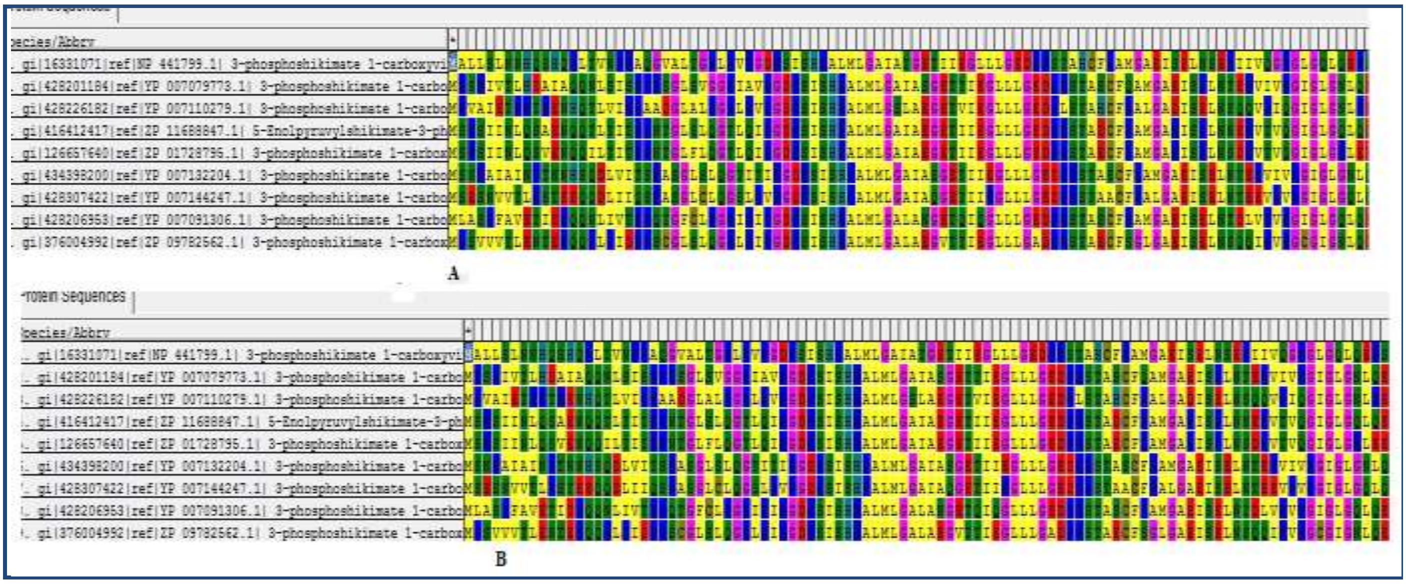
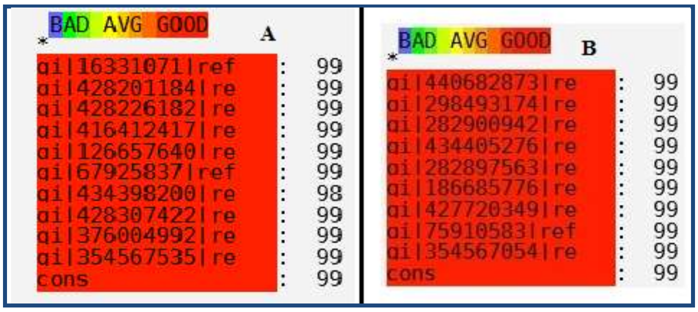


Figure 1: Shows the result of Multiple sequence Alignment using MUSCLE for (A) 3-phosphoshikimate 1-carboxyvinyl transferase protein of *Synechocystis* PCC 6803); **B)** 3-dehydroquinate synthase protein of *Anabaena cylindrica*.



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gi|126657640|re  :  99
gi|67925837|ref  :  99
gi|434398200|re  :  98
gi|428307422|re  :  99
gi|376004992|re  :  99
gi|354567535|re  :  99
cons             :  99
```

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gi|434405276|re  :  99
gi|282897563|re  :  99
gi|186685776|re  :  99
gi|427720349|re  :  99
gi|75910583|ref  :  99
gi|354567054|re  :  99
cons             :  99
```

Figure 2: Shows T-COFFEE result of **(A)** 3-phosphoshikimate 1-carboxyvinyltransferase protein of *Synechocystis* PCC 6803; (**B)** 3-dehydroquinate synthase protein of *Anabaena cylindrica*.

MAAs are water soluble cyclohexane or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol having absorption maxima ranging from 310 to 360 nm. MAA is synthesized by shikimate pathway (prokaryotes) and by pentose phosphate pathway (eukaryotes) **[7, 8].** The UV radiation in cyanobacteria alter protein, DNA, surviving rates, pigmentation, motility, oxygen photoevolution, carbon di-oxide, and nitrogen fixation and phycobiliprotein content **[9].** There are number of MAAs compound like M-tau, dehydroxylusujirene, M-343, Mycosporine glycine, Shinorine etc. are produced in number of cyanobacterial species like *Anabaena species, Synechocystis species, Nostoc species, Plectonema species, Aphanothece species* **[10].** In this present work, we have selected two cyanobacterial species *Anabaena cylindrica* and *Synechocystis* PCC 6803 which are good UV-stress tolerant species along with salinity, heat, oxidative, alkali, drought, water stress , radioactive resistance tolerant ability. Our aim in this present work is comparative analysis of 3-dehydroquinate synthase, key enzyme for the synthesis of shinorine compound in *Anabaena cylindrica* and 3-phosphoshikimate-1-carboxyvinyl transferase a key enzyme for the synthesis of mycosporine glycine production in *Synechocystis* PCC 6803 species with the help of bioinformatic tools and to find out the level of conservation and variability regions in the other 16 (sixteen) cyanobacterial species along with these 2(two) species (*Anabaena cylindrica* and *Synechocystis* PCC 6803) for proteins evolved in MAA production pathway.

Methodology:

Selection of protein sequences from cyanobacteria Synechocystis PCC 6803 and Anabaena cylindrica

The Cyanobacterial protein sequence 3-phosphoshikimate 1-carboxyvinyltransferase for producing mycosporine glycine in *Synechocystis* PCC 6803 and 3-dehydroquinate synthase for producing shinorine in *Anabaena cylindrica* was retrieved from the National Centre for Biotechnology Information (NCBI) database through their entrez search. The work started by performing Basic Local Alignment Search Tool (BLAST) on base protein sequence (3- dehydroquinate synthase) of *Anabaena cylindrica* and (3-phosphoshikimate-1-carboxyvinyl transferase) of *Synechocystis* PCC6803, then with the help of BLAST results and another parameters like Max.score , E-value / Max.identity of the similar sequences related to protein base sequence were found and were successfully downloaded from NCBI databases. After creating a FASTA file of all those sequences that were used the data obtained was incorporated into MEGA5 software for further analysis. The alignment of all protein sequences was done by using MUSCLE algorithm. The alignment result was saved in MAS file with the help of which further work was carried out. The overall mean distance was counted by using "Poisson Model" and on the basis of that overall mean distance the dendogram was generated. The number of conserved sites, variables sites, singleton sites and parsimony information sites was calculated by using explore data file. Finally the sequence MAS file was used in finding relative rate test. Here, in present analysis the Tree Newick rate test was used. The rate test was found by using base sequence and other sequences one by one, with the help of which identical sites, divergent sites and unique differences among the sequence was found and consequences of which free analysis was done. Using T-COFFEE (verson_685) tool a library of pair wise alignments was produced to guide the multiple sequence alignment and then protein variability tool variability plot was obtained.

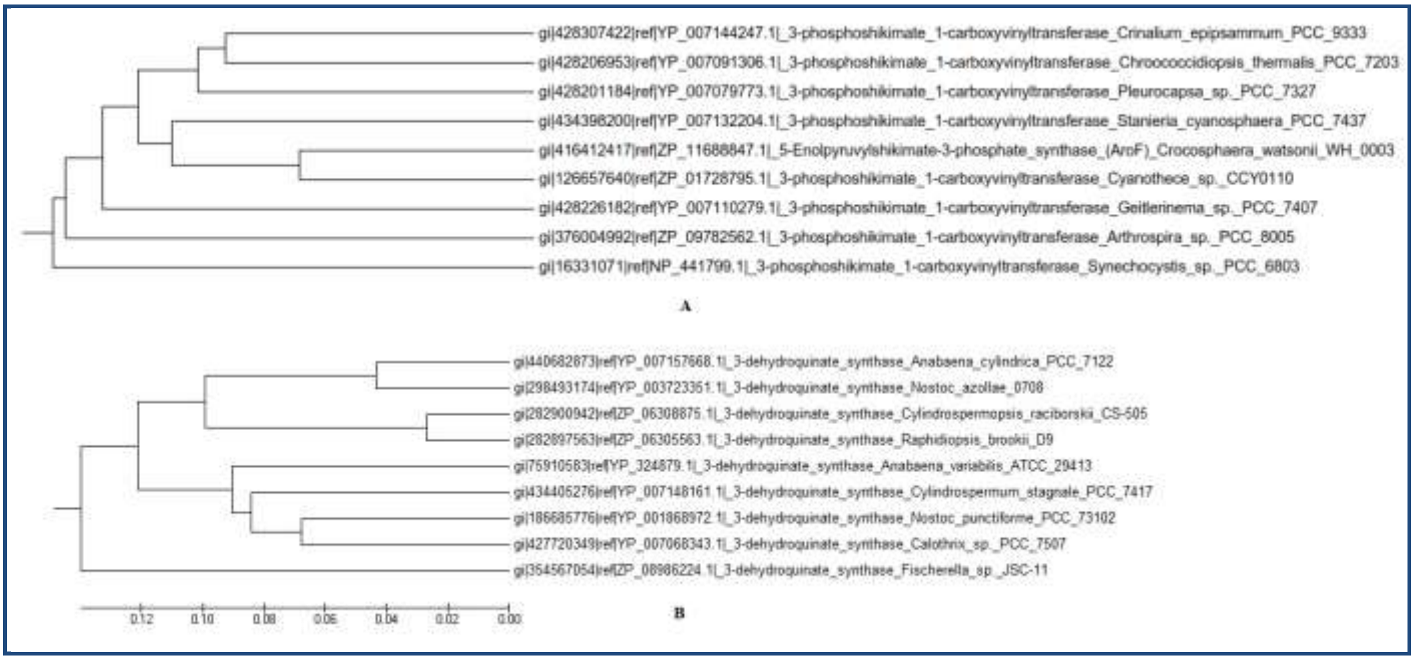

Figure 3: Dendogram showing the closest similarity between different **(A)** cyanobacteria for 3- phosphoshikimate 1-carboxyvinyl transferase protein; **(B):** cyanobacteria 3- dehydroquinate synthase protein.

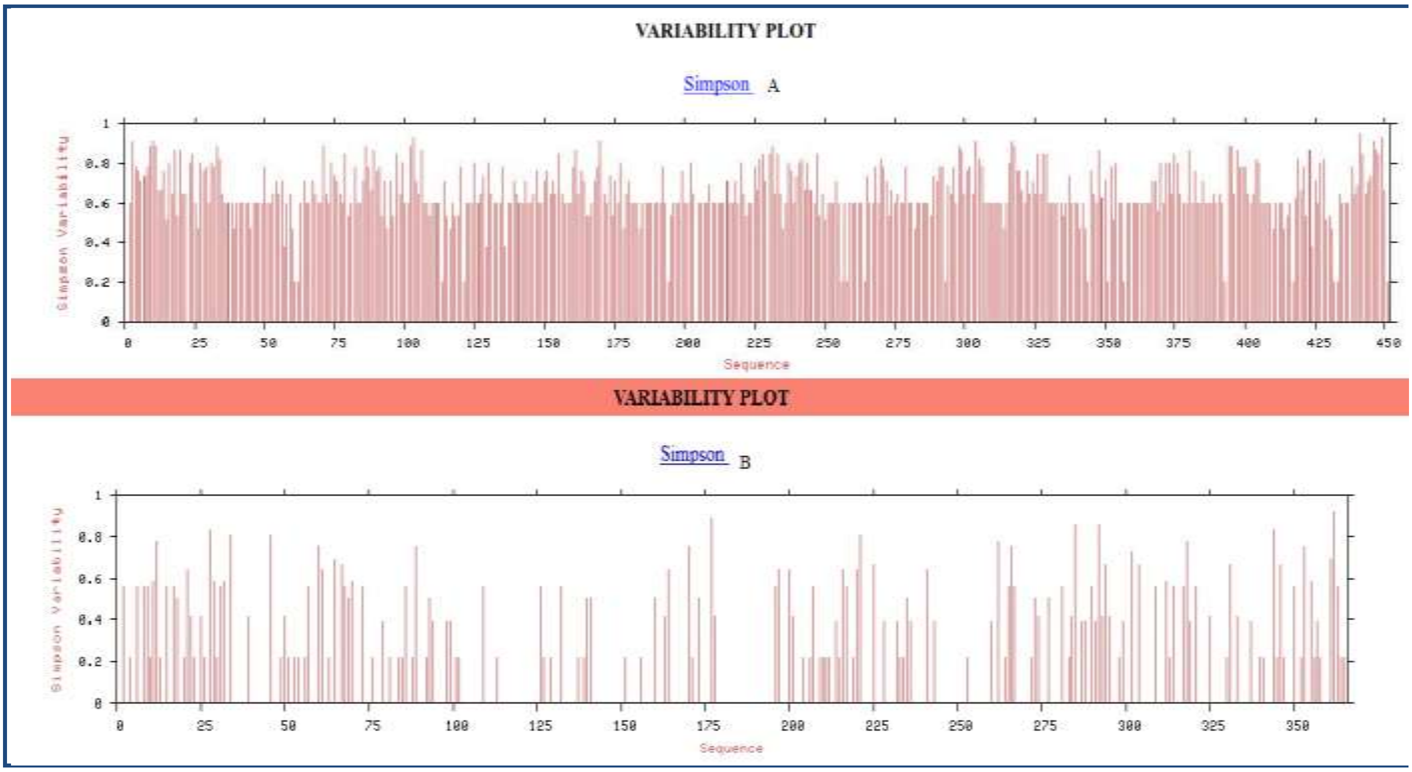

Figure 4: Shows the Simpson's Variability Plot of **(A)** 3-phosphoshikimate 1-carboxyvinyl transferase protein of *Synechocystis* PCC 6803**; B)**: 3-dehydroquinate synthase protein of *Anabaena cylindrica*.

The computational analysis was performed *in silico* using web based software and servers. The alignment of cyanobacterial protein sequences with the other 16 related sequences to find out the identical sequences in other cyanobacteria with respect to 3-dehydroquinate synthase, key enzyme for the synthesis of shinorine compound and 3-phosphoshikimate-1-

carboxyvinyl transferase a key enzyme for the synthesis of mycosporine glycine. Also analysis of the similarity and variability using phylogenetic analysis was done which shows the evolutionary relationship in phylogenetic tree.

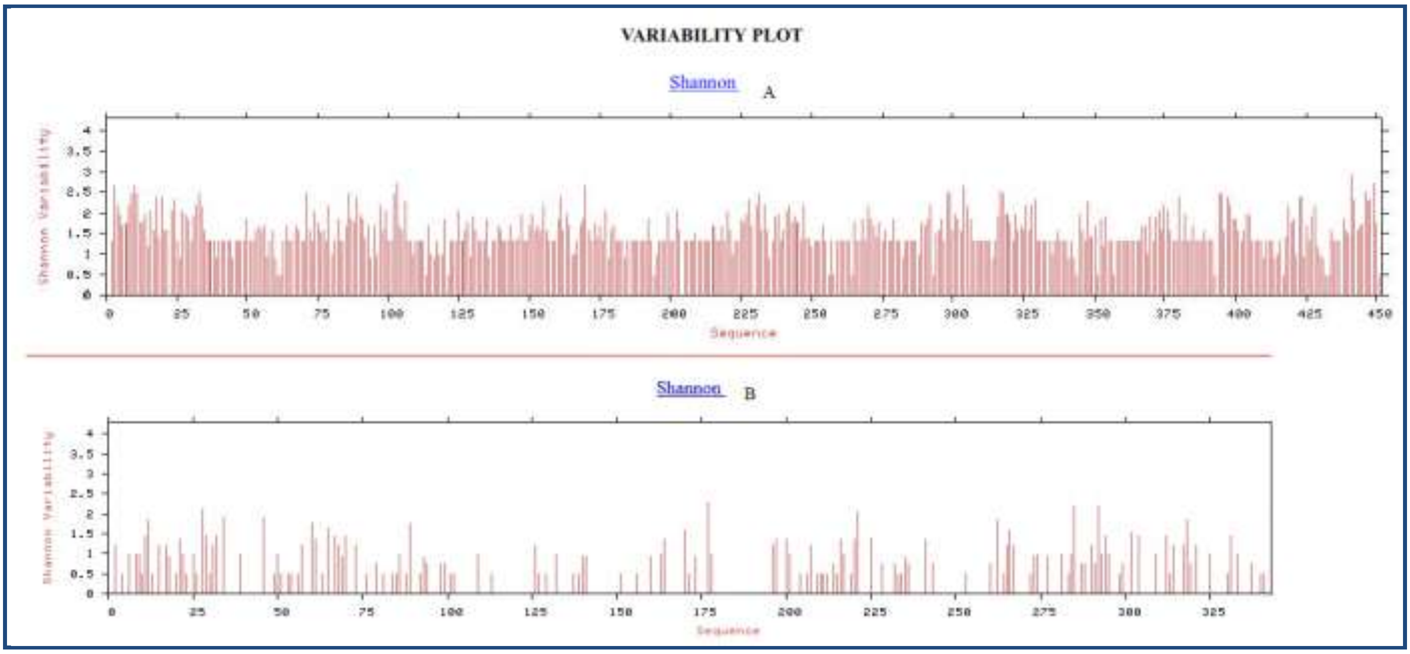

Figure 5: Shows the Shannon's Variability Plot for **(A)** 3-phosphoshikimate 1-carboxyvinyl transferase protein of Synechocystis PCC 6803; **(B)**: 3-dehydroquinate synthase protein of Anabaena cylindrica.

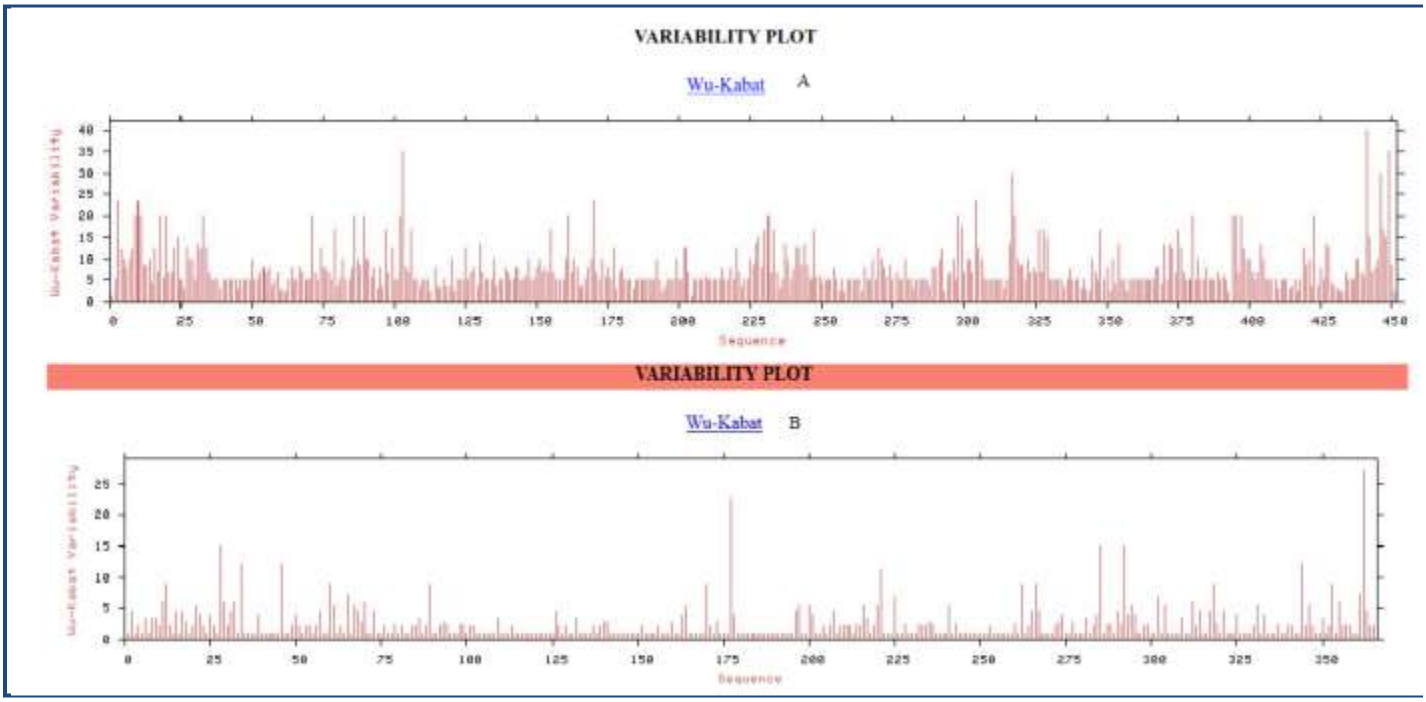

Figure 6: Shows the Wu-Kabat Variability Plot for **(A)** 3-phosphoshikimate 1-carboxyvinyl transferase protein of *Synechocystis* PCC 6803; (**B):** 3-dehydroquinate synthase protein of *Anabaena cylindrica*.

Results & Discussion:

The Cyanobacterial protein sequence 3-phosphoshikimate 1-carboxyvinyltransferase for producing mycosporine glycine of *Synechocystis* PCC 6803 and 3-dehydroquinate synthase for producing shinorine of *Anabaena cylindrica* was retrieved from the NCBI database. The sequence was converted into FASTA format. Then the BLAST software from NCBI was used. It gave us the similar searches related to our base protein sequence which shows max. score, E- value and maximum identity.

On the basis of max. identity and max. score with base protein sequence 3-phosphoshikimate 1- carboxyvinyltransferase of cyanobacteria *Synechocystis* PCC 6803 (gi|16331071|ref|NP_441799.1) eight protein sequences of following different cyanobacteria (given below) were selected.

1-gi|428201184|ref|YP_007079773.1| [*Pleurocapsa* sp. PCC 7327]
2-gi|428226182|ref|YP_007110279.1| [*Geitlerinema* sp. PCC 7407]
3-gi|416412417|ref|ZP_11688847.1| [*Crocosphaera watsonii* WH 0003]
4-gi|126657640|ref|ZP_01728795.1 [*Cyanothece sp.* CCY0110]
5-gi|434398200|ref|YP_007132204.1| [*Stanieria cyanosphaera* PCC 7437]
6-gi|428307422|ref|YP_007144247.1|[*Crinalium epipsammum* PCC 9333]
7-gi|376004992|ref|ZP_09782562.1| [*Arthrospira sp.* PCC 8005]
8-gi|354567535|ref|ZP_08986704.1| [*Fischerella sp.* JSC-11]

On basis of max.identity and max.score with base protein sequence 3-dehydroquinate synthase of cyanobacteria *Anabaena cylindrica* (gi|440682873|ref|YP_007157668.1|) eight protein sequences of following different cyanobacteria (given below) were selected.

1>gi|298493174|ref|YP_003723351.1| [*Nostoc azollae* 0708]
2>gi|282900942|ref|ZP_06308875.1|[*Cylindrospermopsis raciborskii]*
3>gi|434405276|ref|YP_007148161.1 [*Cylindrospermum stagnale]*
4>gi|282897563|ref|ZP_06305563.1| [*Raphidiopsis brookii*]
5>gi|186685776|ref|YP_001868972.1| [*Nostoc punctiforme]*
6>gi|427720349|ref|YP_007068343.1| [*Calothrix sp*.PCC7507]
7>gi|75910583|ref|YP_324879.1 [*Anabaena variabilis* ATCC29413]
8>gi|354567054|ref|ZP_08986224.1|[*Fischerella sp.*JSC-11]

Multiple sequence alignment was performed with MUSCLE separately for the respective enzymes with the selected cyanobacterial species **Figure 1 (A & B).** Phylogenetic and bioinformatic analysis of Mycosporine like amino acids producing protein sequence in *Anabaena cylindrica* and its blast searched eight protein sequences provide a useful framework to understand the relationship of the different forms and how they have evolved from a common ancestor. T- COFFEE (verson_685) and protein variability plot shows the maximum similarity in Mycosporine like Amino acids producing protein sequences in the selected cyanobacterial strains.

The scores of Multiple Sequence Alignment of enzyme sequences obtained from 17 species strains of cyanobacteria associated with Mycosporine like amino acids have been found to be between 72 to 75 which predict a high level of homology and conservation and negligible percentage of gaps amongst the amino acid residues. Similarly T-COFFEE (version_685) results show high level of identity, similarity and positives towards the Good (Red) then towards Average (Yellow) and least towards Bad (Green) which indicate high level of conservation amongst residues **Figure 2 (A & B).** The Cladogram analysis also shows vicinity among the proteins during the process of evolution since nodes are very close to each other. The cladogram is smaller in length, it has fewer homoplasies and it is more parsimonious **Figure 3 (A & B).** The Shannon Variability coefficient seems to be high in few residues which shows that these amino acid residues are prone to mutations during evolution while others seem to be less variable which confirms that variability is less and conservation is more among these residues **Figure 4 (A & B).** To confirm our findings we found that Shannon Variability coefficient comes out to be less than 2 in some residues which again indicate conservation amongst the residues where as in some it comes to be less than 1 which means that these residues are highly conserved while few other residues are variable with values more than 2. The same is confirmed by Simpson **Figure 5 (A & B)** and Wu- Kabat variability index **Figure 6 (A & B**).

Conclusion:

Different cyanobacteria contain different proteins to combat the problem of UV stress. These proteins seem to be conserved but the residues are prone to variations which means different cyanobacteria might have evolved different physiological processes to combat Ultraviolet stress. The location of MAAs within cell is not well defined. Therefore, further study is needed to localize the intracellular distribution of MAAs in different organisms and the regions which express MAA's. The occurrence of MAAs in phylogenetically related members can also be used for chemotaxonomic purposes.

Acknowledgement:

Md. Akhlaqur Rahman is thankful to University Grants Commission, New Delhi, for financial assistance under Maulana Azad National Senior Research Fellowship to carry out this work. Sukrat Sinha is thankful to DST Fast track Fellowship for young scientists and Shailendra Kumar Singh is thankful to DBT Research Associateship

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Edited by P Kangueane
Citation: Rahman *et al.* Bioinformation 10(7): 449-453 (2014)