International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 1639-1645

Received: 09-03-2018
Accepted: 14-04-2018

Yattapu Prasad Reddy
Centre for Conservation and Utilization of BGA, ICAR-Indian Agricultural Research Institute, New Delhi, India

Ravindra Kumar Yadav
Centre for Conservation and Utilization of BGA, ICAR-Indian Agricultural Research Institute, New Delhi, India

G Abraham
Centre for Conservation and Utilization of BGA, ICAR-Indian Agricultural Research Institute, New Delhi, India

Correspondence
Yattapu Prasad Reddy
Centre for Conservation and Utilization of BGA, ICAR-Indian Agricultural Research Institute, New Delhi, India

Screening of cyanobacterial isolates from Rann of Kutch for the production of mycosporine like amino acids

Yattapu Prasad Reddy, Ravindra Kumar Yadav and G Abraham

Abstract
Isolation and characterization of cyanobacteria from extreme environment is important in identifying novel molecules and understanding the mechanisms underlying the tolerance. Therefore in the present study an attempt was made to isolate, identify and characterize cyanobacteria from Runn of Kutch experiencing high levels of salinity. Random soil samples have been collected from Rann of Kutch area from the Kutch District of Gujarat from Western India. The soil samples were used for the isolation of cyanobacteria and the morphological and cultural characteristics were used for identification. The identified strains belong to *Anabaena*, *Nostoc*, *Phromidium*, *Westiellopsis* and *Synechocystis*and were maintained at various salinity levels. The morphological identity was further confirmed using 16S rDNA technique and all the strains examined showed an amplification product of 1500 bp. BLAST analysis was performed and it showed close similarity with cultured cyanobacterial species. The isolates were screened for the presence of mycosporine like amino acids and out of the ten isolates seven were found positive for the presence of MAAs. Heterocystous forms produced more MAAs as compared to non-heterocystous forms. Growth as well as the cellular constituents of the cyanobacterial species producing mycosporine like amino acids showed variability. Positive correlation was found to exist between cellular constituents and MAA content in the isolates.

Keywords: cyanobacteria, extreme environment, 16 S r DNA, mycosporine like amino acids

Introduction
Cyanobacteria are gram negative oxygenic photo autotrophic microorganisms originated during pre Cambrian times and having a unique distribution pattern. They have been found to colonize successfully in several diverse types of ecosystems. The long evolutionary history holds the key to their competitive success to thrive in a wide range of environment. They have the ability to tolerate high temperature, UV desiccation, water and saline stress [1-3]. The cyanobacteria are the simplest photosynthetic organisms and are the only prokaryotic organisms to carry out a higher plant type oxygen evolving photosynthesis.
Hence, they made the atmosphere oxygenic and allowed other organisms to develop. Their chloroplasts are remarkably similar to higher plant chloroplasts in structure and function. They are important as primary producers in soil, fresh water and marine environments.
Sustainability of ecosystems has been maintained by the cyanobacteria since time immemorial and improved soil fertility and crop production. Further advancement in the field of microbiology and biotechnology helped in the exploration of cyanobacteria for industrially important and valuable products from them. The cyanobacteria are also rich sources of industrially important compounds. Burja *et al.*, [4] reported a variety of cyanobacterial products from diverse cyanobacteria. In this context exploration of newer habitats, especially the extreme ones is important from a biotechnological prospective. Hence, search for these organisms from extreme environments would be an important strategy to isolate and identify potential organisms for exploitation. The cyanobacterial isolates from extreme habitats may constitute an important component of the vast an unexplored biological potential. The cyanobacteria are able to survive not very high salt concentrations prevailing in diverse hyper saline environments. Oren [5] reported that cyanobacteria from extreme environment can contribute significantly to the productivity of such environments. However, there have been very few reports on the cyanobacteria from extreme environment such as Runn of Kutch till date.

Cyanobacteria from extreme environments show the presence of several important compounds involved in conferring stress tolerance. The cyanobacteria from extreme environments synthesize several compounds and metabolites to cope with the situation arising out of this complexity [6]. One such compound that has gained considerable often is mycosporine like amino acids According to Portwich and Pichel [7] some cyanobacteria exposed to osmotic stress conditions have been shown to synthesize unique amino acids known as mycosporine like amino acids (MAAs). Oren [8] reported about mycosporne like amino acids which can function as an osmolytes in cyanobacteria from extremely saline environment. In view of this, the present study was undertaken to analyze cyanobacterial isolates from Runn of Kutch for mycosporine like amino acids and growth.

Materials and Methods

Soil samples were collected randomly from five different sites from the Runn of Kutch region of Western Gujarat during June, 2011. These soil samples were used to isolate cyanobacterial strains following standard enrichment culture techniques [9]. BG-11 medium (+N for non heterocystous and –N for heterocystous strains) was used for the isolation procedure. The medium used for the isolation was made saline by the addition of sodium chloride at a concentration of 1-5% and the pH was adjusted to 8.0. The isolated and identified cultures were maintained in the culture room having light intensity of 52-55µmol photon/m^2/S, 16/8 light and dark period and 28±.2 ° C temperature. Strains were examined microscopically and the morphological characters were compared according to Desikachary [10]. For molecular identification, the technique 16S r DNA gene sequencing was used for the identification of cyanobacterial strains. DNA extraction was carried out using DNeasy Tissue Kit and by following the Manufacturer's Protocol (Genetics, USA). Amplification of 16S rDNA gene fragment was done according to [11, 12]. The amplified PCR products were electrophoresed, stained and visualized. The amplified product was sequenced partially. The 16S partial gene sequence was subjected to search for highly similar sequence using mega blast and sequences with the highest similarity indicated by BLAST were identified.

Exponentially growing (15th day of incubation) cultures were used for the determination of growth and other physiological variables. The dry weight determination was done according to Sorokin [13]. Chlorophyll was determined by the protocol given by McKinney [14]. Total protein was estimated by the method of Lowry *et al.,* [15]. Total carbohydrates were estimated by the method of Spiro [16]. The isolated cyanobacterial isolates were screened for mycosporine like aminoacids by the method developed by Sinha *et al.,* [17]. Presence of MAAs were identified by comparing the absorption spectra and retention times and the quantification of MAAs was performed spectrometrically (260nm, 325nm, 327nm) and calculations were made according to Garcia Pichel and Castenholz [18]. The results were analyzed by using the statistical package SPSS 10.0. Duncan's multiple range test (DMRT) was employed to compare the mean performances of different treatments for the parameters used in the study. The rankings have been denoted by superscripts in appropriate tables.

Results

The morphological attributes of the cyanobacterial isolates from Rann of Kutch identified on the basis of cultural parameters and microscopic observations is shown (Table 1). Cyanobacterial forms identified on the basis of morphology belongs to *Anabaena, Nostoc, Phormidium* and *Westiellopsis*. The cyanobacterial strains of *Anabaena* sp., *Nostoc* sp., *Phormidium* and *Westiellopsis sp.* (YPR-4, YPR-6, YPR-8 and YPR-10) grew well at 1% NaCl. However, at 2% NaCl, strains of *Westiellopsis* (YPR-5) were recorded. The strains found to grow at 3% salinity consisted of *Phormidium* sp., *Anabaena* sp. and *Nostoc* sp (YPR-1, YPR-2, YPR-3, YPR-7 and YPR-9) (YPR-1, YPR-2, YPR-3, YPR-7 and YPR-9). 16S rDNA gene sequencing was used for the identification of cyanobacterial isolates. The extracted and quantified DNA from cyanobacterial isolates was subjected to 16S rDNA gene amplification with the primers FD1 and RP2 (Plate 1). Single amplified product of 1500 bp for 16S rDNA was observed in the all the strains examined (Plate 2). The amplified product was used for sequence analysis and the BLAST analysis established the homology in terms of % similarity (Table 2). The cyanobacterial strains of *Synechocystis, Phormidium, Anabaena*, *Nostoc* and *Westiellopsis* showed close similarity with *Synechocystis* sp (98%), *Phormidium inundatum* (98%), *Phormidium priestleyi*ANT.LACV5.1 (99%), *Anabaena bergii* 09-02 (99%), *Anabaena oryzae* (97%), *Nostoc elgonense* TH3S05 (97%), *Nostoc commune* VB516200 (99%), *Nostoc punctiforme* (99%), *Westiellopsis prolifica* (99%), *Westiellopsis* sp. 1590-2 (97%) respectively.

The cyanobacterial isolates were screened for the presence of mycosporine like amino acids (MAAs). Among the ten strains MAAs was detected in seven strains such as *Anabaena*, *Nostoc*, *Phormidium* and *Westiellopsis*, and *Synechocystis* on the basis of retention time and λ_{max} (Fig 1 and Table 3). Heterocystous forms were found to be better producers of MAAs as compared to non-heterocystous forms. Highest amount of MAAs was observed in YPR-9 whereas lowest was observed YPR-6. These strains have been subsequently analyzed for growth and physiological parameters. The growth (dry weight) of these cyanobacterial isolates showed significant variation amongst them (Table 4).

Discussion

Several cyanobacteria, *Anabaena, Nostoc, Westiellopsis* and *Phormidium* have been identified microscopically in the soil samples collected from Rann of Kutch. Although, there are several reports on the occurrence of cyanobacteria in saline soils the type of cyanobacteria found in such saline soils are different [19-21]. In less saline conditions, the heterocystous cyanobacteria have advantage over non heterocystous cyanobacteria due to the presence of glycolipid envelope of the heterocyst [22]. According to Srivastav *et al.,* [23] low salinity favoursheterocystous cyanobacteria where as high salinity supports growth of non-heterocystous genera. It is difficult to characterize the cyanobacteria on the basis of observing structures such as hormogonia, heterocysts and akinites [24] and hence the molecular identification is important. In the present study to support the morphological identification of the cyanobacteria, 16SrRNA gene amplification was carried out. BLAST analysis was employed to establish homology in terms of percentage similarity and revealed that the identified cyanobacteria had close similarity with cultured cyanobacteria. Rudi *et al.,* [25] used 16S rRNA for individual strain characterization and identification of cyanobacteria. Similar observations were also made by Srivastav *et al.,* [26].

Seven out of the ten cyanobacterial isolates were found to show the presence of mycosporine like amino acids. This

could be one of the protection mechanisms to protect the cells against salinity and high rates of insolation. Garcia Pichel and Castenholz [18] reported widespread occurrence of MAAs in cyanobacterial strains isolated from habitats exposed to strong insolation and they are important in cyanobacterial adaptation. These secondary metabolites are capable of preventing the oxidative damage [27]. According to Singh *et al*., [28] the expression and production of these compounds have been found to be influenced under environmental conditions. Thus, the enhanced level of osmoprotectants observed in the cyanobacterial isolates in the present study is probably related to survival of these organisms under such habitats.

The variation observed among the different strains showing the presence of mycopsorine like amino acids with respect to the growth related attributes could be due to the genotypic differences. Variation in the growth rate time of several species of *Anabaena* collected from a geographical habitat was observed by Meeks *et al*., [29]. Ambient physiological conditions also play a role in the growth and cellular constituents. Biochemical constituents of cyanobacteria depend upon the nature of strains, physiological conditions and the environment [30, 31].

The studies therefore showed that several cyanobacterial strains isolated from Runn of Kutch exhibited the presence of mycosporine like amino acids. These isolates on further characterization showed distinct patterns of growth and cellular constituents. Pattanaik *et al*., [32] observed specific effect on growth, phorosynthesis and mycosporine like amino acids in the cyanobacterium *Microcoleus chthonoplastes*. Mycosporine like amino acids probably provide protection against salinity and insolation in these cyanobacteria. The responses are adaptive in nature and help the organism to grow and survive under inhospitable habitats. Further detailed are to be conducted to decipher the exact nature of tolerance mechanisms studies using molecular tools.

Table 1: List of Cyanobacterial isolates and growth from Rann of Kutch

Sl.No.	Salt concentration (%)	Strain	Taxonomic group
1	3	YPR-1	*Anabaena* sp.
2	3	YPR-2	*Anabaena* sp.
3	3	YPR-3	*Phormidium*sp.
4	1	YPR-4	*Westiellopsis*sp.
5	2	YPR-5	*Westiellopsis*sp.
6	1	YPR-6	*Synechocystis*sp.
7	3	YPR-7	*Anabaena* sp.
8	1	YPR-8	*Phormidium*sp.
9	3	YPR-9	*Nostoc*sp.
10	1	YPR-10	*Nostoc*sp.

Table 2: Morphologically identified Cyanobacterial strains and their close match as revealed by BLAST analysis

Salt (%)	Strain	Morphologically identified genera	Close match based on 16SrDNA sequence	Score (%)
3	YPR-1	*Anabaena* sp.	*Anabaena bergii*	98%
3	YPR-2	*Anabaena* sp.	*Anabaena oryzae*	97%
3	YPR-3	*Phormidium*sp.	*Phormidium inundatum*	98%
1	YPR-4	*Westiellopsis*sp.	*Westiellopsis prolifica*	99%
2	YPR-5	*Westiellopsis*sp.	*Westiellopsis* sp.	97%
1	YPR-6	*Synechocystis*sp.	*Synechocystis sp*	98%
3	YPR-7	*Anabaena* sp.	*Nostoc elgonense TH3S05*	99%
1	YPR-8	*Phormidium*sp.	*Phormidium priestleyi ANT.LACV5*	99%
3	YPR-9	*Nostoc*sp.	*Nostoc punctiforme VB62229*	95%
1	YPR-10	*Nostoc*sp.	*Nostoc commune VB516200*	99%

Table 3: Screening and quantification for mycosporine like amino acids the in the cyanobacterial isolates from Rann of Kutch

Sl.No.	Strain	Taxonomic group	MAAs	MAAs (mg/g dry weight)
1	YPR-1	*Anabaena* sp.	Positive	0.0610
2	YPR-2	*Anabaena* sp.	Positive	0.0443
3	YPR-3	*Phormidium*sp.	Positive	0.0736
4	YPR-4	*Westiellopsis*sp.	Negative	-
5	YPR-5	*Westiellopsis*sp.	Positive	0.0145
6	YPR-6	*Synechocystis*sp.	Positive	0.0040
7	YPR-7	*Anabaena* sp.	Negative	-
8	YPR-8	*Phormidium*sp.	Positive	0.0018
9	YPR-9	*Nostoc*sp.	Positive	0.1676
10	YPR-10	*Nostoc*sp.	Negative	-

Table 4: Selected physiological attributes of the cyanobacterial isolates from Rann of Kutch showing mycosporine like amino acids

Strains	Dry weight (mg/ml)	Protein (µg/ml)	Sugar (µg/ml)	Chlorophyll (µg/ml)
YPR-1	255.62^{fg}	773.84^{b}	605.9^{b}	0.658^{e}
YPR-2	332.02^{ab}	949.61^{a}	563.79^{b}	2.219^{a}
YPR-3	316.93^{bc}	716.64^{b}	1467.9^{a}	0.372^{f}
YPR- 4	349.68^{a}	106.55^{d}	1377.8^{a}	1.499^{b}
YPR-5	259.28^{fg}	327.54^{c}	118.89^{b}	0.276^{f}
YPR- 6	276.94^{ef}	144.93^{d}	1363.8^{a}	0.118^{g}
YPR-7	307.51^{cd}	716.64^{b}	371.90^{b}	0.778^{d}
YPR-8	248.48^{g}	86.33^{d}	1413.4^{a}	0.359^{f}
YPR-9	291.52^{de}	724.95^{b}	563.79^{b}	1.020^{c}
YPR10	266.97^{fg}	79.665^{d}	497.11^{b}	0.010^{g}

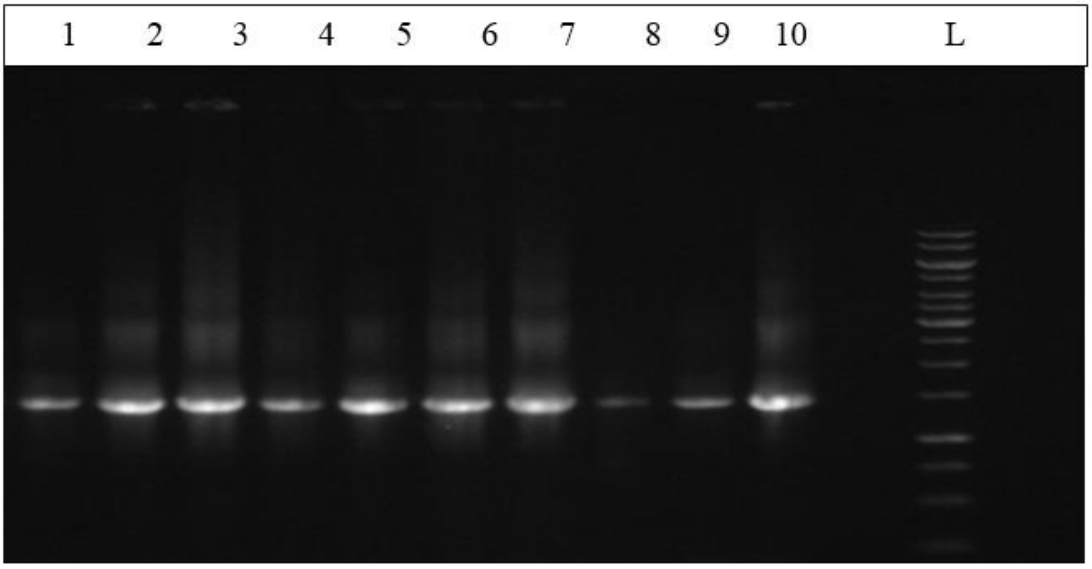


Plate 1: Isolated genomic DNA by Dneasy Tissue kit Manufacturer's protocol with certain modifications L= 1 Kb ladder 1.YPR-1, 2.YPR-2, 3.YPR-3, 4.YPR-4, 5.YPR-5, 6.YPR-6, 7.YPR-7, 8.YPR-8, 9.YPR-9, 10.YPR-10

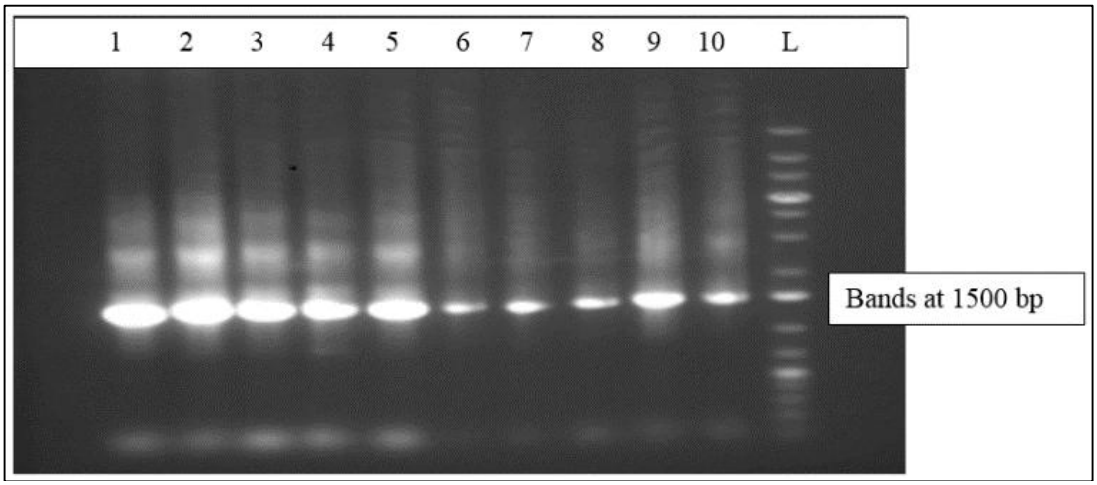
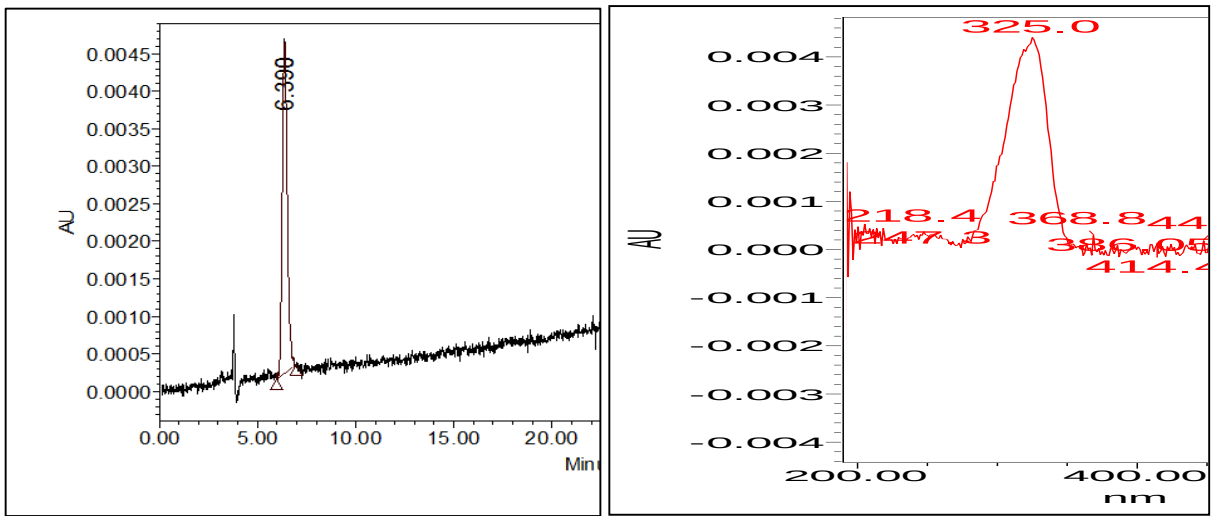


Plate 2: 16S rDNA amplified product in cyanobacterial isolates from Rann of Kutch 1.YPR-1, 2.YPR-2, 3.YPR-3, 4.YPR-4, 5.YPR-5, 6.YPR-6, 7.YPR-7, 8.YPR-8, 9.YPR-9, 10.YPR-10, L= ladder

YPR-1



YPR-2

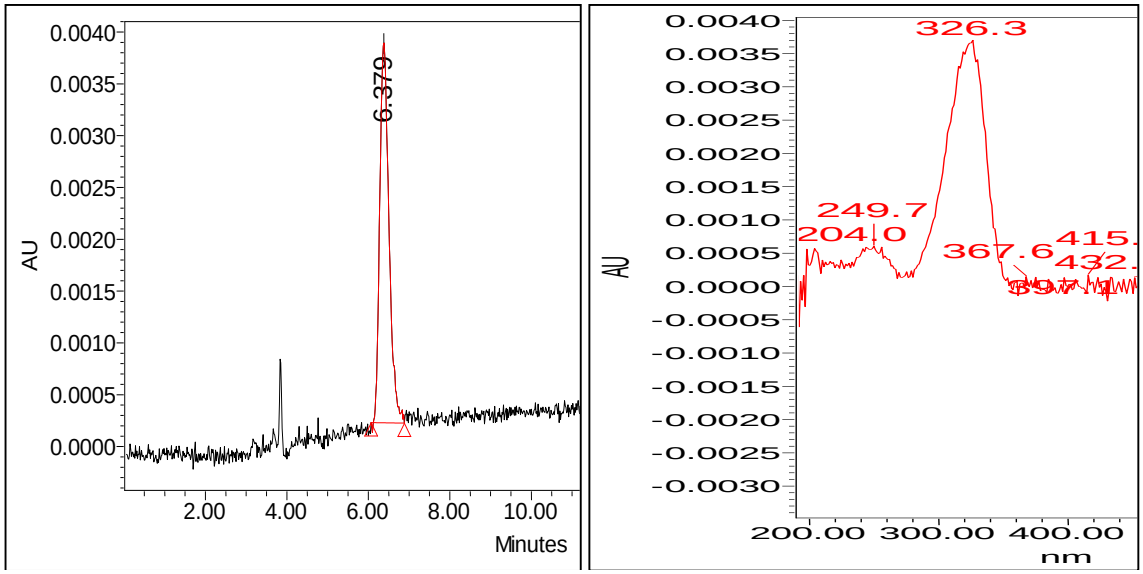
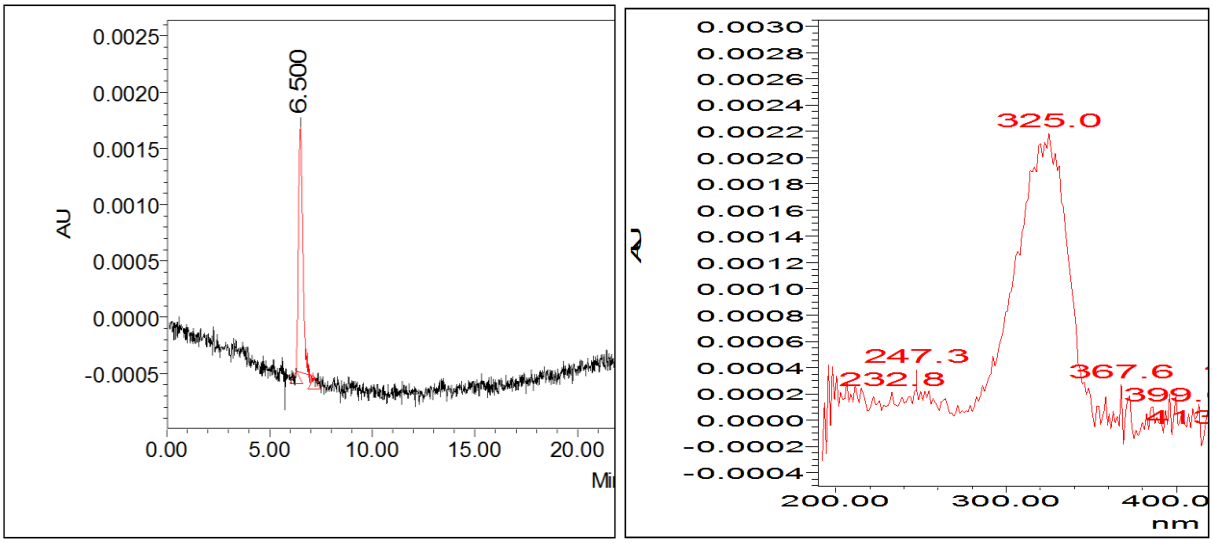
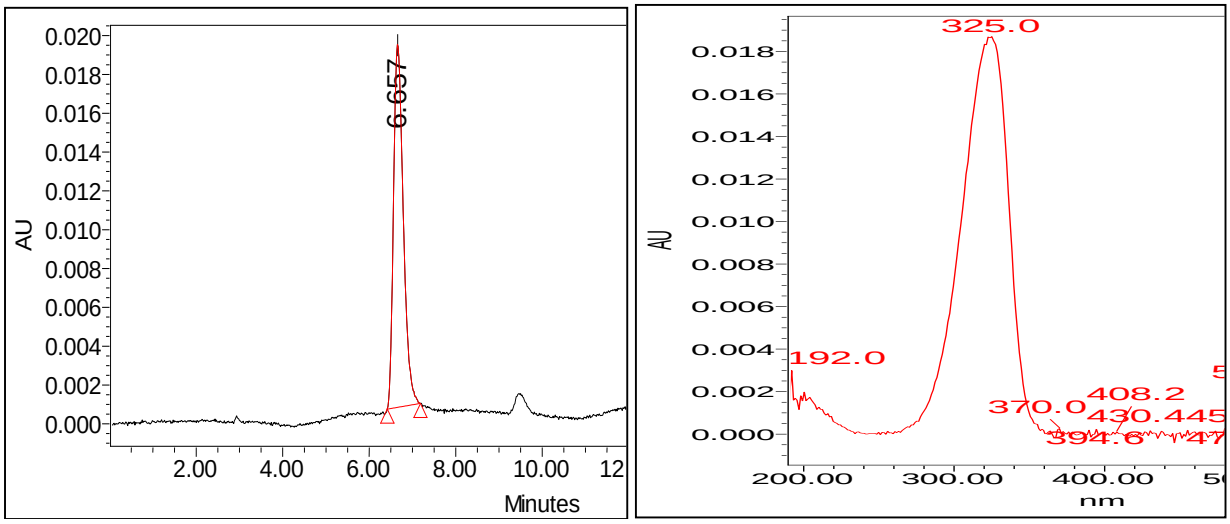


Fig 3: HPLC chromatogram showing the presence of mycosporine like amino acids in cyanobacterial isolates from Rann of Kutch

YPR-3



YPR-5



YPR-6

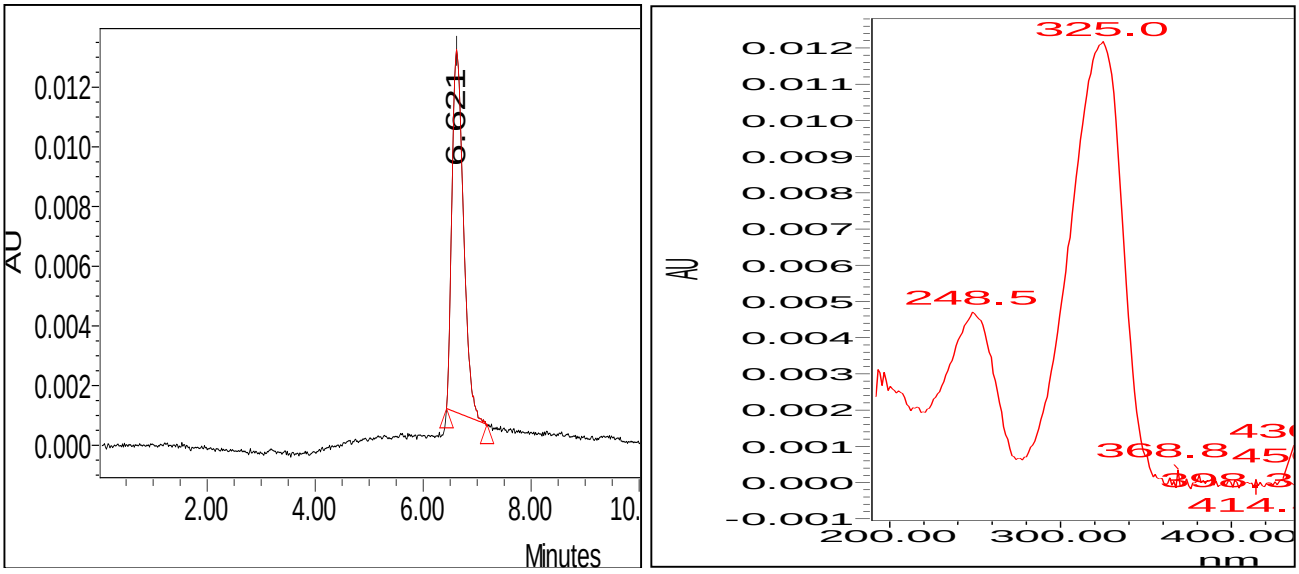
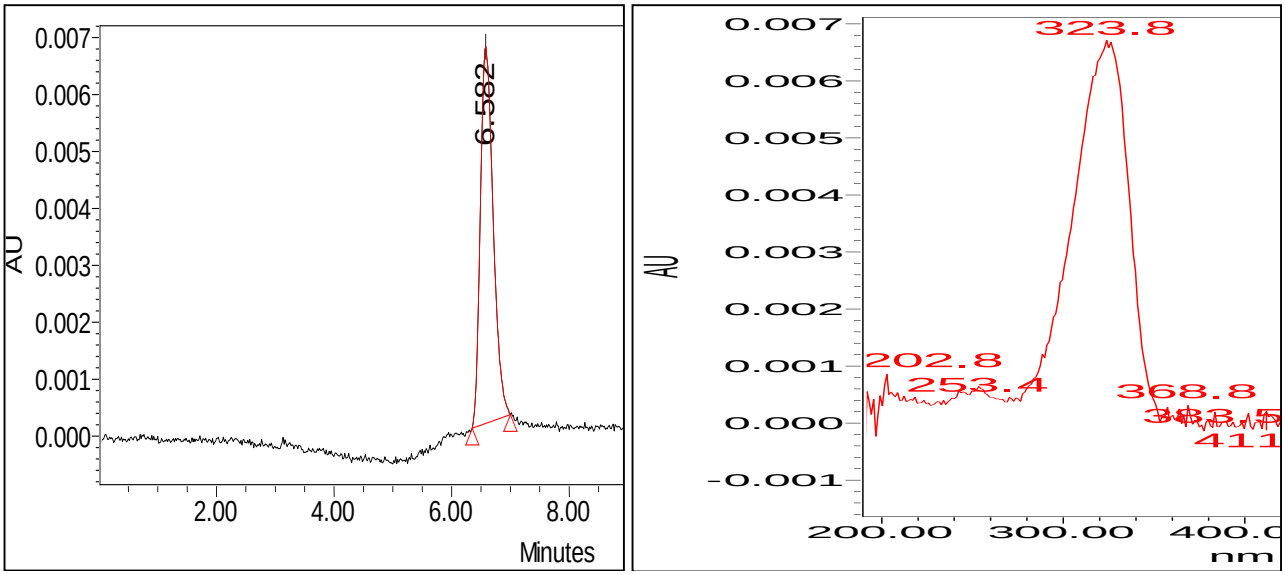


Fig 4: HPLC chromatogram showing the presence of mycosporine like amino acids in cyanobacterial isolates from Rann of Kutch

YPR-8



YPR-9

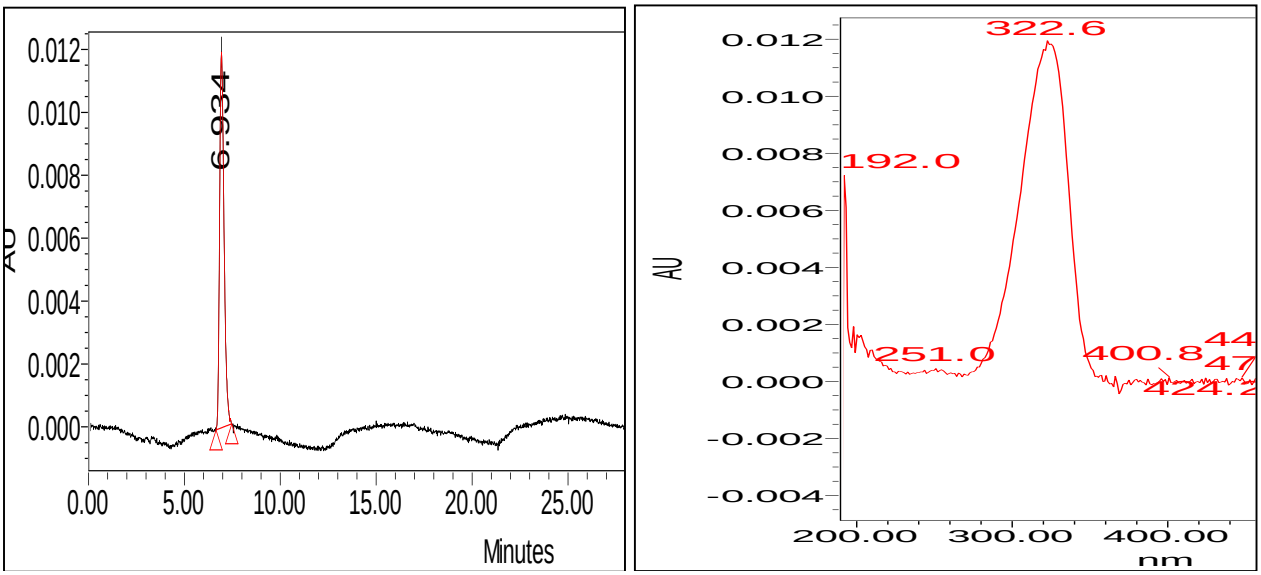


Fig 5: HPLC chromatogram showing the presence of mycosporine like amino acids in cyanobacterial isolates from Rann of Kutch

References

1. Gröniger A, Hader DP. Stability of mycosporine-like amino acids. Recent Res Devel Photochem Photobiol, 2000; 4:247-252.
2. Whitton BA, Potts M. Introduction to the Cyanobacteria. *In*: The Ecology of Cyanobacteria: Their Diversity in Time and Space, Whitton BA, Potts M (*eds*) Dordrecht, Kluwer Academic Press, 2000, 1-11.
3. Herrero A, Flores E. The Cyanobacteria*:* Molecular Biology, Genomics and Evolution. Caister, Academic Press, 2008.
4. Burja AM, Banaigs B, About-Mansour E, Burgess JG, Wright PC. Marine cyanobacteria a prolific source of natural products. Tetrahedran. 2001; 57:9347-9377.
5. Oren A Salts, Brine. The Ecology of Cyanobacteria. Kluwer Academic Publishers. Netherlands. 2000, 281-306.
6. Pandhal J, Wright PC, Biggs CA. Proteomics with a pinch of salt: A cyanobacterial perspective. Saline Systems. 2008; 4:1-18
7. Portwich A, Garcia-Pichel F. Biosynthetic pathway of mycosporines (Mycosporine-like amino acids) in the cyanobacterium*Chlorogloeopsis*sp. strain PCC 6912. Phycologia. 2003; 42:384-392.
8. Oren A. Availability uptake and turnover of glycerol in hypersaline environments. FEMS Microbiol Ecol. 1993; 12:15-23.
9. Stanier RY, Kunisawa R, Mendel M, Cohen-Bazire. Purification and properties of unicellular blue-green algae (Chroococcales). Bacteriol Rev. 1971; 35:171-205.
10. Desikachary TV. Cyanophyta, Indian Council of Agricultural Research, New Delhi, 1959.
11. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991; 37:463-464.
12. Lyra C, Hantula J, Vanio E, Rapala J, Rouhiainen L, Sivonen K. Characterization of cyanobacteria by SDS-PAGE of whole cell proteins and PCR/RFLP of the 16S r RNA gene. Arch Microbiol. 1997; 168:176-184.
13. Sorokin C. Dry weight packed cell volume and optical density. *In*: Stein JR (*ed*), Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge University Press, Cambridge. 1973, 321-343.
14. McKinney. Absorption of light by chlorophyll solutions. J Biol Chem. 1941; 140:315-332.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagents. J Biol Chem. 1951; 193:265-275.
16. Spiro RA. Analysis of sugars found in glycoproteins. Methods Enzymol. 1966; 8:3-26.
17. Sinha RP, Klisch M, Hader DP. Induction of a mycosporinelike amino acid (MAA) in the rice-field cyanobacterium Anabaena sp. by UV irradiation. J Photochem Photobiol B. 1999; 52:59-64.
18. Garcia-Pichel F, Castenholz RW. Occurrence of UV-absorbing, mycosporine- likecompounds among cyanobacteriail isolates and an estimate of their screening capacity. Appl Environ Microbiol. 1993; 59:163-169.
19. Ali S, Sandhu GR. Blue-green algae of the saline soils of the Punjab. Oikos 1972; 23:268-272.
20. Kaushik BD. Reclamative potential of cyanobacteria in salt-affected soils. Phykos. 1989; 28:101-109.
21. Shah V, Ray A, Garg N, Madamwar D. Characterization of the extracellular polysaccharide produced by a marine cyanobacterium *Cyanothece* sp. ATCC 51142, and its exploitation toward metal removal from solutions. Curr Microbiol. 2000; 40:274-278.
22. Stal LJ. The metabolic versatility of the mat-building cyanobacteria *Microcoleuschthonoplastes* and *Oscillatorialimosa* and its ecological significance. Algol Studies. 1991; 64:453-467.
23. Srivastava A, Bhargava P, Kumar A, Rai LC, Neilan B. Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India. Saline Systems. 2009; 5:1-17.
24. Nubel U, Garcia-Pichel F, Clavero E, Muyzer G. Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ Microbiol. 2000; 2:217-226.
25. Rudi K, Skulberg OM, Larsen F, Jakobsen KS. Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequence from the variable region V6, V7 and V8. Appl Environ Microbiol. 1997; 63(7):2593-2599
26. Srivastava A, Bhargava P, Kumar A, Rai LC, Neilan B. Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India. *Saline Systems.* 2009; 5:1-17.
27. Oyamada C, Kaneniwa M, Ebitani K, Murata M, Ishihara K. Mycosporine-like amino acids extracted from scallop (*Patinopectenyessoensis*) ovaries: UV protection and growth stimulation activities on human cells. Mar Biotechnol. 2008; 10:141-150.
28. Singh PS, Sinha RP, Klisch M, Häder DP. Mycosporine-like amino acids (MAAs) profile of a rice-field cyanobacterium *Anabaena doliolum*as influenced by PAR and UVR. Planta. 2008a; 229:225-233.
29. Meeks JC, Wycoff KL, Chapman JS, Enderlln CS. Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena*species.*Appl Environ Microbiol* 1983; 45:1351-1359.
30. Maslova IP, Mouradyan EA, Lapina SS, Klyachko-Gurvich GL, Los DA. Lipid fatty acid composition and thermophilicity of cyanobacteria. Russ J Plant Physiol. 2004; 51:353-360.
31. Rosales N, Ortega J, Mora R, Morales E. Influence of salinity on the growth and biochemical composition of the cyanobacterium *Synechococcus* sp. Cienc Marin. 2005; 31:349-355.
32. Pattanaik B, Roleda MY, Schumann R, Karsten U. Isolate-specic effects of ultraviolet radiation on photosynthesis, growth and mycosporine-like amino acids in the microbial mat-forming cyanobacterium Microcoleuschthonoplastes. Planta. 2008; 227:907-916.