Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Evaluation of Antioxidant Activity and Total Phenolic Content in Cyanobacteria Isolated From Different Regions of India.

Biomedical

KEYWORDS : cyanobacteria, neutraceuticals, phenolic content, antioxidants, DPPH assay.

Roshan Kumar — Centre for Conservation and Utilisation of Blue Green Algae, I.A.R.I., New Delhi -12. INDIA. & Department of Plant Biology and Biotechnology, Presidency College, Chennai-05. INDIA

S. Elumalai — Department of Plant Biology and Biotechnology, Presidency College, Chennai-05. INDIA

Sunil Pabbi — Centre for Conservation and Utilisation of Blue Green Algae, I.A.R.I., New Delhi -12. INDIA.

ABSTRACT

Twenty cyanobacteria isolated from different regions of the country were evaluated for antioxidants and total phenolic content at different time of incubation. The isolates were very diverse in both antioxidant (0.320 – 25.86 m mole Trolox/g dry weight) and total phenolics activity (1.415 – 42.32 µg GAE/g dry weight). Anabaena sp. (SP12), Anabaena sp. (SP13), Calothrix sp. (SP9) were promising strains in terms of antioxidants and in case of total phenolic content Anabaena sp. (SP12), Nostoc sp. (SP20), Nostoc sp. (SP2) were found to have the highest total phenolics content. Most of the isolates recorded maximum antioxidant activity as well as total phenolics content after 14 days of incubation with decrease thereafter. The similarity matrix and the dendrogram made on the basis of these activities depicted inter/intra generic similarity in terms of antioxidants and total phenolics when assigned equal importance stating that antioxidant activity and total phenolics production by cyanobacteria varies among different isolates and is independent of any inter or intra generic boundaries.

Introduction

Cyanobacteria are a highly diverse group of microscopic organisms comprising both unicellular and multicellular forms that occur naturally in different habitats. Almost 20,000 species of algae have been identified and grouped into different divisions. Three of these divisions comprise algae that are frequently grown in large-scale cultivation for commercial purpose. These have been exploited to contribute in a number of areas including nutrition, aquaculture, agriculture and pharmaceutical. Since the last decade, much attention has been focussed on cyanobacteria (Blue green algae) as a source of novel biologically active compounds such as phycobilins, antioxidants, phenols, terpenoids, steroids and polysaccharides (Li et al. 2006; Baky et al. 2008). The synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene are toxic in nature and cause serious health risk. Therefore, these need to be replaced with natural antioxidants (Jamuna and Rai, 2011; Costa et al. 2007). Although, the occurrence of antioxidants and phenolic compounds in cyanobacteria is less documented than that in higher plants but these organisms are excellent source of natural antioxidants which include a range of enzymes (for example superoxide dismutase, glutathione peroxidase), coenzyme Q, melatonin, iron binding proteins (for example transferrin, lactoferrin), vitamins C and E as well as carotenoids, flavonoids and other plant phenolic compounds (Halliwell and Gutteridge, 2007). Several epidemiological studies revealed that antioxidants and phenolic compounds present in diet are helpful in treating coronary heart disease, osteoporosis and other degenerative diseases (Maatela et al. 1996; Kromhout et al. 1996). Polyphenols is a group of phenolic compounds (flavanols, flavonols, anthocyanins, phenolic acids, etc.) that possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Chanda and Dave 2009; Salazar et al. 2008). Furthermore, antioxidants and phenols have been reported to exhibit pharmacological properties such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory or anti tumoral (Sawa et al. 1995). Algal antioxidants and phenolic compounds have also been reported to be a potential candidate to combat free radicals, which are harmful to human body and food systems (Jacobs et al. 1999; Villar et al. 2001). Realizing the importance of phenolic compounds present in cyanobacteria, this study was planned to evaluate the antioxidant and phenolic production potential of cyanobacterial cultures isolated from different locations.

Materials and Methods

Organisms and Growth Conditions

Cyanobacteria were isolated from soil samples collected from different region of India viz. Tamil Nadu, Haryana, Delhi, Kashmir and Bihar (Table.1). The cultures were isolated following standard enrichment culture technique using BG-11 medium (Stanier et al. 1995) in a culture room maintained at 28 ± 2°C temperature and 52- 55 µmole photon/m2/s light intensity with 16/8 light and dark period. Identification of the isolates was confirmed based upon the keys given by Desikachary (1959) for microscopic parameters. For experimental purpose, the cultures were grown in triplicate in 100 mL autoclaved BG-11 medium contained in 250 mL ehrlenmeyer flasks in growth room at 28±2° C and light intensity of 55-60 µmol photon m-2 s-1 with light/dark period = 16h:8h. The 14-day old culture was used as inoculum and a 2% inoculum was used to inoculate the experimental flasks. These were studied for production of antioxidants and phenolic compounds at different incubation periods viz. 7, 14, 21, 28 and 35 days.

Extraction and sample preparation

Extracts of cyanobacterial samples were prepared using the modified method of Lim et al. (2002). For antioxidants, ~0.2 g of dried algal sample was extracted with methanol (10 mL) for 30 minutes at 40° C followed by centrifugation (4500 × g, 10 min). The extract was removed. The extraction process was repeated thrice and all extracts were pooled together. These were washed with chloroform to remove pigments. For total phenolics, dried cyanobacterial culture was extracted with 2 mL of acetone for 30 min at room temperature (24° C) followed by centrifugation. The extraction process was again repeated thrice and all extracts were pooled together and volume noted.

Trolox equivalent antioxidant capacity (DPPH) assay

The DPPH assay was done according to the method of Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and stored at -20° C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1± 0.02 units at 515 nm. Cyanobacterial extracts (150µL) were allowed to react with 2850µL of DPPH solution for 24 h in the dark. The absorbance was then taken at 515 nm. The standard curve was linear between 25 and 800 mM Trolox. Results were expressed in m mole Trolox equivalent (TE)/g dry weight. Additional dilution can be made if the DPPH value measured was over the linear range of the standard curve.

Determination of total phenolics

Total phenolic content was estimated by the Folin–Ciocalteu method of Singleton and Rossi (1965). 200 µL of diluted sample was added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 µL of saturated sodium carbonate (75 g/L) was added. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm. Gallic acid (0–500 mg/L) was used for the standard calibration curve. The results were expressed as gallic acid equivalent (GAE)/g dry weight.

Statistical analysis

For studying the similarity/dissimilarity between the cyanobacterial strains, cluster analysis was performed using SAS 9.3. The Dendrogram was constructed using unweighted pair-group method using arithmetic averages (UPGMA) based on Euclidean distance. Three basic statements were used. viz. PROC DISTANCE (to calculate the distance matrix between species), PROC CLUSTER (to calculate the clusters) and PROC TREE (to draw the dendrogram).

Results and Discussion

Antioxidant activity as well as phenolics production in different cyanobacterial isolates was estimated at different times of incubation viz. 7, 14, 21, 28 and 35 days. The maximum antioxidant activity (25.86 m mole TE/g dry weight) was recorded in *Anabaena* sp. (SP12) followed by *Calothrix sp.* (SP9) (14.81 m mole TE/g dry weight) and *Anabaena sp.* (SP13) (14.67 m mole TE/g dry weight). *Phormidium sp.* (SP14) recorded least activity of 1.49 m mole TE/g dry weight after 7 days of incubation (Fig. 1) whereas, Phormidium sp. (SP11) showed least value of 2.306 m mole TE/g dry weight after 14 days of incubation. Most of the isolates showed maximum activity on day 14 or day 21 followed by decrease thereafter. The activity reduced drastically by end of incubation i.e. after 35 days of incubation. Considering the time course study, *Anabaena* sp. (SP12) also recorded maximum activity at all stages of incubation/observation as compared to other isolates. Among different *Nostoc* sp., there was immense variability in terms of antioxidant activity. Comparing the activity on day 14, isolate SP16 recorded maximum activity (11.72 m mole TE/g dry weight) among the ten *Nostoc* spp. and the least activity was shown by isolate SP10. As stated earlier, maximum activity was observed on day 14 or 21 with decrease on further incubation but *Anabaena* sp. (SP13) showed relatively stable antioxidant activity till 28 days of incubation which decreased considerably at 35 days. The isolates that sustained an activity of more than 4.55 m mole TE/g dry weight after 35 days of incubation were *Calothrix* sp. (SP9), *Anabaena* sp. (SP12) and *Nostoc* sp. (SP19). Statistical analysis of the data confirmed that *Anabaena* sp. (SP12) was best among all the strains for the production of antioxidants. *Calothrix* sp. (SP9) and *Anabaena* sp. (SP13) were close second and were on par statistically. Suhail et al. (2011) reported antioxidant potential of the methanol extracts of different cyanobacteria viz. *Plectonema boryanum, Scytonema* sp., Osillatoria sp., Chroococcus sp., *Anabaena* variabilis, and *Nostoc* sp. Abd El-Baky et al. (2008) observed pronounced antioxidant activity in a crude extracts of Spirulina maxima. Spirulina and its antioxidant activity have been well documented (Abd El-Baky 2003; Khan et al. 2005; Athukorela et al. 2006) and many products based on Spirulina are available in the market based on whole cell or the extracts recommended as antioxidants. Similarly, phenolic compounds are a class of antioxidant agents which act as free radical terminators (Kedage et al. 2007). These compounds have been extensively studied for their antioxidant properties not only in fruits and vegetables but also in green algae and cyanobacteria (Yoshino and Murakami 1995; Duval and Shetty 2001). The cyanobacterial isolates used in this study showed great variation with respect to the production of total phenolic compounds (Fig. 2). Like the antioxidant activity, total phenolics were maximally produced by cyanobacterial isolates at 14 days with decrease thereafter. It was interesting to note that unlike antioxidant activity where maximum activity among different isolates fluctuated between 14, 21 or 28 days, the total phenolics production was maximum on day 14 in all the twenty isolates studied without any exception. *Anabaena* sp. (SP12) showed maximum amount of phenolics (42.32 µg GAE/g dry wt.) and dominated throughout the incubation recording maximum activity at each stage of observation. This was followed by *Nostoc* sp. (SP20) and *Nostoc* sp. (SP2) whereas another *Nostoc* sp. (SP15) recorded minimum amount of phenolics production i.e. 1.535 µg GAE/g dry wt. at 7 days. Phormidium sp. (SP14), however, showed minimum activity (8.05 µg GAE/g dry wt.) after 14 days of incubation. The variations could also be observed among *Nostoc* isolates with *Nostoc* sp. (SP20) showing maximum phenolics production (35.18 µg GAE/g dry wt.) followed by SP2 (28.80 µg GAE/g dry wt.) and *Nostoc* sp. (SP10) was having the least phenolics production of 9.24 µg GAE/g dry wt. as observed after 14 days of incubation.

The similarity matrix (Fig. 3) prepared based on both antioxidant activity and total phenolics production revealed that there was no similarity based on genera among the isolates and the isolates showed lot of variation among each other for both the parameters that extended beyond generic differences. The isolates SP1 to SP11 were distinct but close to each other whereas isolates SP13-SP20 showed more distinctness among each other. All these isolates showed better degree of similarity with SP12. The dendrogram prepared divided the isolates into two major clusters (Fig. 4) with 19 isolates in cluster 1 and only one isolate (SP12) in cluster 2. This also confirms our results of similarity matrix where SP12 was a standalone isolate with maximum production. The cluster 1 was further divided into two sub clusters with sub cluster 1 having 16 isolates and sub cluster 2 having 3 isolates. Sub cluster 1 again bifurcated the isolates to two main clusters with 13 and 3 isolates respectively. Each cluster/sub cluster was represented by isolates belonging to different genera and there was no intrageneric similarity observed among the isolates for these two parameters viz. antioxidant activity and total phenolics production.

Conclusion

Antioxidants and phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of an organism. These compounds also possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities. These activities may be related to their antioxidant activity (Lin et al. 1998). The antioxidants and total phenolic content of cyanobacterial isolates belonging to different regions was evaluated using the DPPH assay and Folin–Ciocalteu method respectively. The isolates had very diverse radical scavenging capacity (0.320 – 25.86 m mole Trolox/g cyanobacterial dry weight) and total phenolics activity (1.415 – 42.32 µg GAE/g dry weight). This was expected because the strains were selected from different families. However, it was surprising that among the same genus, different isolates could exhibit very different radical scavenging ability as has been stated earlier (Hua et al. 2007). Among the 20 cyanobacteria evaluated, *Anabaena* sp. (SP12), *Anabaena* sp. (SP13), *Calothrix* sp. (SP9) are the promising strains in terms of antioxidants and in case of total phenolic content *Anabaena* sp. (SP12), *Nostoc* sp. (SP20), *Nostoc* sp. (SP2) were found to have the highest total phenolics content and these isolates can be exploited for commercial production of these natural phenolics for their possible use as nutraceuticals. The results in the dendrogram depicted inter/intra generic similarity in terms of antioxidants and total phenolics when assigned equal importance. Thus, it can be concluded from this study that antioxidant activity and total phenolics production by cyanobacteria varies among different isolates and is independent of any inter or intra generic boundaries. The findings open a new aspect for further study on cyanobacteria, particularly the high producing strains where the antioxidant and phenolic compounds produced by these can be characterized to look for novel compound and their optimum utilization.

Acknowledgement
Authors are grateful to CCUBGA, I.A.R.I., New Delhi for support and to the authorities of Department of Plant Biology & Biotechnology, Presidency College (Autonomous), Chennai for providing necessary facilities.

Table 1. List of cyanobacteria isolated from different regions.

S.No	Origin/site	Name of Cyanobacteria
SP1	IARI fields, New Delhi	*Nostoc* sp.
SP2	IARI fields, New Delhi	*Nostoc* sp.
SP3	IARI fields, New Delhi	*Nostoc* sp.
SP4	IARI fields, New Delhi	*Syctonema* sp.
SP5	IARI fields, New Delhi	*Hapalosiphon* sp.
SP6	IARI fields, New Delhi	*Nostoc* sp.
SP7	IARI fields, New Delhi	*Nostoc* sp.
SP8	IARI fields, New Delhi	*Oscillatoria* sp.
SP9	IARI fields, New Delhi	*Calothrix* sp.
SP10	Areraj,Motihari district of Bihar	*Nostoc* sp.
SP11	Areraj,Motihari district of Bihar	*Phormidium* sp.
SP12	Karnal District of Haryana	*Anabaena* sp.
SP13	Karnal District of Haryana	*Anabaena* sp.
SP14	Baramulla distict of Kashmir	*Phormidium* sp.
SP15	Baramulla distict of Kashmir	*Nostoc* sp.
SP16	Baramulla distict of Kashmir	*Nostoc* sp.
SP17	Kanchipuram district of Tamilnadu	*Lyptolyngbya* sp.
SP18	Kanchipuram district of Tamilnadu	*Microcoleus* Sp.
SP19	Kanchipuram district of Tamilnadu	*Nostoc* sp.
SP20	Kanchipuram district of Tamilnadu	*Nostoc* sp.

Fig: 1 Antioxidants production by different cyanobacteria

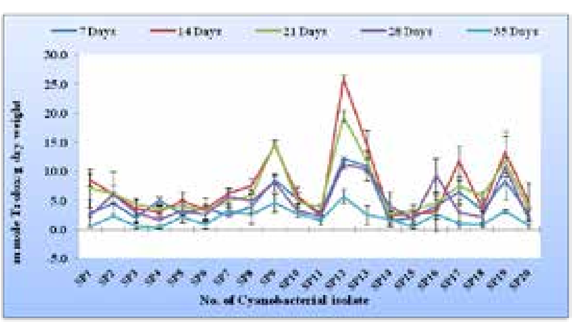


Fig: 2 Total phenolics production by different cyanobacteria

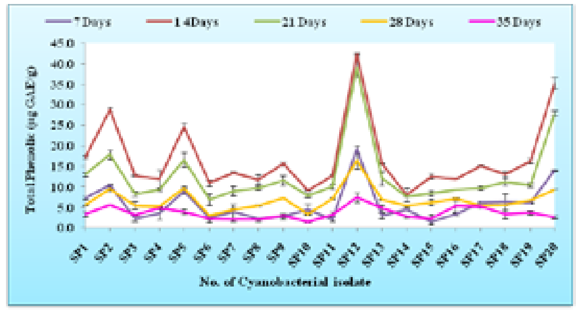


Fig: 3 Similarity matrix based on antioxidants and total phenolics parameters.

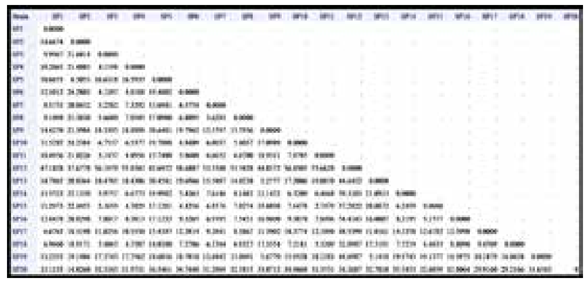
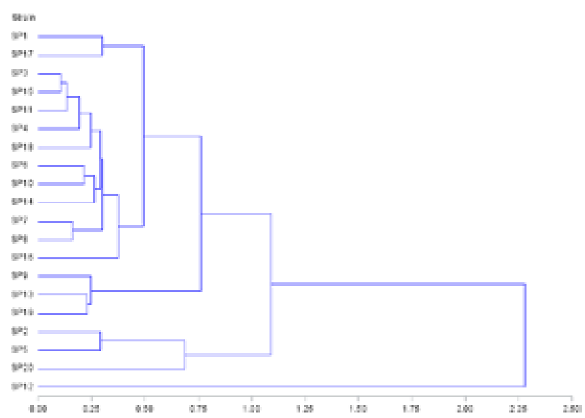


Fig: 4 Dendrogram based on Antioxidants and total Phenolics.



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