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Biochemical analysis of a few selected cyanobacterial species isolated from Deepor beel Ramsar site

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Abstract
With the emerging knowledge of biochemical composition, cyanobacteria are now considered as one of the potent group of organisms to be used in different industries. Though rich in diversity, the cyanobacterial flora of North-Eastern region is yet to be analyzed biochemically. The present study was an attempt to generate a baseline data for pigments, protein and lipid contents of seven selected species isolated from Deepor beel, a Ramsar site of Assam.
Keywords: Cyanobacteria, protein, lipid, carotenoids, chlorophyll-a

Introduction

Cyanobacteria are microscopic photosynthetic organisms that are ubiquitous in their distribution [1]. The nutritional composition containing carotenoids, essential polyunsaturated fatty acids, minerals and protein with balanced amino acid profile makes them suitable for many fields of application [2] for which the organisms are widely used in food industries as well as in few biotechnological applications [3].

The biotechnological applications of cyanobacteria in diverse areas such as agriculture, aquaculture, bioremediation, bioenergy and biofuels, cosmetics and nutraceuticals have already been well-documented [4]. Cyanobacterial feed are currently used mainly for the culture of larvae and juvenile shell and finfish, as well as for raising the zooplankton required for feeding of juvenile animals [5]. Some species of *Nostoc*, *Anabaena* and *Calothrix* are known to increase the body weight of telapia hybrid fish with excellent food conversion ratio [6]. *Phormidium valderianum* has also been used in India as aquaculture feed for high nutritional value and non-toxic in nature [7].

Among the pigment groups of cyanobacteria, the commercially sought are carotenoids and phycobilins. Manufacture of carotenoids via cyanobacteria has undergone a greater scientific and commercial importance in the cosmetic, poultry and aquaculture industry [8] and is responsible for the yellow, red and orange colour of egg yolk, feathers, crustacean shells, fish flesh and skin [9]. Several researchers have demonstrated that carotenoids contribute significantly to the total antioxidant capacity of cyanobacteria [10] due to which they have a high demand in nutraceuticals and pharmaceuticals industry. Lipids are essential for all living organisms as components of membranes, energy storage compounds and as cell signaling molecules [11], and have been recognized as essential components in human and animal nutrition. It is used as feed additives in aquaculture too.

Protein quality is generally one of the most important aspects used to define the nutritional value of novel feed ingredients and is largely dependent on amino acid profile [12]. *Chlorella* and *Spirulina* were the first two microalgae to be commercially used as ‘health food’ in Japan,

Taiwan and Mexico [13]. In addition to being consumed as a food product, *Spirulina* is known to have therapeutic implications against diabetes, arthritis, anemia, cardiovascular diseases and cancer [6]. Potential protein source from microalgae and algal co-products for nutrition applications has long been recognized, but commercial success has only been realized to a small extent for a few species e.g. *Spirulina*, *Chlorella* and *Dunaliella* till date [12]. Keeping the enormous diversity of algae in mind in North Eastern region of India which is considered as a part of biodiversity hotspot, biochemical screening of potential cyanobacterial species become the need of the hour. The present work was therefore aimed to study the quantification of pigments and other biochemical constituents of a few indigenous cyanobacteria species isolated from Deepor beel Ramsar site.

Materials and Methods

Selection of algae

Out of the 37 number of species isolated from Deepor beel Ramsar site (91°35′-91°43′ E longitudes and 26°05′-26°11′ N latitudes), seven cyanobacterial species were selected based on their performance in growth and abundance. The species selected are *Anabaena circinalis*, *Calothrix javanica*, *Nostoc calcicola*, *N. ellipsosporum*, *N. microscopicum*, *Rivularia hansgirgi* and *Westiellopsis prolifica*. Identification of the species was done following the taxonomical characterization of keys provided by Desikachary [14].

Cultivation and growth condition of the organisms

Once collected, the cyanobacterial samples were allowed to grow in a 250 ml Erlenmeyer flask containing 100 ml BG-11 medium, maintaining the temperature at 27 ± 1 °C with continuous photoperiod under a light intensity of 140 µmol photons $m^{-2}s^{-1}$ provided by cool white fluorescent lamps. The medium was sterilized in an autoclave at 121°C for 90 minutes before inoculation. Frequent streaking and subculture was done in order to obtain pure form of the culture. For each species, 10 ml of the mother culture was diluted in a 250 ml Erlenmeyer flask containing 100 ml of the fresh sterile media as mentioned above. Each sample was grown in triplicates for each experimental test. Cyanobacterial cells were harvested by centrifugation at 4000 rpm for 10 minutes at room temperature after 20 days for biochemical analysis. All inoculums and experimental cultures were carried out under aseptic laboratory conditions.

Lipid estimation

The lipid concentration of the cyanobacterial biomass was analyzed by a procedure adapted from Folch *et al*. [15]. This method involves using chloroform: methanol 2:1(v/v) as solvent mixture. Known amount of the algal sample (dry weight) was homogenised with 5 ml of chloroform: methanol. The extract was then filtered through Whatman GF/C filter paper and transferred into a separating funnel. 3 ml of saline water (1% NaCl) was added to the filtrate and allowed to stand undisturbed in room temperature for few minutes. A clear biphasic layer was formed. The lower organic phase ($CHCl_3$) layer was collected in a clean pre-weighed (W_1) small beaker. The solvent was left overnight in a dessicator to evaporate the solvent near to dryness, leaving lipid at the bottom of the beaker. The weight of this beaker with the lipid extract was reweighted (W_2). The difference between the initial weight and the final weight (W_2-W_1) gives the total lipid content of the sample.

Protein estimation

The estimation of total protein of the cyanobacterial samples was done as per the method proposed by Lowry *et al*. [16]. Extraction of protein was carried out by homogenizing known volume of the sample in 5 ml of Tris buffer. The homogenate was then centrifuged at 6000 rpm for 10 minutes and the supernatant was collected. Alkaline copper reagent and Folin-ciocalteau reagent were added to the supernatant and mixed well. It was then incubated at room temperature in the dark for 30 minutes. The absorbance of the blue coloured solution produced was read at 660 nm in a UV-Visible Spectrophotometer 119 (SYSTRONICS) against the Bovine Serum Albumin as blank.

Estimation of chlorophyll-a and carotenoids

Chlorophyll-a estimation: Estimation of chlorophyll-a was done according to Arnon [17]. 10 ml of 80% acetone was added to known volume of the sample and placed on a water bath at 60°C for 30 minutes. The suspension formed

was centrifuged at 3000 rpm for 5 minutes and the supernatant was collected. The absorbances of the supernatant were recorded at 645 and 663 nm against 80% acetone as blank using UV-Visible Spectrophotometer 119 (SYSTRONICS).

Carotenoid estimation: The estimation of caroteinoids was done spectrophotometrically by the method of Myers and Krantz [18]. 10 ml of homogenized algal suspension was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the pellet obtained was extracted with 3 ml of 80% acetone and homogenized. The contents were incubated overnight at 4°C. The suspension formed was centrifuged and absorbance of the supernatant was read at OD 480 nm to measure the caroteinoids content.

Statistical analysis

The entire test was conducted in triplicate independent cultures to confirm their reproducibility and the results of the entire analysis were expressed as mean ± standard deviation of three experiments.

Results and Discussion

The biochemical composition of *A. circinalis*, *C. javanica*., *N. calcicola*, *N. ellipsosporum*, *N. microscopicum*, *R. hansgirgi* and *W. prolifica* were analyzed with the harvested biomass generated in the laboratory condition in BG-11 medium under the light intensity of 140 µmol photons $m^{-2}s^{-1}$ for 20 days. Pigment concentrations were determined in terms of fresh weight (FW) at the end of the experimental test i.e., on the 20th day. Results revealed that both chlorophyll-a and total carotenoid contents vary considerably amongst the tested species (Table 1). The highest chlorophyll-a content was found in *N. calcicola* (3.17 ± 0.26 mg ml^{-1}) and the lowest in *C. javanica* (0.86 ± 0.18 mg ml^{-1}) per 30 mg fresh biomass. It is well known that carotenoids are important contributors to the antioxidant activity in microalgal/cyanobacterial biomass besides their photosynthetic role [19]. Cyanobacteria are also potential sources of carotenoids of interest. In the present study, the highest amount of total carotenoid content was exhibited by *N. ellipsosporum* with 5.58 ± 0.17 mg ml^{-1} FW, followed by *N. calcicola* (5.43 ± 0.69 mg ml^{-1}), *W. prolifica* (5.39 ± 0.05 mg ml^{-1}), *A. circinalis* (4.03 ± 0.58 mg ml^{-1}) and *N. microscopicum* (3.17 ± 0.4 mg ml^{-1}). It was recorded to be lowest in *C. javanica* (2.75 ± 0.38 mg ml^{-1}) per 30 mg of fresh biomass.

The total protein content on percentage dry weight (% DW) basis after 20 days of incubation revealed that protein was highest in *N. ellipsosporum* (12.78 ± 1.05 %), followed by *N. calcicola* (11.65 ± 0.88 %), *N. microscopicum* (9.84 ± 1.31 %) and *R. hansgirgi* (9.62 ± 1.26 %) respectively. The lowest was observed in *C. javanica* (7.01 ± 1.19 %). The total lipid content of the seven tested cyanobacterial species ranged from 11.6 ± 1.43 to 4.28 ± 0.94 %. Highest total lipid content was exhibited by the cyanobacterium *N. calcicola* with 11.6 ± 1.43 %, followed by *N. ellipsosporum* (7.14 ± 1.21 %) and *R. hansgirgi* (5.51 ± 0.79 %) respectively. Of them, species with the lowest lipid content was *W. prolifica* (4.28 ± 0.94 %). The relatively high lipid content obtained (11.6 ± 1.43 %) from *N. calcicola* in the present work was much higher than that of the value obtained (3.6 ± 0.23 %) by Shinde *et al.* [20] in the same species. It may therefore be concluded that chlorophyll content, carotenoid content, lipid content and protein content are highly species specific and their composition are influenced by culture media and environmental factors such as nutrient availability and light intensity and is also known to change with age of a culture [19].

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Table 1: Pigments, lipid and protein content of the studied cyanobacterial species

Species Name	Total lipid content (% DW)	Total protein content (% DW)	Chlorophyll-a (mg ml^{-1})	Carotenoids (mg ml^{-1})
Anabaena circinalis	7.14 ± 1.21	7.15 ± 1.26	1.38 ± 0.28	4.03 ± 0.58
Calothrix javanica	6.5 ± 0.56	7.01 ± 1.19	0.86 ± 0.18	2.75 ± 0.38
Nostoc calcicola	11.6 ± 1.43	10.65 ± 0.88	3.17 ± 0.26	5.43 ± 0.69
Nostoc ellipsosporum	7.73 ± 1.64	12.78 ± 1.05	2.52 ± 0.34	5.58 ± 0.17
Nostoc microscopicum	6.65 ± 1.3	9.84 ± 1.31	1.11 ± 0.42	3.17 ± 0.4
Rivularia hansgirgi	5.51 ± 0.79	9.62 ± 1.09	1.08 ± 0.15	2.93 ± 0.21
Westiellopsis prolifica	4.28 ± 0.94	8.23 ± 0.96	1.88 ± 0.21	5.39 ± 0.05

Each value represents mean of three independent experiment ± standard deviation (n=3).

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