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Microalgal carotenoids: Potential nutraceutical compounds with chemotaxonomic importance

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ABSTRACT

There are more than 600 different carotenoids which perform a range of functions in various organisms including microalgae. In the present study, chemosystematics approach was followed to segregate 57 microalgal strains based on their carotenoid composition using principal component analysis (PCA) and hierarchical clustering. The present findings suggest that lutein and violaxanthin can be effective chemotaxonomic markers for Chlorophyta members with an average content of 1.26 mg g^{-1} and 0.14 mg g^{-1} dry cell weight (DCW), respectively. Similarly, myxoxanthophyll and echinenone can be used as markers for Cyanophyta members with average contents of 0.23 mg g^{-1} and 0.32 mg g^{-1} DCW, respectively. The total carotenoid content ranged from 0.23 to 7.2 mg g^{-1} DCW. Our method combining PCA and artificial hierarchical clustering has been proposed as an alternative method for identification of carotenoids as biomarkers for classifying unknown microalgal strains based on their pigment profiles.

1. Introduction

Carotenoids are a group of diverse lipophilic pigments with over 600 members that play a central role in light harvesting as well as photoprotection in plants and microorganisms. However, given their diverse and ubiquitous nature, they have been used for many years as important tools for identifying the presence of certain microalgal groups in different aquatic ecosystems all over the world [2].

These microalgal groups in various aquatic habitats have been found to display a fixed pattern of carotenoids during specific growth stage, which is often useful for their identification [16,25]. The constituent pigments of these groups are considered excellent chemotaxonomic biomarkers due to their specificity. HPLC characterization of such pigments can lead to a wealth of information about the taxonomic composition and prevailing physiological conditions [15]. Often, these studies give an indication about the influence of climatic and anthropogenic activities on phytoplankton response on a large geographical area [5].

Several research groups have recorded their observations on the prevailing phytoplankton populations in specific areas based on such pigment profiles. Fietz and Nicklisch [5] studied the phytoplankton population in Lake Baikal using a rapid HPLC and CHEMTAX based method to identify the different groups. A similar strategy was utilized by Madhu et al. [15] for the characterization of phytoplanktonic community structures in Gulf of Mannar and Palk Bay areas. Alternatively, Paerl et al. [20] utilized HPLC analysis followed by photodiode array spectrophotometry to identify areas of eutrophication in coastal areas. The usage of CHEMTAX algorithmic approach has been widespread for these kinds of studies as it utilizes data matrices for calculating the abundance of various algal classes based on the HPLC profiles of their pigments [14].

However, to the best of our knowledge, a statistical approach for identifying carotenoid biomarkers as representatives of specific phytoplankton groups represents a void that can be addressed. Statistical methods utilize a smaller dataset of pigment concentrations to predict representative carotenoid molecules as biomarkers of specific phytoplankton groups in an ecosystem. We have proposed a statistical analysis of the major pigments in 57 different strains of microalgae and cyanobacteria isolated from coastal waters of western India. Hierarchical clustering and principal component analysis (PCA) enabled us to identify certain representative carotenoid molecules by utilizing a far smaller dataset than utilizing CHEMTAX.

2. Materials and methods

2.1. Microalgae identification

57 different microalgal species, belonging to the different phylum (Chlorophyta and Cyanophyta) were isolated from Indian waters. The

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cultures were identified based on their morphological characteristics [10].

2.2. Culture conditions

All microalgae strains were cultured in a 100 ml Erlenmeyer flask with 50 ml of their respective culture medium (Table 1). The cultures were maintained in uniform conditions at 25 ± 2 °C under a light intensity of 60 μmol m^2 s^{-1} and 12:12 h light dark period with manual shaking twice a day. Cultures were harvested after 30 days by centrifugation at 10,000*g* for 5 min. The pellet was lyophilized and stored at −20 °C until further use.

2.3. Extraction of carotenoids

The carotenoids were extracted from pre-weighed lyophilized microalgal biomass in 5 ml of 99.9% pure methanol. The cellular extracts were mixed thoroughly to increase the solvent contact time for maximum carotenoid extraction and were later incubated at 45 °C for 24 h in dark, as described in Pancha et al. [19]. After 24 h, the extract was centrifuged at 10,000*g* for 5 min to remove the cell debris and its

Table 1
Freshwater microalgal isolates with different medium of cultivation and their respective collection sites.

Phylum	Class	Order	Genus species	Location
Chlorophyta	Chlorophyceae	Chlamydomonadales	*Grasiella emersonni* CCNM 1001[a]	Diu
			Grasiella emersonii CCNM 1011[b]	Bhad Road
			Grasiella emersonii CCNM 1015[b]	Bhavnagar
			Chlorococcum sp. CCNM 1023[b]	Kumbarwada
			Chlorococcum sp. CCNM 1025[b]	Gautameshwar
			Chlorococcum sp. CCNM 1027[a]	Bhavnagar
			Desmodesmus subspicatus CCNM 1008[b]	Gautameshwar
			Scenedesmus sp. CCNM 1028[b]	Gautameshwar
			Anikistrodesmus sp. CCNM 1031[b]	Diu
			Monoraphidium minutum CCNM 1042[c]	Hazira
		Sphaeropleales	*Acutodesmus dimorphus* CCNM 1045 [c]	Ankleshwar
			Monoraphidium sp. CCNM 1046[b]	Chennai
			Scenedesmus sp. CCNM1053[b]	Gautameshwar
			Scenedesmus sp. CCNM 1061[a]	Bagdana
			Bracteacoccus pseudominor CCNM 1018[c]	Mithapur
			Chlorella sp. CCNM 1002[a]	Adri road
			Chlorella sp. CCNM 1004[a]	Okha
			Chlorella sp. CCNM1005[a]	–
			Chlorella sp. CCNM 1007[b]	Salt farm, Bhavnagar
			Chlorella sp. CCNM 1014[b]	Bhavnagar
			Chlorella variabilis CCNM 1017[c]	–
			Chlorella sp. CCNM 1019[b]	Diu
			Chlorella sp. CCNM 1021[c]	Okha
	Trebouxiophyceae	Chlorellales	*Chlorella* sp. CCNM 1030[a]	Tadd
			Chlorella sp. CCNM 1036[a]	Bhavnagar
			Chlorella sp. CCNM 1040[a]	Narayan sarovar
			Chlorella sp. CCNM 1043[c]	Ankleshwar
			Chlorella sp. CCNM 1052[b]	Gautameshwar
			Chlorella sp. CCNM 1074[a]	Bagdana
			Micractinium sp. CCNM 1006[b]	Diu
			Micractinium sp. CCNM 1041[c]	Lakpath
			Dictyosphaerium sp. CCNM 1047[b]	Chennai
	Ulvophyceae	Trentepohliales	*Trentepohlia* sp. CCNM 1073[b]	Calcutta
	Eustigmatophyceae	Eustigmatales	*Nannochloropsis* sp. CCNM 1012[b]	Andhra pradesh
Cyanophyta	Cyanophyceae	Pseudanabaenales	*Geitlerinema* CCNM 2010[c]	Mithapur
			Oscillatoria sp. CCNM 2007[b]	–
			Microcoleus sp. CCNM 2011[b]	Thalaja
			Oscillatoria sp. CCNM 2012[b]	–
			Phormidium sp. CCNM 2019[c]	Kodinar
			Phormidium sp. CCNM 2032[b]	Diu
		Oscillatoriales	*Phormidium* sp. CCNM 2034[c]	Saltfarm, Bhavnagar
			Phormidium sp. CCNM 2043[b]	Samakhyali Bridge
			Phormidium sp. CCNM 2046[b]	Hazira
			Phormidium sp. CCNM 2055	–
			Plectonema sp. CCNM 2031[b]	Anand
			Lyngbya sp. CCNM 2050[c]	Bhavnagar
			Anabaena sp. CCNM 2016[b]	–
		Nostocales	*Anabaena* sp. CCNM 2029[b]	Anand
			Nostoc sp. CCNM 2017[b]	–
			Westiellopsis prolific CCNM 2030[b]	Anand
			Synechococcus lividus CCNM 2503[b]	–
			Synechococcus sp. CCNM 2508[b]	Kutch
		Synechococcales	*Synechocystis* sp. CCNM 2513[b]	Okha
			Synechococcus sp. CCNM 2514[b]	Salt farm, Bhavnagar
			Synechococcus sp. CCNM 2519[b]	Porbandar
			Synechocystis pevalekii CCNM 2520[b]	Calcutta
		Chroococcales	*Chroococcus* sp. CCNM 2507[b]	–

[a] Bold's Basal Medium.
[b] BG11 Medium.
[c] Zarrouk's Medium [7].