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Full Length Research Paper

Macromolecular and fatty acid profile studies on symbiotic cyanobacterial isolates of cyanolichens

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The cellular carbohydrate, protein and fatty acid content of three cyanobacterial strains belong to the genera *Aphanocapsa* sp. (NTK28) and *Nostoc* species (NTK29 and NTY30) isolated from cyanolichens analyzed. Among the three cyanobacterial species, *Nostoc* sp. (NTK 29) showed the maximum of total carbohydrate, protein and lipid content about 25, 15 and 14%, respectively. Gas chromatographic analysis showed that, three cyanobacterial isolates has an array of fatty acids. A total of 17 fatty acids both saturated and unsaturated were detected from three cyanobacterial isolates. Among these, 8 types fell under saturated and 9 types unsaturated fatty acids which comprise mono and polyunsaturated fatty acids including ω9, ω6 and ω3 found in the organisms. Single fatty acid stearic acid (C18:0) was commonly present in three cyanobacterial isolates whereas, eicosedienoic acid (20:1) ω9 was present in single cyanobacterium *Nostoc* sp (NTK29).

Key words: Fatty acids, cyanobacteria, carbohydrate, protein, lipids.

INTRODUCTION

Cyanobacteria constitute an ancient group of photosynthetic prokaryotes, which has existed continuously since early evolution of the biosphere (Bhargoorn, 1992). Recent descendents of cyanobacteria thrive in variable aquatic and terrestrial environment, exhibiting adaptive morphological biochemical and metabolic properties (Agarwal and Singh, 2000, 2002). Furthermore, studies on macromolecular, fatty acid and hydrocarbon composition are a useful analytical tool in taxonomy (Rezanka et al., 1982; Welch, 1991; Emblay and Wait, 1994). Moreover, the filamentous cyanobacterium *Spirulina platensis* has also been used as a food for centuries by native peoples from Lake Chad in Africa and Lake Texcoco in Mexico (Vonshak, 1997; Henrikson, 1994), an observation which has led to the use of cyanobacterium Spirulina as a food supplement for undernourished people in many parts of the world (Henrikson, 1994) due to its high protein content (65%), high digestibility (Henrikson, 1994) and specific amino acid content.

Preparations of Spirulina, sold in capsule form or in foods such as beverages and pastes, have been shown to have therapeutic properties in the treatment of conditions such as hypercholesterolemia and atherosclerosis (Ramamoorthy and Premakumari, 1996), pre-menstrual tension and arthritis and as an auxiliary in weight loss (Henrikson, 1994). The cyanobacteria (Spirulina*)* components which are responsible for these therapeutic properties are thought to be compounds with antioxidant abilities, such as polyunsaturated fatty acids, phycocyanin (Estrada et al., 2001) and phenolics (Miranda et al., 1998). Gamma-linolenic acid (C18:3, *ω*6, GLA) and phycocyanin are those which have received most attention from researchers. Phycocyanin was first studied as a food colorant (Sarada et al., 1999), while GLA has mainly been studied in respect to its therapeutic properties, such as its ability to decrease blood cholesterol levels (Ishikawa et al., 1989).

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Cyanolichens contain cyanobacterium as the primary photobiont or in cephalodia. They are sensitive to air pollution but they can fix the atmospheric nitrogen and they can be physiologically activated by moisture and air. They can grow in the shaded places with moisture. Generally, lichens produce a variety of secondary metabolites including some of fatty acids which are unique to lichens. Lichenologists have studied lichen chemistry for the past hundred years and have found over 800 compounds (Müller, 2001) considered as secondary metabolites (Elix, 1996). Polyketide-derived aromatic compounds include: depsides and depsidones, dibenzofurans, anthraquinones, xanthones, and naphthaquinones, esters, terpenes, steroids, terphenylquinones, and pulvinic acid (Fahselt, 1994; Cohen and Towers, 1995; Brunauer and Stocker-Wörgötter, 2005). Cyanobacteria also produce a wide range of peptides and other bioactive compounds and are a rich source of mixed peptide–polyketides (Burja et al., 2001). Twenty metabolites with commercially promising bioactivity, such as anticancer, antibiotic, antifungal, and antiviral activities, including fatty acids were reported from seven Nostoc strains, the genus most common in terrestrial cyanobacterial symbioses. So, the study of biomolecular components is much more important in medical, biotechnological, value addition and taxonomy. Hence, the objective of the research presented in this paper was to evaluate the presence of macromolecular constituents and fatty acid profile of three cyanobacterial species isolated from cyanolichens.

MATERIALS AND METHODS

Collection and identification of cyanolichens

Lichen samples were collected from Yerkaud and Kollihills area of Tamil Nadu, India. Samples were carefully collected with their hold fasts and neatly kept in poly bags and brought to the laboratory. Morphological features were studied using powerful lenses and trinocular zoom dissection microscope (Meiji optics, Japan). Cross sections were made by the use of sharp knives and microtomes in various planes at a thickness up to 15 µm blocks were made using paraffin wax and teased out preparations were also made to examine asci and ascocarp for some samples. K, C, KC and P tests (Santesson, 1973), which are important for identification of lichens and cyanolichens, were made. Microcrystal tests (Awasthi, 1975) were also carried out for some specific samples.

Isolation and identification of cyanobacteria

The lichen thalli were washed in a washing chamber (Renner, 1982) for about 30 min. Sections of 30 to 40 µm thickness were cut with a microtome, placed in 6 cm Petri dishes on BG11 medium containing 1.5% agar (Waterbury and Stanier, 1978; Boissiere, 1987) and incubated at 20°C under continuous light at 2,000 lux (Osram, universal white, fluorescent light, 40 W). After 6 to 8 weeks, the first free colonies of the cyanobiont were observed within the disintegrating thallus sections. They were transferred to fresh agar plates and incubated under the same conditions. Colonies of the phycobiont was harvested after approximately 4 months.

Cyanobacterial isolates were identified morphology (presence or absence of sheath, apical region and colonial forms and etc.,) size (micrometric analysis for measuring the size), structure and etc., using the taxonomic publications of Geitler (1932), Desikachary (1959) and Starmach (1966).

Preparation of biomass

BG11 medium was used for cultivation of symbiotic cyanobacteria (Rippka et al., 1979). Culture medium was provided with proper light (2000 lux) source. After 15 to 20 days of incubation period, homogenous culture was obtained and used further for biochemical studies.

Estimation of carbohydrate

Finely ground cyanobacterial sample (0.2 g) was taken with 20 ml of hot 80% alcohol, mixed well for 5 to 10 min and was centrifuged at 3000 rpm for 10min the supernatant was decanted. After cooling, 6.5 ml of 52% perchloric acid was added stirred with a glass rod and kept for 15 min prior to centrifugation at 4°C. Supernatant was collected and made up to a volume of 100 ml with water. From this 5 ml of aliquot (extract), was taken and to this 10 ml of freshly prepared cool anthrone reagent was added. It was transferred to boiling water bath for 7.5 min. After cooling the absorbance was read at 630 nm. The amount of sugar in the solution was calculated using standard sugar curve (Carroll et al., 1956).

Estimation of protein

Homogenized cyanobacteria were centrifuged at 5000 rpm for 10 min and the pellet was washed twice in distilled water. To the pellet 5 ml of 10% TCA was added and left for half an hour in boiling water bath. Contents were cooled and centrifuged at 5, 000 rpm for 5 min. The resulting pellet was dissolved in 1 ml of 1 N NaOH. From this 0.1 ml was taken and made up to 1 ml with distilled water. To this 5 ml of alkaline reagent was added and incubated for 3 min. To this 0.5 ml of Folin Ciocalteu reagent was added and mixed thoroughly and allowed to stand for 30 min. The absorbance was read at 750 nm in a spectrophotometer (Lowry et al., 1951).

Estimation of total lipids

5 g of substance (dry weight) was dissolved in 5 ml of chloroform: methanol mixture (2:1 v/v) and transferred into an airtight glass stoppered iodometric flask. The content of the flask was shaken well and filtered. The extraction procedure was repeated twice or till the colour was completely removed. The solvent was removed from the residue by distillation under vacuum. The crude lipids were re-dissolved in 10 ml of chloroform: methanol mixture containing 1 ml of 1% sodium chloride. The sample was transferred into the separating funnel, and thoroughly shaken. Lipid was recovered in the lower chloroform layer while soap, glycerol and other water insoluble impurities moved into the upper layer. Lower lipid layer was collected and lipid content was estimated. The sample was further subjected to the sample used for gas chromatographic analysis to find the fatty acid profile (Folch et al., 1957).

RESULTS AND DISCUSSION

Identification of cyanolichens and cyanobacteria

Collema auriforme (With.) Coppins and J.R. Laundon.

Thallus isidiate, granular, globular simple, distinctly foliose and wide lobed, thallus striate, riged, wrinkled, corticolous, dark olive green lobes 200 to 500 μm thick.

Collema rugosum Kremp.
Thallus isidiate, globular, terriform simple, fenestrate, sub fruticose distinctly foliose, ridged or pustulate, irregularly arranged coarse ridges blackish green lobes up to 15 mm wide 40 to 150 μm thick scleroplctenchymatous cortex.

Leptogium javanicum Mont.
Thallus lacking tomentam of multicellular hypal hairs, rarely unicellular hyphae, muriform, apothecia lacking isidia lobes not anastomosing, grey spores 16 to 30 thalline exciple vertically wrinkled and lobed.

Cyanobacteria
Nostoc sp. (Kutz.) Hariot (NTK 28)
Thallus sub-globose up to 2 mm diameter, scattered sheath delicate, hyaline, mucous; trichome 3 to 4μ broad, cells short barrel shaped or ellipsoidal, spores sub spherical, or oblong, 5 to 6μ broad and long (symbiotic form).

Nostoc sp. Vaucher ex. Born.et. flah. (NTK 29)
Thallus firm, gelatinous, globose, laterally flattened, expanding, undulated, membranous or leathery irregularly torn, entangled; sheath mostly distinct only at periphery, thick, trichome 4.5 to 6 μ broad, cells short barrel – shaped or nearly spherical, heterocyst nearly spherical, about 7 μ broad (symbiotic form).

Aphanocapsa Nag. (NTK 27)
Cells spherical or nearly so, many loosely arranged without an order, forming a formless gelatinous mass, often few centimeter (cm) in diameter, mucilage homogeneous colourless, cells often with thin gelatinized individual sheaths, divisions in two directions. This genus has three species represented on lichen species. *Aphanocapsa* sp. (NTK28), *Nostoc* sp. (NTK29) and *Nostoc* sp. (NTY30) were subjected to find macromolecular constituents including carbohydrate, protein and fatty acid analysis and results are shown in (Table 1). Protein content is more in these three isolates 24, 26 and 23%. The total carbohydrate content is 15, 17 and 14%; total lipid content is 1, 2 and 1.2%, respectively with NTK28, NTK29 and NTY30 (Figure 1). Totally, 17 fatty acids both saturated and unsaturated were detected in the tested cyanobacteria. Of these, 8 were saturated fatty acids and 9 were unsaturated fatty acids. Among 17 fatty acids, only two fatty acids that is, stearic and palmitoleic acids were commonly present in all the three cyanobacteria. Three types of fatty acids namely tridecanoic, myristoleic and linolenic acids are common with *Nostoc* sp. (NTK29) and *Nostoc* sp. (NTY30). Four types of fatty acids such as oleic, linolenic, eicosapentaenoic and γ-Linolenic acids are commonly present in *Aphanocapsa* sp. (NTK28) and *Nostoc* sp. (NTK29). The unsaturated fatty acids are belonging to mono, and omega polyunsaturated group and has ω9, ω6, ω3 type of fatty acids. Blue-green algae have a prokaryotic cell structure, a property that they share with bacteria. Reportedly, the major cellular lipids of the blue-green algae include the three glycolipids characteristic of the chloroplast: monogalactosydiglyceride, digalactosyl-diglyceride and sulfoquinovosyldiglyceride (Stransky and Hager, 1970). The chloroplast fatty acids of eukaryotic algae and vascular plants are largely polyenoic acids, of which α-linolenic acid almost exclusively found in chloroplasts, is a major constituent in the leaf tissues of higher plants and in certain groups of algae (Stransky and Hager, 1970).

The plasma and thylakoid membranes in cyanobacteria, responsible inter alia for absorption of light energy, electron transfer and ATP synthesis, function similarly to the chloroplasts in eukaryotic algae (Gombos et al., 1999; Sato and Murata, 1988). Although, the major lipid classes in cyanobacteria and photosynthetic eukaryotes are quite similar, fatty acid profiles were demonstrated by ample evidence to be affected by physicochemical factors and showed great heterogeneity even in different strains of the same species (Caudales and Wells, 1992; Rathore et al., 1993). Fatty acid compositions have been shown to affect the susceptibility of the cells to low temperature stress. Alteration of the glycerolipids composition and degree of fatty acid unsaturation may lead to consequent changes in physiological metabolisms and adaptation to environmental conditions such as temperature and irradiance. Changes in the profile and proportions of fatty acids are considered a key process in temperature acclimation in thermophilic organisms. From an ecological point of view, the relationship between temperature and fatty acid profile could partially reflect the distribution and interaction of the organism with environmental factors (Kiseleva et al., 1999). Thus, studies on fatty acid compositions of cyanobacteria are also of significant importance in elucidating their adaptability to the environment.

In this present investigation, 17 different saturated and unsaturated fatty acids were found in three different cyanobacterial isolates. Two type of fatty acids namely stearic and palmitoleic acids commonly present in all these three isolates such as *Aphanocapsa* sp.(NTK28), *Nostoc* sp. (NTK29) and *Nostoc* sp. (NTY30). A higher number of fatty acid was present in *Nostoc* sp. (NTK29) (Table 1). Four types of fatty acids namely oleic, linolenic, eicosapentaenoic, γ-linolenic acid commonly present in *Aphanocapsa* sp. (NTK28) and *Nostoc* sp. (NTK29) two other types of unsaturated fatty acids and one saturated fatty acid are commonly seen in *Nostoc* sp. (NTK29) and *Nostoc* sp. (NTY30). Kenyon and Stainer (1970) reported that, the bacterial type of fatty acid composition is relatively common among unicellular blue-green algae,

Table 1. Fatty acid profile of symbiotic cyanobacterial isolates.

S/N	Name of the fatty acid	*Aphanocapsa* sp. (NTK28)	*Nostoc* sp. (NTK29)	*Nostoc* sp. (NTY30)
Saturated fatty acids				
1.	Tridecanoic acid (C13:0)	-	0.3659	0.1447
2.	Pentadecanoic acid (C15:0)	-	-	0.0557
3.	Heptadecanoic acid (C17:0)	-	-	0.0199
4.	Stearic acid (C18:0)	0.0292	0.1105	0.1241
5.	Non-decanoic acid (C19:0)	0.1036	-	-
6.	Heneicosenic acid (C21:1)	-	0.0099	-
7.	Myristoleic acid (C14:1)	-	0.0249	0.0440
8.	Plamitoleic acid (C16:1)	0.3075	0.7308	0.5114
Unsaturated fatty acids				
9.	Elaidic acid (C18:1)	-	-	0.0621
10.	Oleic acid (C18:1)	0.0179	0.1067	-
11.	Linodelaidic acid (C18:1)	-	0.0239	0.0271
12.	Linolenic acid (C18:2)	0.0034	0.0546	-
13.	Linoleic acid (C18:2)	0.0207	-	0.1414
14.	Eicosenoic acid (C20:1)	0.0055	-	-
15.	Eicosedienoic acid	-	0.0094	-
16.	Eicosapentaenoic acid (C20:5)	0.0030	0.0036	-
17.	γ Linolenic acid (18:3)	0.0148	0.0263	-

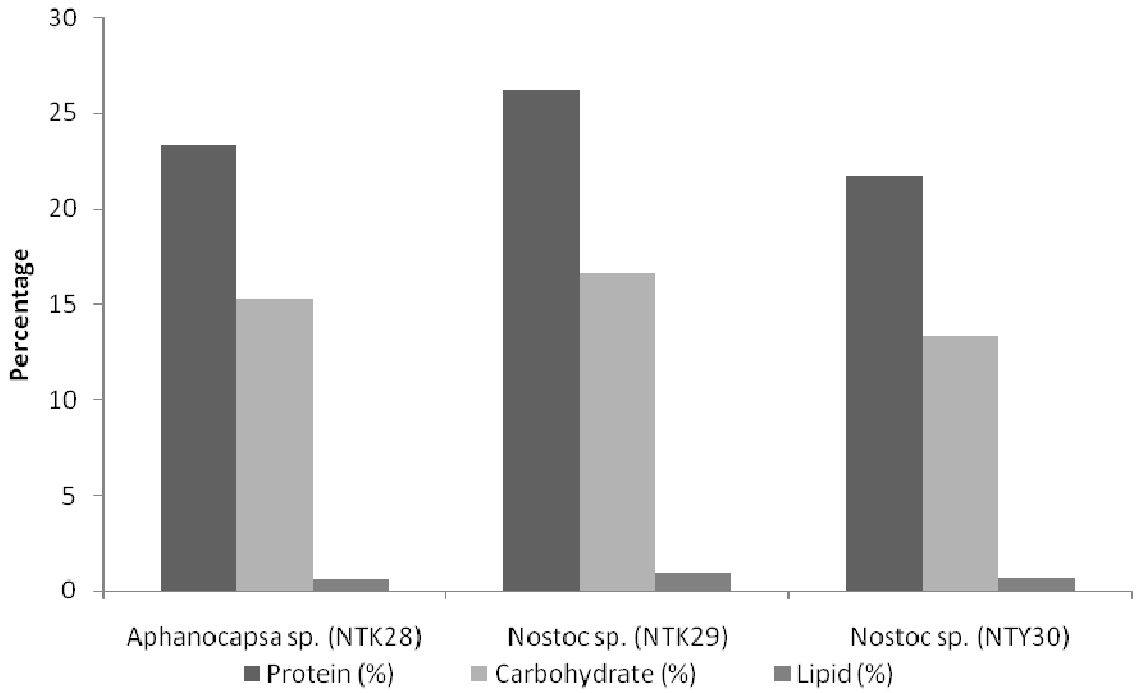

Figure 1. Macromolecular constituent in cyanobacteria.

whereas the presence of large quantities of polyenoic fatty acids is characteristic of most filamentous blue-green algae. Based on the compositional variation of fatty acids, 34 strains of unicellular blue-green algae have been characterized (Kenyon, 1972). Fatty acid composition of filamentous strain was analysed by Kenyon et al.

(1972), in which all the filamentous strains except two contained a relatively large amount of polyunsaturated fatty acids (25 to 60%). It is notable that significant quantities of γ-linolenic acid occur rarely among the filamentous cyanobacteria, so far analysed.

In contrast, γ-linolenic acid was the predominant polyunsaturated fatty acid in unicellular blue-green algae. Similarly, present investigation revealed the presence of a higher number of unsaturated fatty acids found in filamentous symbiotic cyanobacteria *Nostoc* sp. (NTK29) compared to the other filamentous *Nostoc* sp. (NTY30) and unicellular form *Aphanocapsa* sp. (NTK28). γ-linolenic acid is commonly present in *Aphanocapsa* sp. (NTK28) and *Nostoc* sp. (NTK29) whereas the same fatty acids are not found in *Nostoc* sp. (NTY30). The time course of fatty acid accumulation in *Nostoc* species has never been challenged, although the phenomenon of cell differentiation and colony alteration of these organisms in liquid suspension culture has been confirmed (Potts, 2000; Liu and Chen, 2003).

Yap and Chen (2001) suggested that, when the algae were grown under unfavourable conditions, such as nutrient depletion and temperature stress, synthesised carbon in photosynthesis tended to be accumulated in the neutral lipids, which were supposed to contain a high proportion of the total monounsaturated and polyunsaturated fatty acids.

Similarly, the occurrence of n- saturated, branched and unsaturated fatty acids was also reported by Rezanka et al. (2003a, b) from three wild terrestrial strains of the genus Chroococcidiopsis (Order: Chroococcales): *Chroococcidiopsis surpralittoralis, Chroococcidiopsis umbratilis and Chroococcidiopsis versatilis* collected from Lake Kinneret, Dead Sea and Ein Kerem (Jerusalem). The cellular fatty acid composition of five of the six genera of unicellular cyanobacteria in Pleurocapsales (Dermocarpa, Xenococcus, Dermocarpella, Myxosarcina and the Pleurocapsa) was examined which contained high proportions of saturated straight-chain fatty acids (26 to 41% of the total) and unsaturated straight chains (40 to 67%). Isomers of 16:1 were the main mono-saturated acid components (11 to 59%). Polyunsaturated acids were present at trace levels (0 to 1%) in Xenococcus and Myxosarcina, at concentrations of less than 7% in Dermocarpa, Dermocarpella, Pleurocapsa and CCMP1489 and high concentrations (35% or more) in Chroococcidiopsis (Caudales et al., 2000). The fatty acid composition of cyanobacterial species have been influenced by a number of factors, thus this kind of studies would help to identify variations, value additions and medical applications.

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