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MORPHOLOGICAL AND BIOCHEMICAL ASSESSMENT OF SEVEN CYANOBACTERIAL SPECIES ISOLATED FROM MANGROVE HABITAT ALONG COASTAL WATERS OF KARACHI, PAKISTAN

AFIFA SARWAR[1], SAIRA BANO[1], MUNAWWER RASHEED[1], PIRZADA J.A. SIDDIQUI[1], SEEMA SHAFIQUE[1] AND ZAIB-UN-NISA BURHAN[1]*

[1]Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan
**Corresponding author Email: zaib.burhan@uok.edu.pk*

Abstract

Cyanobacteria contribute significantly by producing high quality nutrition which could be utilized through biotechnological applications to combat health challenges. This research aimed to identify the cyanobacterial isolates on the basis of morphological features and explore their capacity as a natural resource through comprehensive assessment of biochemical constituents. In the present investigation cyanobacterial species were isolated from microbial consortium proliferated on sediment surface in mangrove habitats at Sandspit and Korangi, along the Karachi coast. A total of seven cyanobacterial isolates were grown in an Artificial Seawater Nutrient (ASN) medium. The isolated species representing filamentous form, belonged to 2 orders and 3 families. The identified species *Leptolyngbya tenuis*, *Leptolyngbya* sp. A, *Leptolyngbya* sp. B, *Limnolyngbya circumcreta*, *Stenomitos frigidus* were affiliated to family Leptolyngbyaceae of the order Leptolyngbyales. Whereas, *Oxynema thaianum* and *Phormidium breve* under the order Oscillatoriales represented family Microcoleaceae and Oscillatoriaceae respectively. The isolates, *Limnolyngbya circumcreta* and *Stenomitos frigidus* are reported for the first time from the Karachi coast, Pakistan. These seven species were analyzed for three major biomolecules namely carbohydrates, proteins and lipids. Overall, total carbohydrates, proteins and lipids constituent were in the range of 6.4-31%, 2.1-6.3% and 2.4-20.7% of dry weight biomass respectively. The highest value of carbohydrate was recorded in *Limnolyngbya circumcreta* (31%). The maximum protein was observed in *Leptolyngbya* sp. A (6.3%). Whereas, high lipid content was found in *Phormidium breve* (20.7%). The findings revealed that the carbohydrates of all these species were detected higher than proteins and lipids. Owing to their potential, it can be speculated that isolated cyanobacterial species could be useful as alternative sources for sustainable feed, food, biofuel and other bioproducts.

Key words: Cyanobacteria, Isolation, Identification, Culture, Carbohydrates, Proteins, Lipids, Microbial mats, Mangroves.

Introduction

Cyanobacteria evolved to possess remarkable capabilities of oxygenic photosynthesis and nitrogen fixation. These cyanobacteria are the only prokaryotes that can fix atmospheric carbon dioxide and molecular nitrogen thus by take-up of inorganic matter and transform into biomass and production of valuable biomolecules, they contribute significantly in aquatic environments (Soo *et al*., 2017; Udayan *et al*., 2017). Marine cyanobacteria are highly versatile and fully adaptable inhabiting almost all ecosystems along with their ability to survive in stressful and harsh environmental conditions (Gaysina *et al*., 2019). They are morphologically diverse and are mostly unicellular and filamentous forms with different levels of complexity, including branched and unbranched types (Herrero *et al*., 2016; Rippka *et al*., 1979). This morphological plasticity creates difficulties, in the cyanobacterial identification (Komárek, 2006). The taxonomy of cyanobacteria has traditionally been based on the morphological features of the species; however, in recent decades, with the active development of molecular biology methods, the systematic position of cyanobacteria has been revised. On the basis of morphological, biochemical, molecular, and phylogenetic studies of Phylum cyanobacteria is divided to 8 orders: Chroococcidiopsidales, Chroococcales, Gloeobacteriales, Nostocales, Oscillatoriales, Pleurocapsales, Synechococcales and Spirulinales (Komárek *et al*., 2014). Cyanobacteria has tendency to show different morphological features under changed environmental conditions. These morphological variations can be due to fluctuation in light, temperature, and nutrients concentrations (Zepernick *et al*., 2023).

Numerous studies have documented the importance of cyanobacteria as a source of valuable bioproducts (Zahra *et al*., 2020). Scientific community has paid attention towards promising role of cyanobacteria and applicability in various fields for example agriculture, aquaculture, food, feed, bio-fertilizers biofuels, biopolymer, and secondary metabolites (Demay *et al*., 2019; Khalifa *et al*., 2021). Several products produced by cyanobacteria are being used as sunscreens, antimicrobial, anti-inflammatory, anticancer, immunosuppressant (Shahid *et al*., 2024).

Cyanobacteria are emerged as good source of supplementary food for high levels of valuable constituents and to its nutritional quality (Andrade *et al*., 2018). In recent years they have been testified extensively for their great potential for high quality productivity, high biomass production and highest capability to grow in hampering conditions (Norena-Caro & Benton 2018). They have been proven as rich source of carbohydrates, protein, fats, minerals, vitamins and other beneficial biologically active components (Arias *et al*., 2021). Carbohydrates are a fundamental product of carbon fixation during photosynthesis which serves different functions as energy reserve depending on their cellular location and their structural characteristics (Usov & Zelinsky, 2013). Moreover, cyanobacteria are also efficient in exopolysaccharides production, the abundant carbohydrates higher than in eukaryotic microalgae (Pierre *et al*., 2019). In addition cyanobacteria are also rich in protein content and along with protein they are considered as a good source of essential amino acids. Cyanobacterial proteins are not only important as dietary source but also a good source of bioactive peptides which

can be employed for therapeutic purposes (Ferrazzano *et al*., 2020). Many cyanobacteria carry high value of protein and other nutritional components. For example *Spirulina* is one of the genera of cyanobacteria which has been used as supplement and provides 60 to 70 percent plant protein (Gogna *et al*., 2023). Furthermore, cyanobacteria are also valuable source of variety of lipids with high lipid content (Rajeshwari & Rajashekhar, 2011; Uma *et al*., 2020). They are also considered as valuable candidate for biofuel production (Sarsekeyeva *et al*., 2015). Cyanobacteria are rich and promising source of lipids and poly-unsaturated fatty acids and essential fatty acids and are very important source for pharmaceutical industry (Anahas & Muralitharan, 2018). Thus due to their great capability of photosynthesis and fast growth even in low nutrients, cyanobacteria can be utilized to produce enriched carbohydrates, proteins and lipids as super food, feed and renewable sources of biofuels and many other possible uses with respect to future perspective.

Coastal waters of Karachi has a dynamic coastal belt, occupied with most productive mangrove ecosystem in pockets with subtropical climatic conditions prevailing both South West and North East monsoons season that can generate new nutrients (Siddiqui *et al*., 2008). Mangrove habitat serves as niche for several inhabitants including microorganisms (Thatoi *et al*., 2013). These mangroves support highly diversified groups of all microbes in water channels, several parts of plant and sediment surfaces. They support the occurrence of various morphotypes of cyanobacteria that inhabit several niches as epiphytic and epileptic in the form of microbial mats consisting of microbial consortia (Alvarenga *et al*., 2015). In spite of ecological importance, high nutritional value and great biotechnological potential and their capabilities for bioremediation, inadequately addressed (Bano *et al*., 2021a, 2021b; Zymanczyk-Duda *et al*., 2022). Very limited and comprehensive information is available on their isolation, identification and cultivation from mangrove ecosystem of Pakistan (Zaib-un-Nisa *et al*., 2000; Ahmed *et al*., 2016; Mansoor *et al*., 2023) Owing to the importance of cyanobacteria the present study was undertaken to isolate, characterize and elucidate biochemical constituents of cultivable cyanobacteria from mangrove habitat. These findings will be helpful to understand their potential as valuable resources which can be used for valuable bioproducts in future.

Material and Methods

Sampling sites and sample collection: Sampling was carried out in mangrove habitat of two different locations, Sandspit (24º83'04" N and 66º92'82" E) and Korangi creek (24º78'99" N, 67º23'83" E) along the coastline of Karachi, Pakistan (Fig. 1).

Samples consisted of microbial consortium forming microbial mats of different colour on sediment surface in rhizopsheric region of mangroves. It is exposed during low tide and fully covered with seawater throughout high tide. The samples were collected through sterilized glass slides, placed in plastic bags with a small amount of seawater, kept in an ice box and transported to the laboratory for further process of isolation and identification.

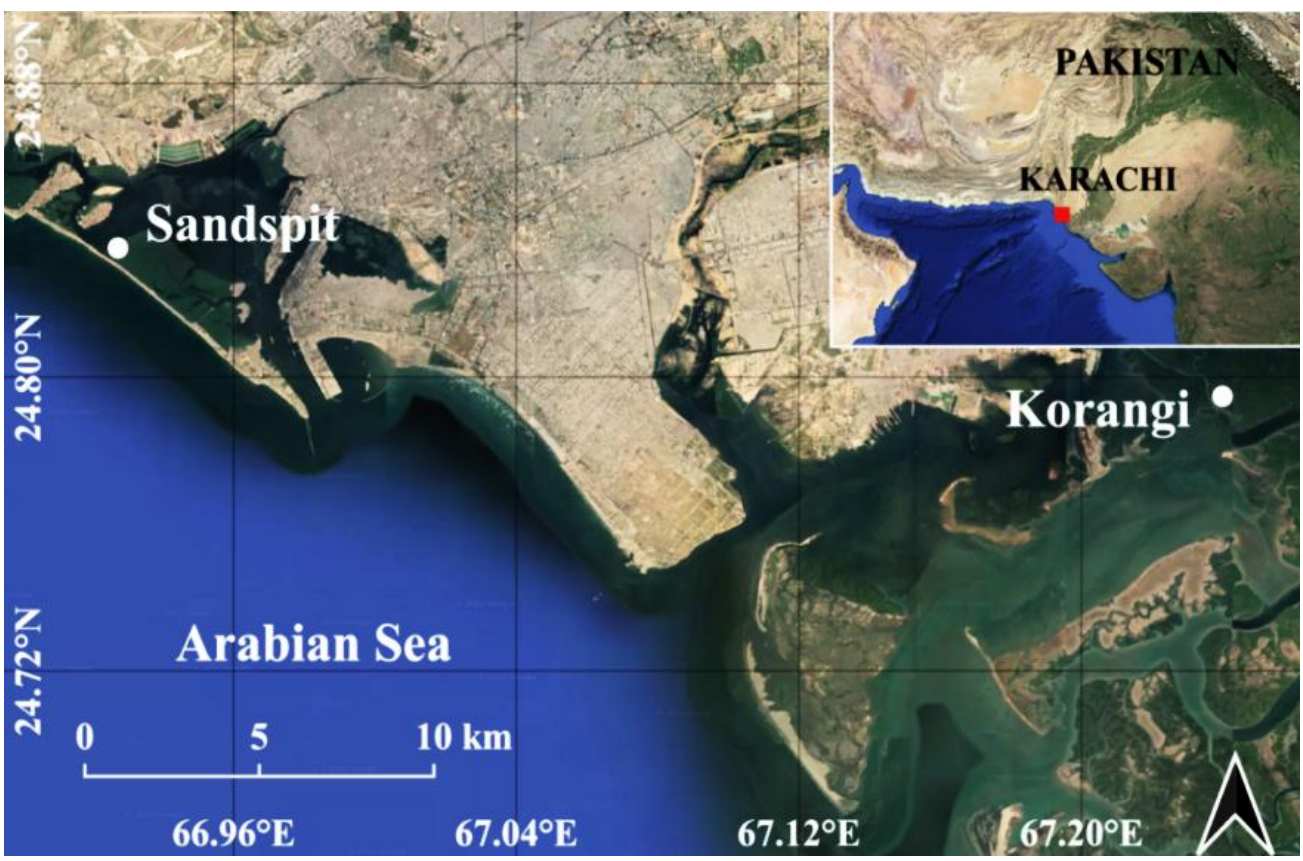

Fig. 1. Map of the study site showing two sampling locations in mangrove ecosystem situated at Sandspit and Korangi creek along Karachi coast.

Isolation and culturing of cyanobacteria: To acclimatize, freshly collected samples were initially introduced in the sterilized seawater collected from same habitat for two weeks. Samples were then inoculated in slightly modified Artificial Seawater Nutrients (ASN III) medium (Rippka *et al*., 1979) by adding extra amount of sodium chloride to increase salinity up to ~37-40 PSU (ambient environment). For isolation of cyanobacterial species, multiple techniques were used including serial dilution (Stainer *et al*., 1971), Pasteur capillary pipette method in liquid medium and streaking plate technique based on solidified medium (Preisig & Andersen, 2005). All the isolated cultures were regularly repeated sub-culturing after every two weeks and maintained at 28±2ºC and kept in an illuminated incubator under controlled white light (~40 µmol $s^{-1}m^{-2}$) with 8 hour light/16 hour dark cycle. Maintenance of stock cultures was performed on daily basis for even distribution of media and to avoid dense aggregates through manual shaking of Erlenmeyer flasks.

Morphological characterization and Identification: For identification, all isolated species of cyanobacteria were observed at 40x and 100x magnifications using light microscope (Olympus, IX-51). The relevant morphological features were recorded and photomicrographs were taken by using a digital camera (Olympus, DP-20) equipped with Cell-imaging software. In the present study traditional method based on morphometric measurements and morphological features was considered for the identification up to the species level in accordance with taxonomic revision by Komárek *et al*., (2014)) and also verified from data base Cyano DB (Hauer & Komárek, 2022) for the current taxonomic status.

Establishment of mass culturing of cyanobacteria: For mass culturing, isolated cyanobacterial species, once obtained after several transfers and grown by maintaining in Erlenmeyer flask (250 ml) containing 100 ml of liquid medium were then harvested for mass culture in Erlenmeyer flask (5000 ml) containing 3000 ml of medium under same conditions as described above.

Biochemical assessment: Biochemical analyses of seven isolated cyanobacterial species were determined for the estimation of major constituents namely carbohydrates, proteins and lipids by standard biochemical methods. All measurements were repeated in triplicate and incorporated as mean value ± S.D.

Total carbohydrates: Total carbohydrates were determined following Dubois *et al*., (1956). For extraction, three aliquots of 100 mg dried and pre-weighed samples were hydrolyzed with 2.5N hydrochloric acid at 100ºC in a water bath for 1h.The extracts were filtered in volumetric flasks (100 ml) and diluted with distilled water up to the 100 ml. Known volume (0.5, 01 and 2 ml) of extracts were taken in glass test tubes and diluted to make final volume of 2 ml and 1 ml of 5% phenol and 5 ml of concentrated sulphuric acid was added. The test tubes were vortexed at 2500 rpm for 20 minutes. The samples were then analyzed on UV-visible spectrophotometer at 480 and 490 nm against the blank. The amount of total carbohydrate present in the sample was calculated using the standard curve obtained from standard glucose solution (concentration from 10 to 100 µg ml^{-1}).

Total proteins: The total proteins were estimated according to Lowry *et al*., (1951). For total protein analysis, dried and pre-weighed (100 mg) triplicate samples of cyanobacterial species were soaked with 10 ml of distilled water. Proteins were extracted by using 0.5% β-mercaptoethanol. Total proteins were precipitated by adding ammonium sulphate until it precipitates but not more than 6.3 gm. bovine serum albumin was used as standard stock solution (Concentration range from 10 to 100 µg or mg ml^{-1}).

Total lipids: To estimate total lipid content in cyanobacterial species, Folch *et al*., (1957) method was applied. Dry weight (100 mg) of samples of cyanobacterial species were then extracted with chloroform-methanol solvent (2:1 [v/v]). The filtrate was transferred to pre-weighed vials for drying. The total lipid was calculated by taking the difference between the initial and final weight and percentage was taken as total lipid content in dried biomass.

Results

Isolation, culturing and characterization of marine cyanobacteria: Seven different filamentous species were isolated from Sandspit and Korangi sites of Karachi coast during winter season; however the cultures growth was fast during summer season. Among seven species of cyanobacteria, six were successfully isolated through serial dilution technique on ASN III medium and only one species i.e., *Oxynema thaianum* was isolated by Pasteur capillary pipette method on same medium. In addition these species were also purified through repeated streaking on solidified agar based ASN III medium. These out of 7 taxa, 5 were identified at species level and 2 were identified at genus level on the basis of their morphological characterization (Table 1).

Morphological characterization of cyanobacteria: A total of seven cultivable cyanobacterial taxa were successfully isolated from the live samples collected from the field as shown in (Table 1).

These taxa were distributed in the orders Leptolyngbyales (5) and Oscillatoriales (2). Among them family Leptolyngbyaceae was lead with 3 genera and 5 species. The genus Leptolyngbya was represented by 3 species (*Leptolyngbya tenuis* (Gomont) Anagnostidis & Komarek, *Leptolyngbya* sp. A and *Leptolyngbya* sp. B) and remaining 2 *Limnolyngbya circumcreta* (G.S.West) X.Li & R.Liand *Stenomitos frigidus* (F.E. Fritsch) Miscoe & J.R. Johansen were the first recorded for Pakistan. From Microcoleaceae and Oscillatoriaceae families *Oxynema thaianum* Chatchawan, Komarek, Strunecky, Smarda & Peerapornpisal and *Phormidium breve* (Kutzing ex Gomont) Anagnostidis & Komarek were the isolated taxa respectively. Detailed morphometric observations and descriptions of isolated species of cyanobacteria are given below. Average and standard deviations for the different sized cell's length and width were calculated (n = 5).

Table 1. List of seven isolated species of cyanobacteria.

Cyanobacterial taxa		
Order	**Family**	**Genus**
Leptolyngbyales	Leptolyngbyaceae	*Leptolyngbya tenuis* (Gomont) Anagnostidis & Komárek
		Leptolyngbya sp. A
		Leptolyngbya sp. B
		Limnolyngbya circumcreta (G.S.West) X.Li & R.Li
		Stenomitos frigidus (F.E.Fritsch) Miscoe & J.R.Johansen
Oscillatoriales	Microcoleaceae	*Oxynema thaianum* Chatchawan, Komárek, Strunecky, Smarda & Peerapornpisal
	Oscillatoriaceae	*Phormidium breve* (Kützing ex Gomont) Anagnostidis & Komárek

Order Leptolyngbyales
Family Leptolyngbyaceae

***Leptolyngbya tenuis* (Gomont) Anagnostidis & Komarek (Fig. 2a-2c):** Thallus was dark blue green in appearance. Mat forming filaments, blue green in colour. Trichomes cylindrical, entangled with each other to form dense colonies. Trichomes slightly constricted at the cross-walls. Granules present at cross-wall of the cell. Cells longer then wider, 3.544±0.259 µm long and 2.538±0.202 µm broad. Apical cell was rounded without calyptra. A very thin, hyaline and colorless sheath was present around the trichomes. Movement was oscillating. Disintegration in trichomes was noticed through fragmentation.

***Leptolyngbya* sp. A (Fig. 2d-2f):** Thallus dark blue green in appearance showing aggregated and colonies densely entangled with each other. Trichomes were also dark blue green in colour, cylindrical in shape and found mostly in paralleled arrangement. Trichomes were not constricted at the cross-walls. Cells longer then wide, 4.664±0.639 µm long and 3.04±0.122 µm broad. Apical cell was rounded without calyptra. Very thin and hyaline sheath was present. No movement was observed. Disintegration in trichomes was through fragmentation.

***Leptolyngbya* sp. B (Fig. 2g-2h):** Dens mat forming colonies, thallus dark green attached with substrate with mucilaginous structure. Filaments blue green in color,. Trichomes were not constricted at the cross-walls. Cells were cylindrical and longer then wider, 5.76±0.899 µm long and 2.538±0.202 µm broad. Granules were present, yellowish in colour and scattered in cytoplasm. Sheath absent. Creeping movement was observed. Filaments were fragmented through necrosis without any separation disc.

***Limnolyngbya circumcreta* (G.S. West) X. Li & R. Li (Fig. 3a-3c):** Thallus was dark blue green in appearance with densely aggregated filaments. Filaments were also dark blue green in colour, forming spirally coiled colonies. Trichomes were cylindrical found mostly parallel to each other. Cells longer then wider, 5.76±0.899 µm long and 2.538±0.202 µm broad, deeply constricted at the cross-wall. Apical cells were mostly rounded and without calyptra, few of them were attenuated and tapered towards the end during movement. Thin and hyaline sheath was present. Movement was gliding. Trichomes were fragmented through hormogonia.

***Stenomitos frigidus* (F.E. Fritsch) Miscoe & J.R. Johansen (Fig. 3d-3f):** Thallus light parrot green in appearance showing dense colonies. Filaments light green in colour. Trichomes straight mostly entangled with each other. Cells were almost isodiametric cell 1.17± 0.059 µm long and 0.804±0.356 µm broad, deeply constricted at the cross-walls. Apical cell was rounded without calyptra. Thylakoids were arranged in the peripheral region and parallel to the cell wall. Sheath was very thin and diffluent. No movement was observed. Fragmentation of trichomes was noticed.

Order Oscillatoriales
Family Microcoleaceae

***Oxynema thaianum* Chatchawan, Komarek, Strunecky, Smarda & Peerapornpisal, (Fig. 4a-4c):** Thallus was olive green in appearance. Filaments were also olive green to pale green in colour. Filaments form dense colonies. Trichomes were cylindrical found parallel to each other. Cells shorter then wide, 3.622±0.328 µm long and 4.808±0.254 µm broad. Trichomes slightly constricted and granulated at the cross-wall. Apical cell was rounded, sometime taper and narrowed, slightly attenuated at the end, terminal cell elongated and bent, without calyptra. Sheath was absent. Movement was gliding. Disintegration of trichomes was observed through necrosis.

Family Oscillatoriaceae

***Phormidium breve* (Kutzing ex Gomont) Anagnostidis & Komarek (Fig. 4d-4f):** Thallus thinly mat forming, switches mode of color from pale yellow to brownish and sometime green. Filaments were light green in colour. Trichomes very slightly constricted at cross wall. Cells shorter then wide, 4.754±0.6215 µm long and width was 4.986±0.284 µm broad. Apical cell tapered and pointed but some time broadly rounded. Thin and translucent sheath was present. Gliding movement was observed with smooth jerking of attenuated cells.

Biochemical Assessment

Total carbohydrates, total proteins and total lipids: Three biochemical constituents including carbohydrate content, protein content and lipid content were analyzed in all 7 isolated species of cyanobacteria (Table 2).

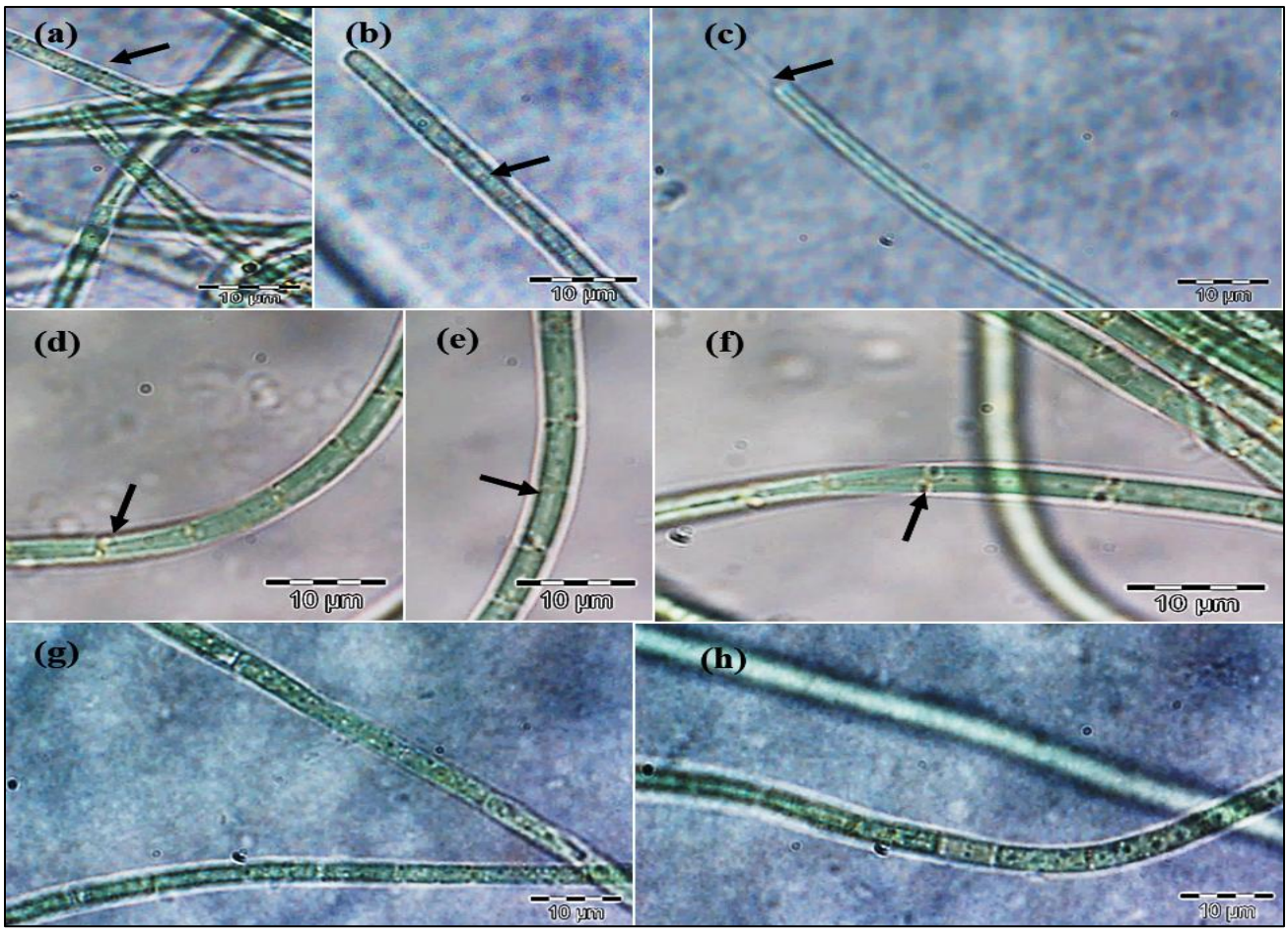

Fig. 2. Micrographs of (a) *Leptolyngbya tenuis,* aggregates of filaments showing presence of granules in the cells (b) trichome with rounded apical cell and constriction at the cross-walls (c) thin hyaline and colour-less sheath (d) *Leptolyngbya* sp. A, presence of aerotops (gas vesicles) at the cross-wall of filament (e) filament with slight constriction(f) filament showing parallel arrangement to each other and presence of granules on each side at the cross-wall. (g) *Leptolyngbya* sp. B, solitary filaments with slight constriction at the cross-walls (h) occurrence of necrosis without any separation disc in the filament. All described features are shown with black arrows.

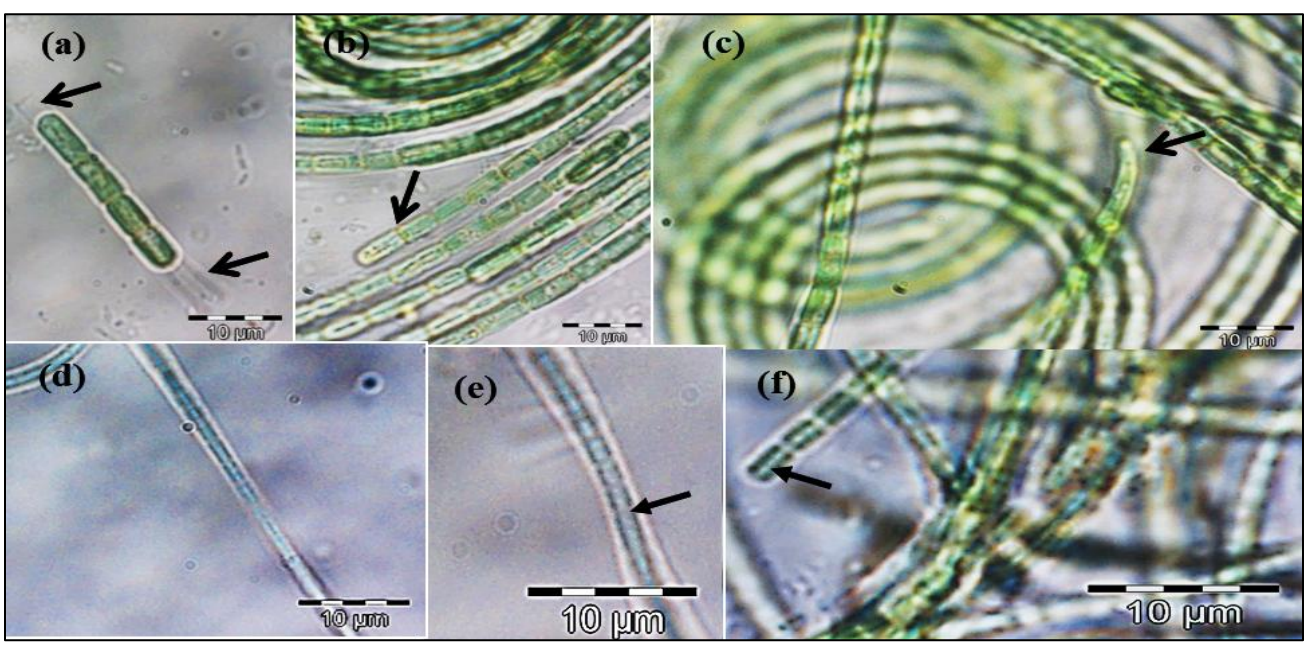

Fig. 3. Micrographs of (a) *Limnolyngbya circumcreta,* transparent sheath visible on both sides of hormogonium (b) filament showing apical cell rounded and deep constrictions at cross-walls (c) filaments aggregated parallel to each other and coiled, apical cell narrowly tapered and pointed. (d) *Stenomitos frigidus*, showing solitary filaments (e) filament with deep constriction at the cross-wall (f) cells with clear thylakoid presence on peripheral region of cell. All described features are shown with black arrows.

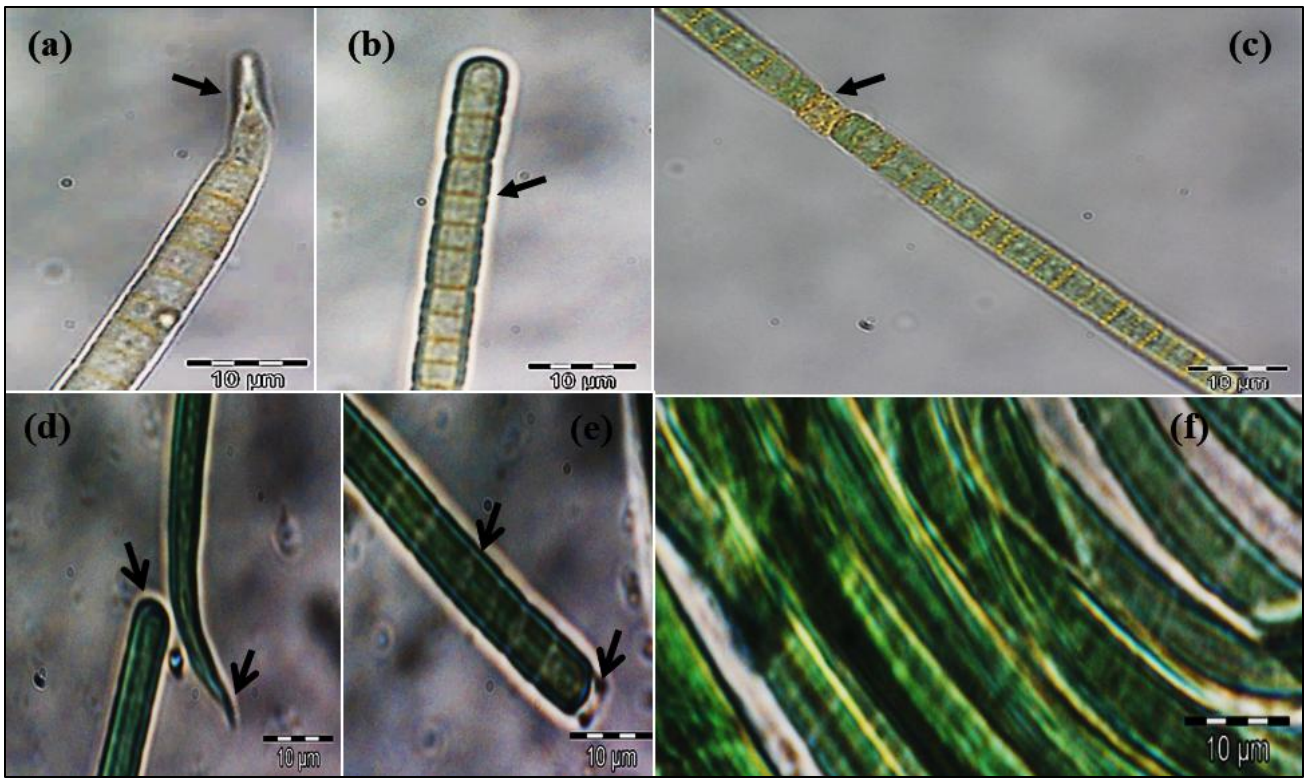

Fig. 4. Micrographs of (a) *Oxynema thaianum,* filament with apical cell tapered and pointed (b) filament with apical cell rounded and deep constriction at the cross-walls (c) filament showing yellow prominent granules at the cross walls and ready to separate through necridic cell between trichomes. (d) *Phormidium breve,* apical cell tapered and pointed and rounded in another filament (e) slight constriction at the cross-walls and presence of facultative sheath in trichome (f) filaments aggregated densely parallel to each other. All features are shown with black arrows on each photograph.

Table 2. Biochemical constituents (% dry weight) of seven cyanobacterial species isolated from microbial mats of mangrove ecosystem.

Cyanobacterial taxa	Species code	Carbohydrates	Proteins	Lipids
Leptolyngbya tenuis	MB. 1013	14.4 ± 0.25	3.8 ± 0.03	2.8 ± 0.14
Leptolyngbya sp. A	MB. 1011	14.3 ± 0.21	6.3 ± 0.2	5.1 ± 0.17
Leptolyngbya sp. B	MB. 1012	16.1 ± 0.04	2.6 ± 0.08	2.4 ± 0.32
Limnolyngbya circumcreta	MB. 1020	31 ± 0.42	4.7 ± 0.1	4.8 ± 0.07
Oxynema thaianum	MB. 1022	14.1 ± 0.06	2.1 ± 0.1	7.2 ± 0.29
Phormidium breve	MB. 1008	6.4 ± 0.34	2.6 ± 0.04	20.7 ± 0.58
Stenomitos frigidus	MB. 1021	10.0 ± 0.43	2.9 ± 0.07	3.6 ± 0.21

Values are the means of three replicates ± standard deviation

The analysis showed that the carbohydrates were accumulated as major component of biochemical composition. Total carbohydrates were estimated in the range 6.4-31% in seven isolates. Highest Carbohydrate content was recorded in *Limnolynbya circumcerata* i.e. (31%), followed by *Leptolynbya* sp.2 (16.1%) > *Leptolynbya tenuis* (14.4%) > *Leptolynbya* sp.1 (14.3%) > *Oxynema thaianum* (14.1%) > *Stenomitos frigidus* (10.0%) > *Phormidium breve* (6.4%). Total protein content of isolated cyanobacterial species was found in the range 2.1-6.3 %. Maximum values for proteins were found in *Leptolyngbya* sp. A as (6.3%) followed by *Limnolyngbya circumcreta* (4.7%) > *Leptolyngbya tenuis* (3.8%) > *Stenomitos frigidus* (2.9%) > *Leptolyngbya* sp.B and *Phormidium breve* (2.6%) and > *Oxynema thaianum* (2.1%). In the present study, total lipids were assessed in the range 2.4-20.7%. The highest lipid content was recorded in *Phormidium breve* (20.7 %) followed by *Oxynema thianum* (7.2%) > *Leptolyngbya* sp.1 (5.1%) > *Limnolyngbya circumcreta* (4.8%) > *Stenomitos frigidus* (3.6%) > *Leptolyngbya tenuis* (2.8%) > *Leptolyngbya* sp.2 (2.4%).

Discussion

Cyanobacteria are morphologically diversified group of organisms and exhibit variation in shape and size. In the present study, 7 isolated cyanobacterial species from microbial mats were grown successfully in the laboratory conditions. These species were filamentous types and belong to two orders namely Leptolyngbyales and Oscillatoriales. It has been noticed during the investigation that members of Leptolyngbyales were

grew easily and frequently when isolation and culture method were applied as oppose to field observations where dominance of order Oscillatoriales persisted in terms of number of species. Among the isolated species, few were previously documented from microbial mats in the mangrove habitat from coastal waters of Pakistan (Zaib-un-Nisa *et al*., 2000; Ahmed *et al*., 2016; Bano *et al*., 2021a, 2021b; Mansoor *et al*., 2023).

The filamentous taxa which were isolated from mangrove habitat and identified as *Leptolyngbya tenuis*, *Leptolyngbya* sp. A, *Leptolyngbya* sp.B, *Limnolyngbya circumcreta*, *Stenomitos frigidus*, *Oxynema thaianum* and *Phormidium breve,* showed fast isolation and cultivation on ASN III medium and particular laboratory conditions. Though laboratory conditions do not fulfill all requirements of natural habitat but all filamentous cyanobacteria adapted to these conditions and grew rapidly in the laboratory. More recently Yadav *et al*., (2021) also emphasized that filamentous cyanobacteria had a tendency to produce high biomass in a short period of time.

In the present investigation three major biomolecules i.e., carbohydrate, protein and lipid were assessed for the first time from seven isolated species of cyanobacteria. It is known that the biochemical composition of cyanobacteria varies from species to species, culture conditions such as temperature, light, pH and salinity, availability of nutrients and several other factors (Thomas & Litchman, 2016; Yang *et al*., 2020; Yadav *et al*., 2022). Numerous studies have emphasized that the environment and physiological conditions may affect the growth of culture and nature of species that affect the biochemical makeup of cyanobacteria. Among them light intensity is an important parameter for cyanobacterial growth and the duration of the light and dark cycle (Maltsev *et al*., 2021). There is a correlation with light intensities and the growth of cyanobacteria. Studies showed that correlation is species specific and due to limitation of light the growth of some taxa may be rendered and excessive light could enhance the growth of other species. Muhetaer *et al*., (2020) evaluated the effect of light intensities and reported that *Pseudanabaena galeata* had a negative effect by light intensities lower than 30 μmol m^{-2} s^{-1} and higher than 50 μmol m^{-2} s^{-1}. During the same study they also showed that on the contrary *Microcystis aeruginosa* showed higher tolerance for extreme light conditions. This phenomenon was also observed during present study with *Stenomitos frigidus* (synonym, *Pseudanabaena frigida*) showed elevated growth when kept under low light intensity.

Various studies highlighted about the nutritional values of cyanobacteria over last two decades, depending on number of factors including culture and growth conditions (Markou *et al*., 2014). A pioneering work done by Vargas *et al*., (1998) emphasized that proteins and lipids were at their highest levels in stationary phase opposed to the carbohydrate level which was maximum during the exponential phase during growth of the culture. Subhashini *et al*., (2003) observed significant variations in protein content of four isolates of *Anabaena azollae* and suggested that biochemical variations may exist at sub-species level, probably due to the effect of environmental factors on protein synthesis.

All cyanobacterial species tested in the present investigation had total carbohydrate values ranging from 6.4-31% which coincided with values (14.6-34.15%) similar to earlier report by Rajeshwari & Rajashekhar, (2011). Several other findings revealed that cyanobacteria secretes exopolysaccharide (carbohydrates) in response to environmental stresses (Parikh *et al*., 2006) which is also true for the current study, hence all the cultured species were isolated from polluted mangrove habitat of Karachi. Therefore, it can be speculated that carbohydrate values were found higher than protein and lipids as reported in some recent findings from same habitat (Bano *et al*., 2021a, 2021b).

Cyanobacteria have gained attention for being used as an alternative food source due to their proteins. The protein content recorded in the present study was in the range of 2.1-6.3% which corresponds well with the values recorded by Rajeshwari & Rajashekhar, (2011). However, some other studies depicted much higher values of protein in cyanobacteria such as 43 - 55% (Fatma *et al*., 1994) and 50 – 70% (Rafiqul *et al*., 2005). In the present study, the total protein was recorded lowest in all seven species as compared to lipid and carbohydrate content which is may be a consequence of culture conditions.

Lipids are major biomolecule stored in thylakoid membrane of the cell of cyanobacteria (Sato & Wada, 2009). Total lipid content observed in the present study ranged from 2.4-20.7% and found within the range given in different studies carried out earlier (Fatma *et al*., 1994; Rafiqul *et al*., 2005; Tedesco & Duerr, 1989). According to a recent study by Yadav *et al*., (2021), *Oscillatoria* sp. has accumulated more lipid (10.2%) compared to other studied taxa. In the current study, the highest lipid content was recorded in *Phormidium breve* (20 % of dry weight), whereas, a cyanobacterium *Oscillatoria calcuttensis* with same high amount of lipid was also reported by Rajeshwari & Rajashekhar, (2011). Studies suggested that total lipids and fatty acids composition of cyanobacteria play a substantial role in tolerance against various environmental conditions (Singh *et al*., 2002). Several reports demonstrated the quality of biodiesel production from algal biomass depending upon high lipid content and fatty acids composition (Ducat *et al*., 2011).

All of the isolated cyanobacterial species were sampled from the area where domestic and industrial effluents and other pollutants released in high concentrations which may affect the biochemistry of these cyanobacteria. During the investigation, it was found that the total carbohydrate content of these species was much higher than protein and lipid content, therefore it can be speculated that it could be due to production of exopolysaccharides. Similarly a recent investigation has also reported the increased levels of carbohydrates in *Pseudanabaena* sp. and *Leptolyngbya* sp., under pesticides stress (Bano *et al*., 2021a). Some of the previous studies undertaken from an area polluted with paper mill effluent have shown that total carbohydrate content of *Oscillatoria* was increased two folds as compared to control (Manoharan & Subramanian, 1992). Role of cyanobacteria in the production of carbohydrates has gained much attention since few decades due to its

contribution as major energy source in food and conversion of their biomass into biofuel (Aikawa *et al*., 2015; Arias *et al*., 2021). It can be concluded that growth of cyanobacteria and biochemical composition could be enhanced by changing culture conditions (Hemlata & Fatma, 2009; Khatoon *et al*., 2010). Their biochemical composition studied during present investigation proposed their potential role as supplementary food and alternative biomass resource for biofuel production.

Conclusion

Cyanobacteria have gained considerable attention due to its great potential for feed, food and many other bioproducts. In present study, seven cyanobacterial species were isolated from the field, grown and culture on large scale in laboratory conditions. In conclusion, the study highlights the dominance of the Leptolyngbya genus, with notable findings such as the first recorded occurrence of *Limnolyngbya circumcreta* and *Stenomitos frigidus* in Pakistan. The biochemical assessment of all cyanobacterial species from the Karachi coast revealed significant variability in terms of carbohydrate, protein, and lipid content. Among them, *Phormidium breve* exhibited the highest lipid content, *Limnolyngbya circumcreta* accumulated the most carbohydrates while Leptolyngbya sp. A contained maximum protein content. The findings of highly diversified cyanobacteria and their capacities to produce broad spectrum bioproducts may provide tremendous opportunity to explore their potential.

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