Current Trends in Biotechnology and Pharmacy
Vol. 12 (1) 7-15 January 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

Biochemical profile of five species of cyanobacteria isolated from polythene surface in domestic sewage water of Silchar town, Assam (India)

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Abstract

Disposal of polythene into waste water poses a serious problem as they get accumulated in the environment. Submerged polythene in waste water offers an ideal substratum for algae to colonize. The present paper highlights the biochemical composition of five cyanobacteria isolated from submerged polythene surface in domestic sewage water, Silchar town, Assam, (India). The carbohydrate, protein, lipid, vitamin C and pigments (Chl*a*, carotenoids, phycobiliproteins) contents of five cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum*, and *Cylindrospermum muscicola* isolated from submerged polythene surface were analysed. Maximum amount of total protein, carbohydrate and lipid content were found in *Oscillatoria subbrevis* and minimum in *Cylindrospermum muscicola*. Vitamin C was found to be highest in *Oscillatoria subbrevis* and *Nostoc carneum* and minimum in *Cylindrospermum muscicola*. The total phycobiliproteins was maximum in *Oscillatoria subbrevis* and minimum in *Cylindrospermum muscicola*.One-way analysis of variance (ANOVA) showed significant differences among the biochemical parameters of cyanobacteria isolated from polythene surface.

Keywords: biochemical; cyanobacteria; domestic sewage water; polythene bags; Assam

Introduction

Cyanobacteria are known to occupy a broad range of habitats across all latitudes and are believed to be the earliest inhabitants of earth. They are not only widespread in freshwater, marine and terrestrial ecosystems but also occur in extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts (1). Besides such natural habitats, algae including cyanobacteria are capable of growing on artificial substrates as well. Made from non-renewable fossil fuel, introduced around 1970s(2), plastic carry bags are indiscriminately dumped into landfills worldwide and emit dangerous methane and carbon dioxide gases during their decomposing stages as well as highly toxic leachates (3). It effectively blocks sewerage pipe lines, litters agricultural lands, canals, rivers and oceans. They are not biodegradable or take incredibly long time to break down into powdery plastic dusts which contaminate the soil and the water adversely affecting all life forms (4). Algae are known to colonise on polythene surfaces submerged in waste water(5-6).Study of growth of algal species on such polythene substrata are important in the context of biodegradation of polythene (7). Biodegradation of polyethylene by algae constitute an attractive environment friendly and cost effective viable option(8).Algae and cyanobacteria are rich source of several bioactive compounds such as

Vol. 12 (1) 7-15 January 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

proteins, polyunsaturated fatty acids (PUFAs), sterols,enzymes,vitamins and pigments(9).Their vast potential in varied applications such as food,feed, fuel, fertilizer, medicine,industry and in combating pollution(10-16) have been explored. Given the huge diversity of algae and the phytochemicals they produce, exploring their biochemical contents has remained a favourite pastime of researchers.Accordingly, the present study addresses the biochemical screening of five cyanobacteria isolated from submerged polythene surface in the domestic sewage water of Silchar town in the state of Assam, India.

Material and Method

Study area : The study was carried out in the urban area of Silchar town of Cachar district located in the state of Assam, India (Fig. 1) during the 2014. The study area lies between latitude $24^0$49Ê North and longitude $92^0$48$^{\hat{E}}$ East and altitude of 114.69 meters above sea level on the banks of river Barak. The domestic sewage drains carries waste from household and medium scale industries. A view of the study site showing algae colonized on polythene bags is presented in Fig. 2

Physico-chemical properties of sewage water : The water samples from domestic sewage drains were collected, transferred into pre-cleaned plastic bottles and stored for further analysis. The pH was measured using a digital pH meter. Biological oxygen demand (BOD) and dissolved oxygen (DO) measured by titrimetric method (17). Chemical oxygen demand (COD) was measured by open reflux method. Alkalinity, free CO_2 and magnesium and calcium were measured by titrimetric method (18). Total dissolved solid (TDS) and suspended solid (SS) were measured by gravimetric method. Chloride was measured by argentometric method (19). Sulphate was estimated by turbidimetric method (17). Nitrate was measured by brucine method (20). Soluble reactive phosphate was estimated by molybdate blue method(21). Ammonia was determined by phenol-hypochlorite method (22).

Isolation of cyanobacteria : A total of 20 dumped waste polythene bags colonised by algae were collected from domestic sewage water drains of Silchar town, Assam and brought into laboratory.

Fig. 1. Map of the study area showing the location of study sites

Fig.2. Close view of algae colonizing on submerged polythene bags (2A-2B)

The polythene bags were cut into 1cm^2 size pieces with a sterilized blade. The algae samples from polythene surfaces were scrubbed with a sterilized brush and observed under microscope. The method used for isolation and purification of cyanobacteria was according to Rippka *et al*. (23). The algal samples were homogenized in sterile water with glass beads, centrifuged at 3000 rpm for 10 minutes with repeated washing.The pellets were suspended in sterilized BG-11 medium and placed onto the agar petri plates by pour plate method. The plates were incubated for 15 days under continuous illumination (2000lux) at 24±1^{0}C. The pure colonies developed in the agar plates were picked upandsub-cultured in 500ml Erlenmeyer flasks. The cultures were observed under microscope and the isolated cyanobacterial species were identified using standard keys (24-25). Photomicrographs of five cyanobacterial species were presented in Fig.3.The BG-11 medium without combined nitrogen source was used for the isolation and maintenance of *Nostoc*and *Cylindrospermum.*

Biochemical analysis : The total carbohydrate was determined according to anthrone method (26). Total protein was estimated by modified method of Herbert*et al*.(27).The chl*a* and carotenoids were estimated by the standard methods of Strickland and Parsons (28) and Parson (29), respectively. Phycobiliproteins estimation has been carried out as per Bennet and Bogorad (30). Lipid content was estimated by the standard method of Bligh and Dyer (31). Vitamin C content was evaluated using the method of Roe and Keuther (32). Growth rate was measured in terms of chl *a* as biomass component(33). Growth kinetics in terms of specific growth rate (K) and generation time(G) were evaluated (34).

Statistical analysis : The statistical analyses were performed using the software Statistical Package for Social Sciences (SPSS Version 21.0). One-way analysis of variance (ANOVA) was used to

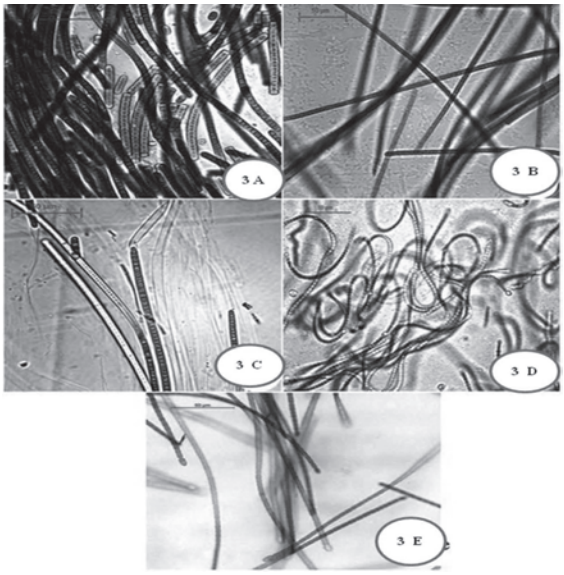

Fig. 3. Photomicrographs of five cyanobacteria isolates
3 A- Phormidium lucidum, 3 B- Oscillatoria subbrevis,
3 C- Lyngbya diguetii, 3D- Nostoc carneum,
3 E- Cylindrospermum muscicola

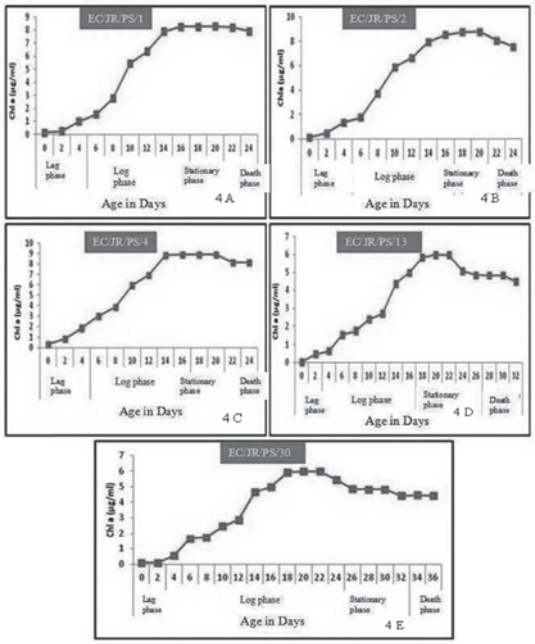

Fig. 4. Growth curve of isolated cyanobacteria (4 A-Phormidium lucidum, 4B- Oscillatoria subbrevis , 3 4C- Lyngbya diguetii, 4D- Nostoc carneum, 4 E-Cylindrospermum muscicola)

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evaluate the differences among the biochemical parameters.The triplicate sets of data were evaluated inaccordance with the experimental design (Completely Randomized Design) with ANOVA (Analysis of Variance). The comparisons between the different means were made usingpost hoc least significant differences (LSD) calculated at p level of 0.05 (5%), and representedas CD (Critical Differences) values in Table 3 and 4 with standard deviations.

Results

The physico chemical propertites of domestic sewage water are presented in Table 1. The colour of domestic sewage water was black to yellowish grey. The temperature ranged from 28 to 34°C. The site 2 recorded maximum water temperature while site 4 recorded the minimum. The pH values of the different sites were quite at variance with each other. The domestic sewage water of site 2 was slightly acidic while that for site 4 was found to be alkaline.The value of BOD varied from 383 to 600 mg/L with site 3 registering maximum value. The COD values were in the range of 1511 to 2189 mg/L with maximum being at site 5. The DO concentrations varied from 1.3 to 2.4mg/Lwith maximum being at site 4 and minimum at site 1. Alkanity of domestic sewage water varied from 9 to 11mg/L. Free CO_2 was in the range 38-42mg/L with maximum at site 2. Nitrate ranged from 12 to 65 mg/L with maximum being at site 4. Magnesium ranged from 25 to 178mg/L, maximum being at site 4 and minimum at site 2. The TDS of sewage water were within the range of 500 to 3210mg/L, it was maximum being at site 2 .The SS of sewage water was in the range of 50 to 200mg/L and being it was maximum at site 2 and minimum at site 3. The chloride concentration were in the range of 35 to 78mg/L, being maximum at site 2 .The calcium content of sewage water was found in the range 54 - 69mg/L, , being maximum at site 4. The sulphate of domestic sewage varied from a minimum of 50mg/L at site 3 to maximum of 897mg/L at site 4.The minimum ammonia value was found to be 28mg/L at site 1 andmaximum at34mg/L at site 4.The phosphate of sewage water varied from a minimum of 58mg/L at site 5 to a maximum of 72mg/L at site 4.

The growth curves of five cyanobacterial species were presented in Fig.4. The maximum growth rate (Table 2)has been shown by *Oscillatoria subbrevis*(0.158μd^{-1})followed by *Nostoc carneum* (0.152μd^{-1}). The growth rate was lowest in *Phormidium lucidum* (0.134μd^{-1}).The generation time was maximum in *Phormidium lucidum* (178.25h) and minimum in *Oscillatoria subbrevis* (151.34h).

The biochemical analysis of five species of cyanobacteria (Table 3) revealed the carbohydrate to be in the range109-370 μgml^{-1}**.**The maximum carbohydrate present in *Oscillatoria subbrevis* (370 μgml^{-1}**)** and minimum in *Cylindrospermum muscicola* (109 μgml^{-1}**)**.The carbohydrate present in *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* were 240 μgml^{-1}**,** 230 μgml^{-1} and 113 μgml^{-1}, respectively**.** The protein range was145-230 μgml^{-1}.The maximum protein content was found in *Oscillatoria subbrevis* (230 μgml^{-1}**)** and minimum in *Cylindrospermum muscicola* (145 μgml^{-1}**)**. The protein present in *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* were 210 μgml^{-1}, 203 μgml^{-1} and 195 μgml^{-1}. The range of vitaminC was 0.3-0.9 μgml^{-1}. The maximum vitamin C content was observed in *Oscillatoria subbrevis*and *Nostoc carneum*(4.2μgml^{-1}) , minimum in *Cylindrospermum muscicola* (1.2 μgml^{-1}). The vitaminC present in *Phormidium lucidum*, *Lyngbya diguetii* were 0.5 μgml^{-1} and 0.8 μgml^{-1},respectively.The range of lipid content in cyanobacterial isolates was 4.2-11.2μgml^{-1}. The maximum lipidcontent was noted in *Oscillatoria subbrevis* (11.2 μgml^{-1}) and minimum in *Cylindrospermum muscicola* (4.2 μgml^{-1}**)**. The lipid content present in *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* were 8.7 μgml^{-1},7.3 μgml^{-1} and 5.1 μgml^{-1} ,respectively.

Phycocyanin (PC) content (Table 4) was maximum in *Lyngbya diguetii* (17.5 μgml^{-1}) and minimum in *Nostoc carneum* (12.1 μgml^{-1}). Phycoerythrin (PE) content was maximum in *Oscillatoria subbrevis* (48.7 μgml^{-1}**)** and minimum

in *Cylindrospermum muscicola* (15.34 μgml^{-1}). Allophycocyanin (APC) was maximum in *Lyngbya diguetii* (25 μgml^{-1}**)** and minimum in *Cylindrospermum muscicola* (15.34 μgml^{-1}**)**. Total phycobiliproteins content was maximum in *Oscillatoria subbrevis* (81.4 μgml^{-1}) and minimum in *Cylindrospermum muscicola* (45.98 μgml^{-1}**)**.

One way ANOVA revealed significant differences among the biochemical parameters, chl*a* ($p = 0.02$), carotenoids ($p = 0.01$), protein ($p = 0.02$), carbohydrates ($p = 0.04$), vitaminC ($p = 0.02$), lipids ($p = 0.04$). Significant variationin phycobiliproteins concentrations, PE ($p = 0.02$), PC ($p = 0.01$) and APC ($p = 0.04$) were observed.It is noteworthy that total phycobili proteins in *Oscillatoria subbrevis* contains almost double the amount of *Cylindrospermum muscicola* **.**

Discussion

The biochemical constituents of cyanobacteria isolated from polythene surface submerged in domestic sewage water showed that *Oscillatoria subbrevis*, *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* contain high cellular constituents of chl*a*, carotenoids, protein, carbohydrate, vitamin C, lipid and phycobili proteins. Significant differences were observed in biochemical constituents species wise. In the present study, *Oscillatoria subbrevis* was found to thrive in an alkaline condition (pH = 8.1, site 4) while *Phormidium lucidum* was collected from an acidic (pH = 5.8, site 2) sewage water. The species *Lyngbya diguetii isolated* was found to grow under slightly acidic sewage water (pH = 6.3, site 3) and *Nostoc carneum* and *Cylindrospermum muscicola* was collected from slightly alkaline condition (pH = 7.3, site 1) and moderately acidic condition (pH = 6.4**,** site 5)**,** respectively.The characteristic morphological and physiological attributes of the species might be ascribed to typical physico-chemical properties of domestic sewage water. It has been reported that the cellular composition of cyanobacteria depend on the nature of strains, physiological state of the isolates and the nutrient conditions of environment from where they have collected (35-38).

The dissolved oxygen of domestic sewage water varied from 1.3-2.4mg/L. The growth of algae was found to be directly proportional to the available nutrients and oxygen level of water which in turnmight alter the oxygen level of sewage water.The cyanobacteria in the present study has been found to adapt well to the oxygen depleted condition of the sewage water and it was presumed that in the absence of additional nitrogen source in domestic sewage water, the flow of carbon fixed in photosynthesis is switched from the path of protein synthesis to that leading to higher production of biochemical constituents of microalgae (39).The extent of dissolved solid and suspended solid of domestic sewage water was quite different in all the five sites. Therefore, the sunlight penetration on submerged polythenes is anticipated to be different.Grossman *et al*.(40) opined that environmental condition of species might alter the composition and abundance of phycobiliproteins. In the present study, the physico chemical parameters were at variance in all the five sites. This, we believe, might have caused a variation in the total phycobiliproteins in the species studied.

In a previous study, the algal species *Phormidium angustissimum*, *Lyngbya holdenii*, *Anabaena doliolum*, *Calothrix marchica* and *Fischerella muscicola* isolated from lime sludge waste of a paper mill in the district showed higher accumulation of chl *a*, phycocyanin, carbohydrates and protein (41). Lime sludge waste is rich in organic carbon and thus contributes to the nutrition of cyanobacterial growth. Lipids content was recorded highest in *Oscillatoria subbrevis* and lowest in *Cylindrospermum muscicola*.In the present study, carbohydrate and protein are found to be highest in *Oscillatoria subbrevis* and lowest in *Cylindrospermum muscicola*, respectively. This is in conformity with the observation made by Zhu *et al.* (42) wherein it has been shown that proteins are present as large fraction of biomass in growing algae.

Table 1: Physico-chemical properties of domestic sewage drain water

Water Parameters	Site 1 (Link road)	Site 2 (Sonai road)	Site 3 (NH road)	Site 4 (Premtola)	Site 5 (Ambikapatty)
Colour and odour	*Black, present*	*Black, present*	*Black, present*	Yellowish grey, present	Yellowish grey, *present*
Temperature	32°C	34°C	29°C	28°C	35°C
pH	7.3±0.23	5.8±0.10	6.3±0.13	8.1±0.23	6.4±0.21
BOD (mg/l)	586.3±0.45	483±0.14	600±0.18	383±1.2	509±2.3
COD (mg/l)	1511±0.67	1520±0.56	1520±0.18	1764±0.24	2189±0.78
DO (mg/l)	1.3±0.12	2.3±0.15	2.2±0.23	2.4±0.21	2.2±0.12
Alkalinity(mg/l)	9±0.34	9.8±0.12	10±1.4	11±0.12	9.8±0.23
Free CO_2 (mg/l)	38±0.13	42±0.13	36.98±0.13	39±0.21	36±1.2
TDS (mg/l)	500±1.2	3210±1.4	500±0.14	1546±2.4	578±2.5
Suspended solids (mg/l)	51±0.56	200±0.13	50±0.35	53±0.13	58±0.23
Chlorides(mg/l)	62±0.21	78±0.34	60±0.06	73±0.21	35±0.12
SO_4^{-2} (mg/l)	880±1.3	876±1.4	50±1.6	897±3.2	783±0.23
Nitrate (mg/l)	43±0.13	44±0.12	12±1.5	65±0.13	46±1.2
Mg (mg/l)	32±0.24	25±0.67	30±1.1	178±1.3	176±2.1
Ammonia (mg/l)	28±0.12	32±0.34	30±1.2	34±0.12	32±0.23
Appearance	Not clear	Not clear	Not clear	Not clear	Not clear

Table 2: Specific growth rate (K) and generation time (G) of the isolates

Sl. No	Cyanobacterial isolates	K (μd^{-1})	G (h)
1	*Phormidium lucidum*	0.134	**178.25**
2	*Oscillatoria subbrevis*	**0.158**	151.34
3	*Lyngbya diguetii*	0.138	173.67
4	*Nostoc carneum*	0.152	157.21
5	*Cylindrospermum muscicola*	0.136	153.89

Vitamin C, a wide spectrum antioxidant not synthesized in the body is obtained from dietary sources (43). In the present study, vitamin C contents was found to be highest in *Oscillatoria subbrevis*, *Nostoc carneum* and lowest in *Cylindrospermum muscicola*. Algae with brighter thalli were reported to be rich in vitamin C (44). In the present study, *Oscillatoria subbrevis* and *Cylindrospermum muscicola* were found to colonize with brighter blue-green and olive green colour thalli on polythene bags. *Oscillatoria subbrevis* formed bright blue-green loop like thallus floating over the liquid medium and *Cylindrospermum muscicola* formed olive green finger like projection on the petri plate surface in laboratory culture.

Conclusion

Five species of cyanobacteria isolated from submerged polythene surface in domestic sewage water are demonstrated to be a rich source of carbohydrate, proteins, lipids, vitamin C and phycobiliproteins.The results are anticipated to be of relevance to biodegradation of polythenes, aquaculture, pharmaceutical

Table 3: Biochemical composition of five cyanobacterial isolates from submerged polythene bags in domestic sewage water

Biochemical parameters	Cyanobacterial isolates					
	Phormidium lucidum	*Oscillatoria subbrevis*	*Lyngbya diguetii*	*Nostoc carneum*	*Cylindros permum muscicola*	CD (0.05%)
Chl*a* (µgml^{-1}	8.47±0.12	7.32±0.23	6.92±0.34	3.02±0.21	4.23±0.42	0.02
Carotenoid (µgml^{-1})	2.10±0.02	2.89±0.01	1.89±0.03	0.89±0.01	1.02±0.02	0.01
Protein(µgml^{-1})	210±o.54	230±0.51	203±0.32	195±0.43	145±0.34	0.02
Carbohydrate (µgml^{-1})	240±0.73	370±1.02	230±0.45	113±0.56	109±0.67	0.04
Vitamin C (µgml^{-1})	0.5±0.01	0.9±0.03	0.8±0.01	0.9±0.01	0.3±0.01	0.02
Lipid (µgml^{-1})	8.7±0.02	11.2±0.21	7.3±0.03	5.1±0.12	4.2±0.12	0.04

Table 4: Phycobiliproteins of five cyanobacterial isolates from submerged polythene bags in domestic sewage water

Phycobiliproteins	Cyanobacterial isolates					
	Phormidium lucidum	*Oscillatoria subbrevis*	*Lyngbya diguetii*	*Nostoc carneum*	*Cylindros permum muscicola*	CD (0.05%)
PE(µgml^{-1})	14.2±0.12	13.1±0.21	17.5±0.12	12.1±0.21	15.3±0.34	0.02
PC(µgml^{-1})	42.4±0.02	48.7±0.23	36±0.23	45±0.04	15.34±0.15	0.01
APC(µgml^{-1})	18±0.01	19.6±0.12	25±0.05	21±0.24	15.34±0.02	0.04
Total Phycobiliproteins (µgml^{-1})	74.6±0.15	81.4±0.56	78.5±0.4	78.1±0.49	45.98±0.51	0.05

applications and biofuel.Due to rich biochemical contents these cyanobacteria may have the potential for use in the food industry as high value nutritional products.

Acknowledgement

One of the authors (PS) is thankful to the University Grants Commission, New Delhi for fellowship. The Department of Biotechnology (DBT-BT/183/NE/TBP/2011 dated 23/04/2012) and Department of Science and Technology (DST/IS-STAC/CO2-SR-164/13 (G), Government of India, New Delhi is sincerely acknowledged for financial support.

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