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RESEARCH ARTICLE

Culturable cyanobacteria from some selected water bodies located in the major climatic zones of Sri Lanka

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Abstract: Cyanobacteria are oxygenic photosynthetic prokaryotes with a wide morphological diversity with some members capable of fixing atmospheric N_2. This paper reports on the taxa isolated and cultured from water in selected reservoirs in the Dry, Intermediate and Wet zones of Sri Lanka with the potential of utilizing them for biofuel and other industries including cosmetics. Water samples were concentrated by filtering through plankton net and re-suspended in 50 mL of either in respective reservoir water samples or in distilled water. Aliquots of these were cultured in blue green 11 (BG 11) and GO (same as BG11 media but N-free) media. Cultures were maintained under 2,000 lux light intensity with shaking at 200 rpm. A total of 64 cyanobacterial cultures, isolated by repeated sub-culturing in liquid and agar media, were observed under a light microscope for identification. While 16 different taxa were isolated from the Dry Zone, 12 and 5 taxa were isolated from the Wet and Intermediate zones, respectively. The frequency of isolating *Oscillatoria* sp. was high (10 reservoirs) followed by *Microcoleus* sp. and *Microcystis* sp. (each from 6 reservoirs). A strong positive correlation was recorded between cyanobacterial occurrences with abiotic properties such as water temperature, pH and secchi depth (turbidity). Samples prepared in water from respective reservoirs gave faster growth during culturing than those prepared in distilled water. The occurrence, availability and culturability of the isolates obtained in the present study will be useful in the development of value-added cyanobacterial products and cyanobacteria-based industries including biofuel.

Keywords: culturable cyanobacteria, fresh water reservoirs, growth conditions, climatic zones of Sri Lanka.

INTRODUCTION

Cyanobacteria (blue-green algae) constitute a morphologically diverse and widely distributed group of gram-negative photosynthetic prokaryotes (Pandey, 2015). They are unique among the prokaryotes in possessing the capacity of oxygenic photosynthesis. In addition, the ability of some of these organisms to fix N_2 either independently or in symbiosis with other organisms not only contributes to natural ecosystems but is used in certain countries as a biofertilizer in rice cultivation (Kulasooriya, 2012). These qualities make cyanobacteria one of the most successful and widespread group among the prokaryotes, occupying a wide range of terrestrial and aquatic environments (Shatheesh, 2013). Cyanobacteria are also characterized with their high morphological diversity not seen among other prokaryotes. This extends from unicellular to filamentous species with a cell volume ranged over more than five orders of magnitude (Whitton, 2000). Their ability to grow in highly polluted environments is also used in treating sewage and industrial effluents (Cepoi *et al*., 2016; Kulasooriya, 2012). Cyanobacteria with an array of light harvesting pigments and superior photosynthetic capabilities are currently looked at as highly attractive candidates for biofuel production, food, feed stocks and high value bioactive compounds (Pereira e*t al.,* 2011). The scarcity of information about cyanobacteria reflects the difficulty in purifying and culturing of these microorganisms (Waterbury, 2006). Photosynthetic cyanobacteria have drawn significant attention as they can serve as important sources for cosmetic, food and pharmaceutical products, industrial materials and even in biofuel production.

In Sri Lanka, some studies have been carried out to examine different aspects of cyanobacteria. Jayatissa *et al.*(2006) investigated the variations in phytoplankton communities in relation to major nutrients, with a particular emphasis on cyanobacteria, sampled from 17 reservoirs from different user categories and climatic zones of Sri Lanka. Another study reported on the occurrence of cyanobacteria in 21

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reservoirs of the Mahaweli river basin in Sri Lanka (Silva and Wijeyaratne, 1999). Perera & Yatigammana (2014) studied the distribution and composition of cyanobacteria in several Sri Lankan reservoirs. Several studies reported so far on cyanobacteria are from fresh water bodies in Sri Lanka with a special emphasis on toxigenic and bloom forming types of cyanobacteria (Idroos and Manage, 2015; Perera *et al.,* 2012; Magana-Arachchi and Wanigatunge, 2013; Magana-Arachchi *et al.*, 2011; Magana-Arachchi *et al.,* 2009; Pathiratne and Saram, 2010; Jayawardene *et al.*,1998; Kulasooriya, 1998; Weerakone *et al*., 1998). Tobschall and Dissanayake (1986) studied heavy metal concentrations in cyanobacterial mats in Mannar lagoon. Hossain *et al.,* (2016) reported on the antioxidant properties of some selected cyanobacterial species of fresh water bodies in Sri Lanka. Several other studies have been carried out since early 20th century on the occurrence of cyanobacteria in Sri Lanka (Abeywickrama, 1979; Crow, 1923; Silva, 1992; Foged and Botanist, 1976; Kulasooriya and Silva, 1981; Holsinger, 1955; Rott and Lenzenweger, 1994; West and West, 1902). The present study reports on the isolation, culturing and identification of cyanobacteria from some selected fresh water reservoirs located in three major climatic zones of Sri Lanka, with the aim of identifying suitable taxa for bio-fuel production and other industries.

MATERIALS AND METHODS

Sampling

Thirty seven fresh water reservoirs were selected for the study (Figure 1). Also the GPS coordinates are provided in the appendix. Water samples were collected from 31 reservoirs in the Dry zone and from 3 reservoirs each from Wet and Intermediate zones. From ancient times, many man-made reservoirs are mainly found in the Dry Zone while a lesser number found in the Wet and Intermediate Zones. GPS locations of the sampling reservoirs were recorded using GPS meter (Garmin, eTrex 30).

Collection of water samples

Water samples were collected using a Ruttner sampler from the photic zone of the reservoir and stored in plastic cans (less than two hours) till the samples were prepared for culturing. The photic zone (turbidity or secchi depth) of the water body was determined by the Secchi disk disappearance technique. At each reservoir, three 2 L samples were collected (two samples from a distance of 3 to 4 m from the edge and one from the middle of the water body). Physical parameters of water bodies such as pH and temperature of water was measured using Hanna pocket pH and temperature meter (model HI 98128) during sample collection.

Sample preparation

Each water sample (2 L) was filtered through 20 µm mesh size planktonic net. The material retained in the mesh was transferred into a screw cap plastic tube before making the final volume into 25 mL by adding distilled water. Another sample was prepared in the same manner but using water from the respective reservoir instead of distilled water. Thus, six separate sub-samples were prepared from each reservoir.

Culturing and sub culturing

Ten ml of the planktonic sample was transferred into 50 mL of BG11 (Stanier *et al.,* 1971) and GO (Rippka *et al.,*1979; 1981) media at pH 7.5 in 100 mL conical flasks and maintained with continuous shaking at 200 rpm in a biological growth chamber under 2,000 lux light intensity. Growth was detected in each medium by the appearance of the bluish green color. Once the growth was detected,100 µl of a sub-sample was transferred on to an agar plate (same media solidified with 1.5% (w/v) bacteriological agar) using spread plate method under a laminar air flow cabinet (Pulz and Gross, 2004). All the samples were cultured in BG11 and GO media and the number of days to produce visible growth in each medium was observed.

Subcultures were carried out by inoculating 100 µl of the vortexed sample on to a petri plate. The purity of the culture was confirmed by repeated plating and regular observations under the microscope (Abou-Shanab *et al*., 2011).

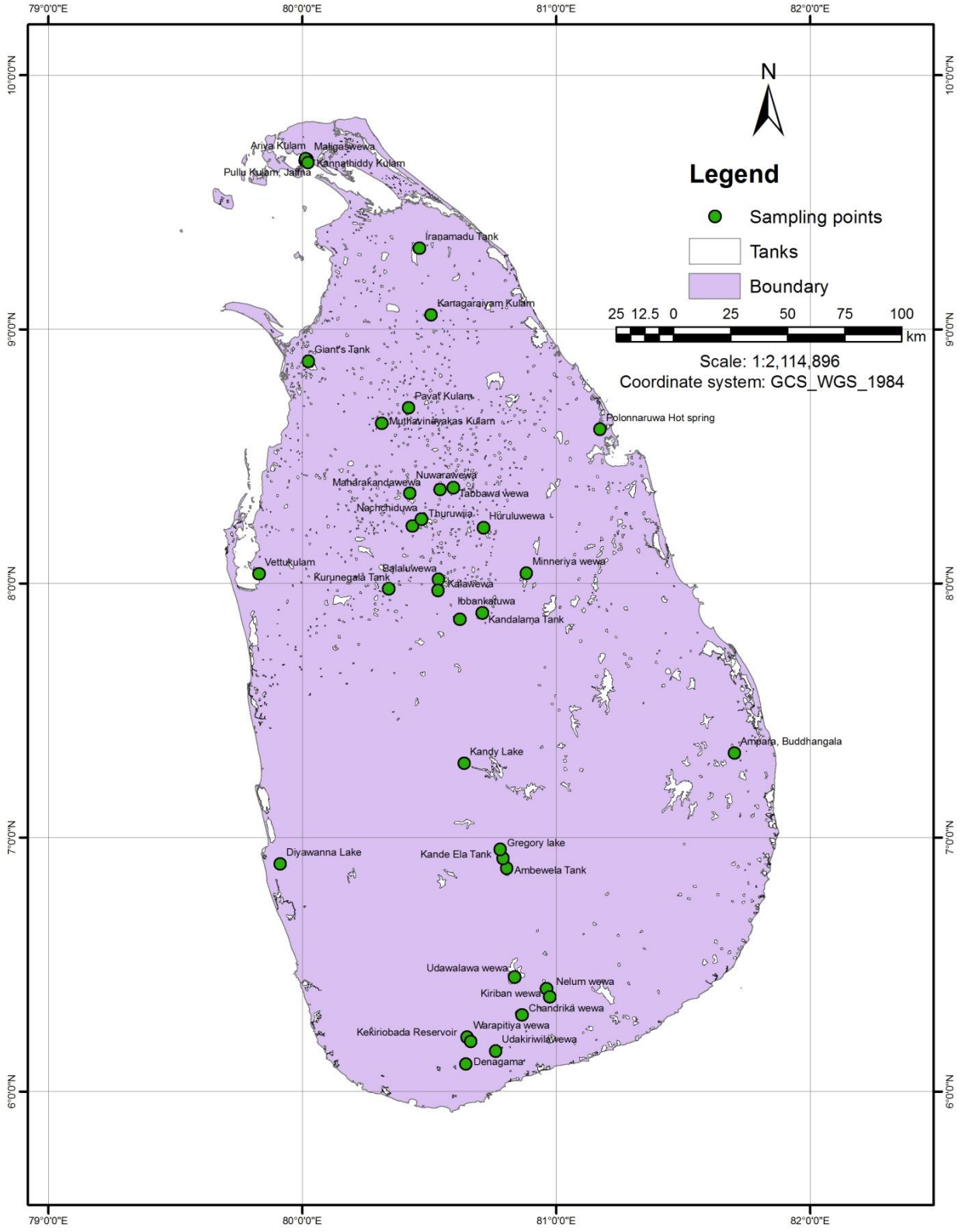

Figure 1: Sampling locations (reservoirs) in Sri Lanka.

Morphological identification

A small aliquot of an isolated culture was observed under a Compound Microscope (Euromex, BioBlue.Lab BB. 1153-PLi) and the identification of specimens was carried out using morphological characteristics described by Desikachery (1959). The following parameters were taken into account during the identification *viz*., length and width of vegetative cells, morphology of the terminal cells, the presence or absence of heterocysts, akinetes and gas vesicles, the distance between heterocysts and the distance between a heterocyst and the nearest akinete and the shape of filaments and their potential aggregation into colonies.

STATISTICAL ANALYSIS

Statistical analyses were done for water temperature, pH and turbidity measurements using ANOVA in SAS 9.1 (SAS, 1999) and MINITAB-16.

RESULTS AND DISCUSSION

In the present study, the cyanobacterial occurrence had a strong positive correlation with abiotic properties of water *i.e*., water temperature, pH and secchi depth (Table 1). Also, the number of sampling reservoirs was found to be positively correlated with number of cyanobacteria isolated from three climatic zones (Table 1). In the present study, a total of 64 uni-algal cultures were obtained and they were distributed as 48, 10 and 6 from reservoirs in Dry, Wet and Intermediate zones, respectively. The identified strains from the Dry Zone were belonged to *Oscillatoria* sp., *Phormidium* sp., *Pseudoanabaena* sp., *Gleocapsa* sp., *Planktolyngbya* sp., *Microcystis* sp., *Limnothrix* sp., *Microcoleus* sp., *Aphanotecae* sp., *Scynechcoccus* sp., *Lyngbya* sp., *Planktothrix* sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Nostoc* sp. and *Microchaete* sp. Ten strains (from 10 uni-algal cultures) including *Anabaena* sp., *Plectonema* sp., *Lyngbya* sp., *Calothrix* sp., *Chroococcus* sp., *Dermocarpa* sp., *Leptolyngbya* sp., *Oscillatoria* sp., *Scynechococcus* sp., *Microcystis* sp., were isolated from reservoirs in the Wet Zone while only four species (from 6 uni-algal cultures) were isolated from the Intermediate Zone which include *Oscillatoria* sp., *Microcystis* sp., *Spirulina* sp. and *Chrococcus* sp. The frequency of isolating *Oscillatoria* sp. was high (10 water bodies) from the reservoirs examined, followed by *Microcleus* sp. and *Microcystis* sp. (each from 6 water bodies) (Figure 2).

According to the present study, the highest water temperature (31.35 ± 1.50^0C), pH (8.20 ± 0.68) and Secchi depth (75.09 ± 26.31 cm) were recorded in dry zone reservoirs whereas the lowest recorded in the wet zone. The abiotic properties of water samples of the three climatic zones are shown in Table 2.

Table 1: Correlation between multiple parameters in the current study.

	Climatic Zone	Water Temp (^{0}C)	pH	Secchi Depth (cm)	Occurrence	Reservoir
Climatic Zone	1.000					
Water Temp (^{0}C)	0.953	1.000				
pH	0.950	0.810	1.000			
Secchi Depth (cm)	0.977	0.865	0.995	1.000		
Occurrence	0.820	0.607	0.958	0.924	1.000	
Reservoirs	0.866	0.673	0.979	0.953	0.996	1.000

Table 2: Abiotic properties of different reservoirs in three climatic zones of Sri Lanka.

Climatic Zone	Water Temperature (^{0}C)	pH	Secchi Depth (cm)
Dry Zone	31.35 ± 1.50^c	8.20 ± 0.68^c	75.09 ± 26.31^c
Intermediate Zone	29.12 ± 1.82^b	7.58 ± 0.44^b	62.60 ± 11.97^b
Wet Zone	21.37 ± 1.27^a	7.41 ± 0.24^a	57.00 ± 39.69^a

Values given are the mean of 93 samples from dry zone and 9 samples from intermediate and wet zone each. Superscript letters in each column indicate significant differences ($p < 0.05$)

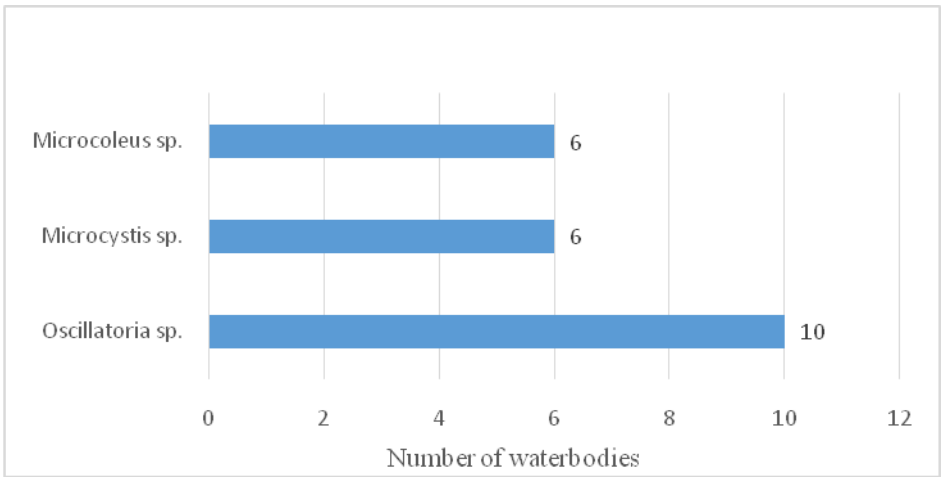

Figure 2: Frequencies of algal species recorded in reservoirs located in three climatic zones in Sri Lanka.

Table 3: Time taken to appear the growth in each media (in days).

Climatic zone	Media with original water		Media with distilled water	
	BG 11	GO	BG 11	GO
Dry zone	8.33±1.53	12.5±3.54**	14.00±40	20.00±1.41**
Intermediate zone	8.33±3.06	8.00±00*	13.33±3.79	13.00±00*
Wet zone	12.67±2.52	18.00±1.41**	19.67±3.06	20.50±2.12**

*Results presented are for the 3 isolates from each climatic zone initially tested for this parameter. *Growth appeared only in one sample out of three and **Growth appeared in two samples out of three.*

Growth of cyanobacteria

Generally, the appearance of cyanobacterial growth took place after about 2 to 3 weeks, mainly depend on the climatic zone and other abiotic parameters of the reservoirs (Table 3). However, the growth in agar supplemented media takes even longer than that of liquid media. According to the results, the samples prepared in water taken from respective reservoirs grew faster than samples prepared in distilled water, regardless of the climatic zone (Table 3). It is most likely that the water from respective reservoirs provided more favorable conditions for the survival and the growth of opportunistic (fast-growing) taxa those having superior survival skills. Also, the cyanobacterial cells can get an osmotic shock once they transfer into distilled water in addition to the changes of other physical and chemical characteristics including temperature, pH, and nutrients.

The results also suggested that cyanobacterial taxa grew faster in BG11 medium compared to the GO medium. In the medium of BG-11, the growth appeared in all samples (Table 3) whereas in the N-free GO medium the isolates were predominantly heterocystous.

Composition of Cyanobacterial taxa

About 40 species of cyanobacteria belonging to 24 genera have been reported so far from reservoirs in Sri Lanka (Silva and Wijeyaratne, 1999). Of these, except for *Microcystis aeruginosa*, other species are either rare or occur only in small numbers (Silva and Wijeyaratne, 1999). However, more recent reports, encompassing several reservoirs, on the occurrence of freshwater cyanobacteria give evidence of *Cylindrospermopsis* as dominant taxa in addition to *Microcystis* (Perera *et al.*, 2011). However, in the present study *Cylindrospermopsis* was not observed in any reservoirs studied. This is perhaps due to the inability of these two media that used in the present study to support its growth or else it could be due to misidentification it as *Planktolyngbya* as a result of their close resemblance under *in vitro* conditions.

Traditionally, the classification of cyanobacteria has been based on morphological characters such as trichome width, cell size, division planes, shape and arrangement, pigmentation and the presence of characters such as gas vacuoles and a sheath (Baker, 1991, 1992; Bourrelly, 1970; Komarek and Anagnostidis, 1986, 1989). However, a considerable expertise is required to identify species with the help of these characters and the subjective judgment by operators can lead to errors resulting or incorrect identification of isolates. Komarek and Anagnostidis (1989) estimated that more than 50% of the strains in culture collections are misidentified. Moreover, some diagnostic characteristics, such as gas vacuoles and/or akinetes, can show variations with different environmental or growth conditions and even be lost during cultivation (Rudi *et al.,* 1997; Lyra *et al.,* 2001). Such limitations of using phenotypic characters in identification of cyanobacterial taxa have highlighted the importance of using more reliable method/s. The molecular approaches in cyanobacterial taxonomy including DNA base composition (Kaneko *et al.*, 1996, 2001), DNA hybridizations (Kondo *et al.*, 2000), gene sequencing (Nubel *et al.*, 1997) and PCR fingerprinting (Rasmussen and Svenning, 1998; Versalovic *et al*., 1991) has been highlighted as a result. Furthermore, cyanobacterial-specific methods not requiring axenic cultures are of utmost importance since such cultures are difficult to obtain (Choi *et al.,* 2008). Some of the representative isolates from the present study are presented in figure 3.

CONCLUSIONS

Though a number of studies were carried to evaluate the occurrence of cyanobacteria in fresh water bodies in Sri Lanka, the present study provided baseline information on culturable cyanobacteria from freshwater bodies. In the present study, a total 64 culturable cyanobacteria were isolated. The study revealed that occurrence of cyanobacteria has a positive relationship with water temperature, pH and turbidity. Also, water from respective reservoirs provides favorable conditions for cyanobacterial growth rather than chemical media alone. Future studies can be carried out with the help of molecular identification of the isolates.

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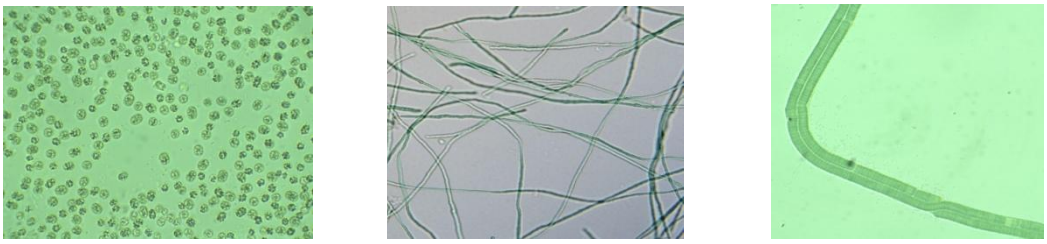

A **B** **C**

Figure 3: A: *Microcystis* sp., B: *Leptolyngbya* sp. and C: *Oscillatoria* sp. recorded in the study.

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