Native Cyanobacteria from Sri Lankan Salt Marsh Ecosystem: A Promising Sustainable Solution to the Prevailing Food Crisis

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***Abstract*—Hyperinflation, poverty, and environmental degradation lead to a sudden deterioration of the nutritional status in Sri Lanka. Crop-based food supply is more challenging due to rapid depletion of arable lands and limited availability of seasonal crops. Native cyanobacteria from extreme environments, specifically adapted with unique, richer nutrient profiles, could be promising alternatives for crop-based food production. Minimal growth requirements ensure their optimal growth in local environments and facilitate convenient utilization with minimal disturbance to the local biodiversity. In line with the above, 08 native cyanobacteria namely; *Leptolyngbya*, *Phormidium*, *Nostoc*, *Pseudanabaena*, *Nodosilinea*-I, *Limnothrix*, *Nodosilinea*-II, and *Oscillatoria* were isolated and morphologically characterized from salt marsh ecosystem in Mannar (8.9810° N, 79.9044° E), Sri Lanka, and nutrient analysis was carried out to identify their potential as promising natural alternatives to overcome food insecurity. Dry biomass of the strains was analyzed for total carbohydrate and protein contents using Dubois' method and Lowry method, respectively. Two strains showed more than 50% of total protein contents with the highest (58.0%) in *Pseudanabaena* sp. Three strains showed more than 40% of total carbohydrate contents with the highest (44.6%) in *Nodosilinea* sp.-II. Two more strains showed more than 25% of total carbohydrate contents. Results were promising and comparable with/higher than the contents found in general protein and carbohydrate food sources, highlighting their significant potential to be developed as macronutrient supplements. Rapid regeneration, easy access and availability throughout the year, make their utilization more promising over plant materials, as a sustainable approach to overcome food insecurity.**

Keywords*—Cyanobacteria, extreme environments, food insecurity, macronutrients

I. Introduction

Poverty, inequality, environmental degradation and climate changes have contributed towards a cumulative impact on the food supply, leading to a rapid deterioration of health and nutritional status of the people. Crop-based food supply is a challenge today, as number of arable lands are rapidly degrading with rapid, ever growing population. Many crops are seasonal with limited availability. Thus, the resulting food production is not sufficient to feed the whole population. It leads to nutritional inequality causing many health issues in different groups of the global population. According to the report on 'The state of the world's children 2019: Children, food and nutrition', malnutrition is more prevalent among children and adolescents from the poorest communities while the overweight in the child population is increasing at an alarming rate in rich countries. This creates a rapid deteriorating pattern of health and nutritional status in the world [1]. With the limitations of crop/plants-based food production, identifying a sustainable approach with other natural and safe alternatives is essential to ensure nutritional equity among communities. Photosynthetic cyanobacteria could be a better alternative for crop-based food production. Cyanobacteria species such as *Spirulina* spp. are rich with proteins contributing to more than 50% of its dry biomass while minerals are contributing to 2.2-4.8% of its dry biomass. Thus, *Spirulina* spp. have been identified as suitable nutritional supplements for vegetarians and are utilized as food and feed additives in food, pharmaceutical and cosmetics industries [2]. *Nostoc* sp. and *Anabaena* sp. have also been identified as nutrient rich sources and are utilized in Chile, Mexico, Peru and Philippines as human food [2]. However, cyanobacteria from extreme environments may have richer nutrient profiles compared to commonly found cyanobacteria as they could be uniquely adapted to ensure their survival in extreme environments. Identification of these potential native strains would be more important as they can be easily grown in local environmental conditions and can be utilized sustainably in industrial applications, without causing any threats to the local biodiversity. Therefore, this study was carried out to investigate and identify the potential of cyanobacteria from Sri Lankan extreme environments in providing a sustainable approach to address the prevailing food insecurity.

II. Methodology

A. Sample Collection, Preparation and Isolation

Water samples were collected from 06 sites of the salt marsh ecosystem in Mannar (8.9810° N, 79.9044° E), Sri Lanka. Samples were cultured in BG-11 medium under the fluorescent light with 2000 lux constant light intensity, in a shaking incubator at a 200-rpm shaking speed. After cyanobacteria growth was observed, they were sub cultured onto agar plates containing the same BG-11 medium solidified with 1.5% (w/v) bacteriological agar [3], using the spread plate method. Frequent sub culturing was carried out using spread plate and streak plate culturing techniques and uni-algal cultures were obtained by transferring isolated colonies repeatedly to newly prepared plates. Then isolated uni-algal cultures were sub cultured in to liquid BG-11 medium in 100 ml and 250 ml conical flasks.

B. *Morphological Characterization of Purified Monocultures of Cyanobacteria*

Prepared slides were observed under the compound light microscope (Euromex Bioblue.lab bb. 1153-pli). Microscopic images were photographed using Image Focus 04 software. Morphological characteristics of the strains were carefully observed, studied, and compared with the morphological characteristics described by reference [4] for identification.

C. *Mass Culturing of Cyanobacteria*

Mass culturing of 08 morphologically characterized strains was carried out in sterile 50 L size fish tanks with one fifth strength of respective BG-11 at pH 7.5 along with aeration, under greenhouse environment with natural light and ambient temperature conditions. The biomass was harvested using filtration methods. The dried biomass was then ground to obtain a fine powder using a mortar and pestle and the powdered biomass was used for nutrient analysis.

D. *Nutrient Analysis*

Total carbohydrate content was analyzed using Dubois' method [5]. Twenty-five milligrams of dry cyanobacteria biomass were used for the analysis. The biomass was first hydrolyzed with 2.5 N HCl and the diluted, hydrolyzed extract was used for the analysis. The total carbohydrate content was calculated using the standard curve plotted with D-Glucose. The total protein content was analyzed using Lowry method [6]. Twenty milligrams of the dry biomass were used for the analysis. The final protein concentration was calculated using the standard curve established with bovine serum albumin dissolved in lysis buffer. Three replicates of the samples were used in each analysis. Data were statistically analyzed and compared using One-way ANOVA Tukey Pair-wise comparison (p=0.05) using Minitab 17 (2016) software version 2.0.

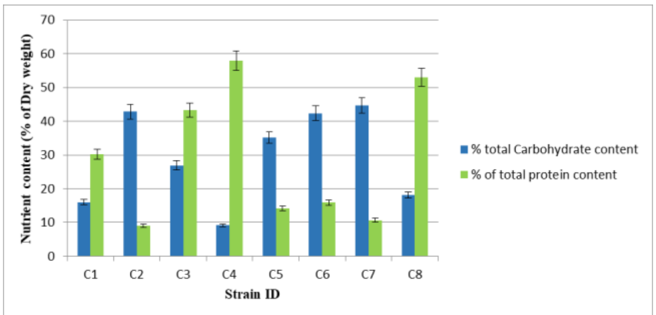

Fig. 1. Total carbohydrate and protein contents as a percentage of dry weight of biomass in eight cyanobacteria strains tested

III. RESULTS

Based on the careful microscopic observations and comparisons carried out with the morphological characteristics described by reference [4], eight isolates were morphologically characterized as; C1-*Leptolyngbya* sp., C2-*Phormidium* sp., C3-*Nostoc* sp., C4-*Pseudanabaena* sp., C5-*Nodosilinea* sp.-I, C6-*Limnothrix* sp., C7-*Nodosilinea* sp.-II, and C8-*Oscillatoria* sp.

Pseudanabaena sp. (C4) and Oscillatoria sp. (C8) were recorded with the highest total protein contents of 58.0% and 53.0%, respectively (Fig. 1). Considerably higher total protein contents found in these strains were closer to the recorded average protein contents (63.0%) of Spirulina plantensis [7] which is widely used as a food source all over the world. Therefore, results highlight the potential of these native strains to be improved as protein supplements.

Moreover, Nostoc sp. (C3) was recorded with significant amounts of total protein (43.3%) and total carbohydrate (27.0%) contents (Fig.1). Nostoc is a well-known blue green alga which is appreciated as a healthy food and traditional medicine all around the world [8]. Specifically, Nostoc commune has been consumed in many countries in Asia and utilized as an ingredient in traditional medicine in China [8]. These strains were isolated and purified from salt marsh ecosystem where they are highly exposed to frequent dehydration and rehydration, osmotic stress, and radiation induced stresses. All these conditions could be favorable for these native strains to produce water stress proteins and it could be a possible reason for them to show remarkably higher protein contents compared to other species. Among tested strains, C7 which was morphologically characterized as Nodosilinea sp.-II was recorded with the highest total carbohydrate content of 44.6% while Phormidium sp. (C2) (42.8%) and Limnothrix sp. (C6) (42.4%) also showed more than 40% of total carbohydrate contents (Fig.1) and these contents were significantly higher than the carbohydrate contents (8%-28.4%) in many previously reported cyanobacteria [9]. As carbohydrate sources, cyanobacteria are more promising as they are also known to be easily digestible due to the absence of complex polysaccharides in their cell wall. Thus, the above graph shows promising results for many of the tested strains, highlighting their higher potential to be developed and utilized as nutrient supplements in nutrient-based applications. Cyanobacteria are naturally occurring botanicals and easily available in any environment. Most of them are fast growing, require less space and nutrients for the growth and they can be cultivated throughout the year. Native cyanobacteria growing in extreme ecosystems can uniquely modify themselves to adjust and survive under fluctuating environmental conditions therefore; they can be grown in any local environment, without causing any threats to the local biodiversity. Also, they can be regenerated in the same space. With all these advantageous characteristics and rich nutrient contents, these native strains from salt marshes could be identified as promising alternatives over plant material to ensure food security through an environmentally friendly, cost effective and sustainable approach.

IV. CONCLUSIONS

Native cyanobacteria strains such as *Pseudanabaena* sp. and *Oscillatoria* sp. with rich protein contents can be improved as protein supplements while *Nodosilinea* sp.-II, *Phormidium sp*. and *Limnothrix* sp. can be identified as easily digestible carbohydrate alternatives to be utilized in nutrient based applications. *Nostoc* sp. with considerable amounts of both proteins and carbohydrates can be improved as macronutrient supplements. However, an extensive molecular characterization is essential to confirm the identity of the strains. Their toxin producing capabilities also need to be analyzed as a future step of ensuring their safe consumption. Nutritionally rich strains which are safe for consumption can be further screened and they will be recommended for commercial applications in food industry such as developing them as macronutrient supplements, protein supplements or incorporating them in different food products. As these native strains from extreme salt marsh ecosystems are uniquely and well adapted to the local environments, they ensure convenient and sustainable utilization in nutrient-based applications without causing any threats to the local biodiversity.

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