



# Studies on the Isolation of Lipids from Mangrove Isolated Cyanobacterial Species

P. Devesh, B. Aishwarya and R. Gyana Prasuna†

Department of Microbiology and Food Science & Technology, GITAM School of Science, Gandhi Nagar, Rushikonda, Visakhapatnam-530045, Andhra Pradesh, India

†Corresponding author: R. Gyana Prasuna; gravi@gitam.edu

Nat. Env. & Poll. Tech.  
Website: [www.neptjournal.com](http://www.neptjournal.com)

Received: 04-09-2021

Revised: 15-11-2021

Accepted: 19-11-2021

## Key Words:

Mangroves  
Cyanobacteria  
Phytoplankton  
Lipids

## ABSTRACT

Cyanobacteria are an important source of food and a primary producer of the aquatic food chains. Cyanobacteria are universally photosynthetic, with a higher plant type of photosynthesis, a large number of those also have the pivotal character of nitrogen fixation. Phytoplankton is made up of mostly cyanobacteria and microalgae. The microalgae store food in the form of lipids and serve as the source of energy as well as lipids for the fish. The lipid content of the fish depends on the diet that they take, and some of the lipids like the omega 3 and omega 6 fatty acids are derived from specific microalgae, like the marine protists and dinoflagellates including *Thraustochytrium*, *Schizochytrium*, and *Phaeodactylum*. Cyanobacteria being nutritionally more independent with nitrogen and carbon fixing ability, are more economical to grow in bulk. Hence the present work was aimed to screen the high lipid-containing cyanobacteria for use as fish feed. Five different cyanobacterial isolates, originally obtained from the mangroves were used as the sample cyanobacteria. The mangroves are a unique and at the same time stressful ecosystem. The significance of choosing cyanobacteria from this area is to allow for the isolation of cyanobacteria with unique characteristics. It is known that microorganisms from harsh or unique environments have even more potential for developing special survival strategies and for the production of more secondary metabolites. These cyanobacteria from stress environments can grow in conditions where other cyanobacteria or microalgae do not survive well. The isolation of lipids was performed by different extraction methods and separation using different solvent compositions was performed. The standard growth and biochemical studies of the cyanobacteria were conducted, followed by the assessment of their lipid content and variability. Out of the five isolates, a higher number of lipids were observed in AS1-(1) and AS2-(2). Lipids were isolated in chloroform-methanol and three variations of TLC were used to separate the lipids. The three are the single mobile phase, two mobile phase systems, and 2D development solvent system. The separation of the lipids gave the best results with the two mobile phase system, in which two different mobile solvent mixtures were used sequentially. The isolates AS1-(1) and AS2-(2) exhibited higher lipids, hence they could be a potentially suitable candidate as a fish feed.

## INTRODUCTION

### Cyanobacteria

Cyanobacteria (blue-green algae) are a group of bacteria showing higher plant-like photosynthesis. Cyanobacteria appeared approximately 2.5-3 billion years old and thus are the oldest oxygenic phototrophs on Earth. Even the development of an oxygen-rich atmosphere on Earth is attributed to cyanobacterial photosynthesis (Kumar et al. 2019, Garcia-Pichel 2009).

The cyanobacteria show oxygenic photosynthesis. However, they differ from the higher plants in having only one type of Chlorophyll, the Chlorophyll *a*, and lacking the chlorophyll *b*. Combinations of Chlorophyll *a*, together with other pigments like phycobilins, and carotenoids, result in the most commonly observed blue-green color of the cyanobacteria. The ability to change the pigment composition, by

altering the proportion of various pigments is another unique character. This allows harvesting light as per the light quality available allowing it to grow even in the polar regions, open ocean, and desert regions. In addition, the cyanobacterial nitrogen fixation can be a significant source of biologically available nitrogen in these ecosystems. Cyanobacteria are also one of the primary colonizers of many new ecosystems

Cyanobacteria is a large, heterogeneous group resembling the eukaryotic algae in many ways, including morphological characteristics and ecological niches. In fact, they were considered a type of algae before being regrouped in the kingdom Monera with the other prokaryotes, including the bacteria (Garcia-Pichel 2009).

Cyanobacteria are structurally simple with only one or two types of vegetative cells. Some of the species form the spores with thick walls called the akinetes, which might allow the cyanobacteria to tide over the stressful conditions.

Despite such simple and unspecialized structural evolution, the cyanobacteria show morphologically diverse forms. They show a very wide occurrence, in both the aquatic and terrestrial habitats (Sompong et al. 2005). They have widespread occurrence due to their physiological diversity, the filamentous species also occur either freely or in groups forming trichomes. Species such as certain *Nostoc* forms synthesize extracellular mucilage embedding the filaments in them and forming large structures like bunches and balls. The filamentous forms include nonheterocystous, and heterocystous genera. Heterocysts are specialized cells harboring enzymes for nitrogen fixation, a process by which atmospheric nitrogen ( $N_2$ ) is converted to a biologically useful form ( $NH_3$ ). All heterocystous and some non-heterocystous coccoid/filamentous cyanobacteria also fix nitrogen. By nitrogen fixation, the cyanobacteria can occupy the most varied ecosystems, including those devoid of reduced nitrogen compounds (Prasanna et al. 2009)

Most cyanobacteria do not grow in the absence of light (they are obligate phototrophs); however, some can grow in the dark if there is a sufficient supply of glucose to act as a carbon and energy source, this could be required for the symbiotic associations with plants where they are harbored away from light or when buried below the soil layers.

In addition to being photosynthetic, many species of cyanobacteria can also “fix” atmospheric nitrogen—that is, they can transform the gaseous nitrogen of the air into compounds that can be used by living cells. Particularly efficient nitrogen fixers are found among the filamentous species that have specialized cells called heterocysts (Garlapati et al. 2020). The heterocysts are thick-walled cells that have special structural features to make them impermeable to oxygen; they provide the anaerobic (oxygen-free) environment necessary for the operation of the nitrogen-fixing enzymes. In Southeast Asia, nitrogen-fixing cyanobacteria often grow abundantly in rice paddies, thereby eliminating the need to apply nitrogen fertilizers (Chittora et al. 2020)

Cyanobacteria reproduce asexually, either utilizing binary or multiple fission in unicellular and colonial forms or by fragmentation and spore formation in filamentous species. Under favorable conditions, cyanobacteria can reproduce at explosive rates, forming dense concentrations called blooms. Cyanobacteria blooms can color a body of water. For example, many ponds take on an opaque shade of green as a result of overgrowths of cyanobacteria, and blooms of phycoerythrin rich species cause the occasional red color of the Red Sea. Cyanobacteria blooms are especially common in waters that have been polluted by nitrogen or phosphorous wastes; in such cases, the overgrowths of cyanobacteria can consume so much of the water’s dissolved oxygen that fish

and other aquatic organisms perish (Ariosa et al. 2003). Two species, which fall under this category, are *Anabaena* and *Microcystis* (Watson et al. 2015, Kumar et al. 2019).

Cyanobacteria are one of the most successful organisms having survived so many ecological and environmental changes, in their atmosphere. There has been very little change in their structure. The physiological adaptations to global change have played a significant role in the success of cyanobacteria. As a group, they can tolerate desiccation, very cold and very hot temperatures, hypersalinity, variable visible light conditions, and high ultraviolet light conditions (Garcia Pichel 2009, Yamamoto 2009).

Cyanobacteria account for a larger proportion of the phytoplankton. Though the cyanobacteria have a simple structural variability, they are limited to only three cell types. They still show a wide variety of morphological, genetic, and ecological diversity in their occurrence in nature (Nayak et al. 2007). The cyanobacteria also present a very wide secondary metabolite production, with applications in the food, feed, pharmaceutical, and nutraceutical industries (Berg & Smalla 2009, Kumar et al. 2019, Carpine & Sieber 2021).

In addition to the large variability of production, the stability of the products of cyanobacterial origin has attracted scientists towards them (Carpine & Sieber 2021). The metabolites are stable over a wide range of pH and temperature and are generally easily soluble in water. There are over 200 genera of cyanobacteria, however, the production of secondary metabolites is more commonly reported from the cyanobacteria belonging to the order Oscillatoriales (49%), Nostocales (26%), Chroococcales (16%), Pleurocapsales (6%), and Stigonematales (4%) (Gerwick et al. 2008).

Foods containing the required amounts of essential nutritional compounds, such as carbohydrates, proteins, fats, vitamins, and minerals, are termed functional foods. They are designed to also contain certain bioactive compounds, exhibiting additional benefits for human health. Such bioactive compounds are generally derived from various plant or microbial sources (Liu et al. 2021).

One of the microorganisms used as a source of bioactive compounds cyanobacteria, has been exploited as a potential food supplement since ancient times. *Spirulina maxima* and some other cyanobacteria have been used as food for more than 600 years (Afnovandra et al. 2021, Alagawany et al. 2021).

The population of the world is on an ever-increasing wave from six billion in 1999 to seven billion in 2011, which may easily reach nine billion by 2050. The population puts pressure on the agricultural resources. Not only the population has to be provided with food, but also it has to be balanced

to overcome the nutritional deficiency. The fish is worldwide recognized as a delicacy with local flavors of each place. In addition, it is a very healthy alternative to providing nutrient-rich food. Fish also occupy a very special place, being one of the richest and in many cases the only way of obtaining omega 6 fatty acids, which helps to provide the crucial balance to the omega 3 fatty acids (Castej6 & Se6or6ns 2020, Jovanovic et al. 2021).

The food needs require that we check into alternative resources for food as well as active principles such as prebiotics, probiotics, or essential fatty acids (Afnovandra et al. 2021, Alagawany et al. 2021).

Despite a large number of plants and other sources being used as a source of active principles of health care or to provide nutrient benefits, cyanobacteria are still underutilized. *Spirulina* are probably the only cyanobacteria that have been utilized to a larger extent (Alagawany et al. 2021).

### Cyanobacteria as A Source of Lipids and Protein For Fish Feed

#### Use of Lipids in Fish Feed

- Lipids play important physiological roles in providing energy, essential fatty acids, and fat-soluble nutrients for the normal growth and development of fish.
- Fish oil, because of its high content of essential fatty acids, is used as the main lipid source in marine fish feeds.

Cyanobacteria are considered to have given rise to the higher eukaryotic chloroplasts through endosymbiosis. This appears true as the cyanobacterial lipids are similar to those in the inner envelope membranes and thylakoid membranes of the chloroplasts of higher plants. On the other hand, they show differences from those of many other bacteria, which generally show phospholipids as a major component of glycerol lipids. The cyanobacteria exhibit monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol, and a phospholipid, phosphatidylglycerol, as major glycerolipids (Oliveira et al. 2018, Uma et al. 2020). They also contain glycolipids. In the absence of reliable morphological and structural characteristics to differentiate between the different cyanobacterial genera, one taxonomic classification is also based on the type of fatty acids in the cyanobacteria. The lipids are also most sought after for commercial uses as the source of essential fatty acids for fish and animals and more vigorously in the recent past as the source of biofuel (Uma et al. 2020).

The ability to act as an important source of lipids and oils is due to the fast growth of many of the cyanobacteria. As also the significant quantities produced by many. The lipids

of cyanobacteria are generally esters of glycerol and long fatty acids. They may be either saturated or unsaturated. In the natural environment, cyanobacteria serve as one of the natural sources of essential fatty acids for animals (Semanti et al. 2021). These are also the same as required in the human diet. These essential fatty acids include the C18 linoleic (18:2 $\omega$ 6) and  $\gamma$ -linolenic (18:3 $\omega$ 3) acids and their C20 derivatives, eicosapentaenoic acids (20:5 $\omega$ 3) and arachidonic acid (20:4 $\omega$ 6). Certain filamentous cyanobacteria show a higher production of the polyunsaturated fatty acids accounting for 25 to 60 % of the total fatty acids. (4-6). Marine microalgae have been studied more with respect to the accumulation of lipids. Some cyanobacteria also show accumulation of lipids as reserve material.

## MATERIALS AND METHODS

### Isolation and Purification of Test Strains

A total of six heterocystous filamentous cyanobacteria were used in this study. All these strains were isolated from rice fields of Visakhapatnam and mangroves of Kakinada, India. The strains were subjected to purification by plating, sub culturing in sterile nitrogen source-free BG-11 agar medium (Rippka et al. 1979).

Cultures were inoculated in 500 and 1000 mL Erlenmeyer flasks containing 40% of the volume as BG-11 nitrogen source free medium and incubated at 28 $\pm$ 2 $^{\circ}$ C, in continuous light intensity from the cool white light source.

### Culture Conditions

The cyanobacteria were maintained on the standard BG 11 broth or agar medium. The medium composition is as under. Citric acid monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>) - 6 g.L<sup>-1</sup>, Ferric ammonium citrate (C<sub>6</sub>H<sub>8</sub>FeNO<sub>7</sub>) - 6 g.L<sup>-1</sup>, dipotassium hydrogen orthophosphate (K<sub>2</sub>HPO<sub>4</sub>) - 40 g.L<sup>-1</sup>, magnesium sulfate heptahydrate (MgSO<sub>4</sub>) - 75 g.L<sup>-1</sup>, calcium chloride dihydrogen (CaCl<sub>2</sub>) - 36 g.L<sup>-1</sup>, sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) - 20 g.L<sup>-1</sup>, ethelene diamine tetra acetic acid (EDTA) - 1 g.L<sup>-1</sup> (0.1g.100 mL<sup>-1</sup>), trace metal solution. After proper mixing of solution, the medium was sterilized in an autoclave at a temperature of 121 $^{\circ}$ C, maintaining 15lb/inch<sup>2</sup> pressure for 15 min. Stock - 2 (ferric ammonium citrate) and stock - 3 (dipotassium hydrogen phosphate solutions) were sterilized separately and added to the medium after cooling as per Stanier et al. (1971).

### Inoculation

**Soil dilutions:** 1 g of soil sample was taken and serially diluted until 10<sup>-9</sup> dilutions. 10<sup>-6</sup> and 10<sup>-7</sup> dilution tubes of each culture were used to inoculate in 5 mL of BG11 broth

medium. An observation for growth was made after 10 to 15 days.

**Direct soil suspension:** For another set, 1g of soil sample was taken and added to 20 mL of saline or BG11 medium in 100 mL conical flasks. Put on a shaker for about 2 h for a complete suspension of the soil sample. Left to settle overnight and then inoculated the supernatant on solid BG 11 medium.

**Direct slide inoculation:** The soil sample was suspended in a minimum amount of water, and allowed to settle. The supernatant was later observed under the microscope. Capillary tubes were used to pick up visible cyanobacterial filaments, on observation and the tubes were put directly into 10 mL of BG11 broth medium in test tubes. The inoculated test tubes were incubated for growth.

**Culture maintenance:** The test cyanobacteria inoculated both on agar and broth medium were subcultured periodically. The colonies on Petri plates were used for restreaking and plating onto agar media to maintain the cultures. Re-streaking was performed monthly.

**Growth:** To assess the growth of various test organisms, a growth curve was made by repeated growth measurement over a period of 10-12 days. For this, the 20 mL culture tubes were used with the 5 mL of BG 11 broth. A homogenized thick suspension of the cyanobacterial samples was prepared by centrifuging actively growing culture suspension followed by homogenization. Homogenization was performed using a mechanical glass homogenizer in laminar airflow to maintain sterile conditions. An equal quantity of the thick suspension was added as inoculum in each of the tubes to a final O.D. to 0.08 -0.09 at 663 nm.

These inoculated culture tubes were transferred to culture racks. Care was taken to ensure all the tubes were equidistant from the light source. Every alternate day 3 test tubes with the suspension were utilized individually for doing growth and biochemical analysis. Growth measurement of samples was carried out at a 2-day interval for 16 days. Absorbance was measured in UV-Visible Spectrophotometer at 670 nm (Plate 13).

**Lipid estimation:** Equal quantity of cultures of the six cyanobacteria cultures AS2(2), AS1(2), AS1(1), AS5(1), AS4(2), and AS3(1) was taken. The cultures were homogenized in mortar and pestle. From this homogenized culture again, equal quantities of each culture were taken in three replicates each.

An equal amount of the homogenized culture was taken in centrifuge tubes and centrifuged at 5000 rpm for 5 min at 16 degrees temperature in high-speed centrifugation. The pellet was extracted in chloroform and methanol (2:1) repeatedly for complete extraction. The extracted solution

was transferred to the preweighed Petri dish and kept the plates in a hot air oven for 1 hour at 45 degrees temperature, for evaporation of the supernatant and collection of lipids. After complete evaporation, the Petri dishes were removed from the hot air oven and the weight of the dish was recorded. The lipids were redissolved in 1 mL of extract solvent and collected and stored in Eppendorf tubes.

### Preparation of Tlc Plates

TLC plates were poured using silica slurry and allowed to dry. After drying the TLC plates were kept in the hot air oven for 3 hours at 80 degrees temperature for activation.

### Lipid separation Using TLC

The separation of isolated cyanobacterial lipids was carried out in 3 ways as follows:

1. Single mobile phase
2. Dual mobile phases
3. 2D direction system

#### Single Mobile Phase

Chloroform, methanol, acetic acid, and water (80 mL: 9 mL: 12 mL: 2 mL) were used in the mobile phase.

#### Procedure

**Single mobile solvent system:** The lipid samples were loaded about 2cm from the bottom end of the TLC plate. For the single mobile solvent system, after a run covering 70% of plates, the plates were dried and developed with iodine crystals in a glass chamber, Followed later with the UV observation.

**Two mobile phase solvent systems:** In this method, the TLC was first run in the mobile phase of chloroform, methanol, and water (60 mL: 30 mL: 5 mL) till the 50% run was over. Whereafter the second mobile phase made of hexane, diethyl ether, and acetic acid was used (80 mL: 20 mL: 1.5 mL).

**2D development system:** The lipid extract from a single organism was taken in the TLC plate and treated with two solvent systems, after the completion of the run in one direction with one solvent system, the run was completed in the second direction after turning the TLC plate by 90 degrees. The first run of TLC plates was run in this solvent after rotating by 90 degrees, so that the run line of lipids in the first direction is towards the bottom, till the solvent front reached about 90% of the TLC plate. After drying the TLC plate, The chromatogram was developed with iodine crystals in a glass chamber.

Protein estimation for the different cultures (AS3-(1), AS2(4), AS1-(2), AS2-(2), AS5-(1), AS1(1)) was done using

Folin's method. 1mg of algal biomass + 1 mL of 1N NaOH was taken in a test tube and placed in a boiling water bath for 10min. The blank/sample tubes were added with reagent A ( 1 mL of freshly prepared Na-K tartrate solution containing 0.5% of  $\text{CuSO}_4$  and 2%  $\text{Na}_2\text{CO}_3$ ) and incubated for 10min. After incubation 0.5 mL of reagent B (Folin reagent) was added and incubated again for another 15min. Finally, the supernatant absorbance was recorded at 650nm. BSA solution standard curve was used to estimate the protein content (Lowry et al. 1951)

### Carbohydrates

Carbohydrates in the six test cultures were estimated by the anthrone method. 1mg of algal sample and 1.25 mL of double distilled water were added to a test tube, to this suspension freshly prepared Anthrone reagent (4 mL) was added and mixed thoroughly in the same way blank/ standard/sample tubes were prepared and these tubes were placed in boiling water bath for 10 min. The absorbance of the supernatant was observed at 620 nm against blank. The carbohydrate content was analyzed with a standard glucose curve (Spiro 1966)

## RESULTS AND DISCUSSION

### Growth Observation

The growth curve of all the six isolates was plotted (Fig. 1). It was observed that all the isolates showed an increase in growth as the time passed however in general an increase in

growth was obtained from the fourth day after inoculation. The highest growth was obtained in AS-2(2). Followed by AS-2(4) and AS 1(1).

### Protein

The protein content of the isolates was also quantified as a standard growth parameter. The protein values were taken after a growth of 15 days for all the cultures (Fig. 2). An equal quantity of the biomass was taken for protein assay. The highest amount of protein was obtained with AS3(1) with 38 micrograms per milligram protein, followed by As-2(2) with less than 37  $\mu\text{g}/\text{mcg}$  per mg. The minimum quantity was observed in AS-5(1) at 28  $\mu\text{g}/\text{mcg}$  per mg.

### Estimation of Carbohydrates

**Carbohydrate analysis:** Carbohydrate content was highest in AS1 (1) followed by AS-2(4) and AS-2(2). The graphs and results show that carbohydrate content was low in AS-5[1] and the highest carbohydrate content was seen in AS-2[4] followed by AS-3[1] (Fig. 3).

### Lipids

**RF value of lipids in single mobile phase:** The five cyanobacterial isolates each showed separation of two lipid spots. With almost similar Rf, in the common range of 0.45 to 0.48 and 0.52 to 0.54.

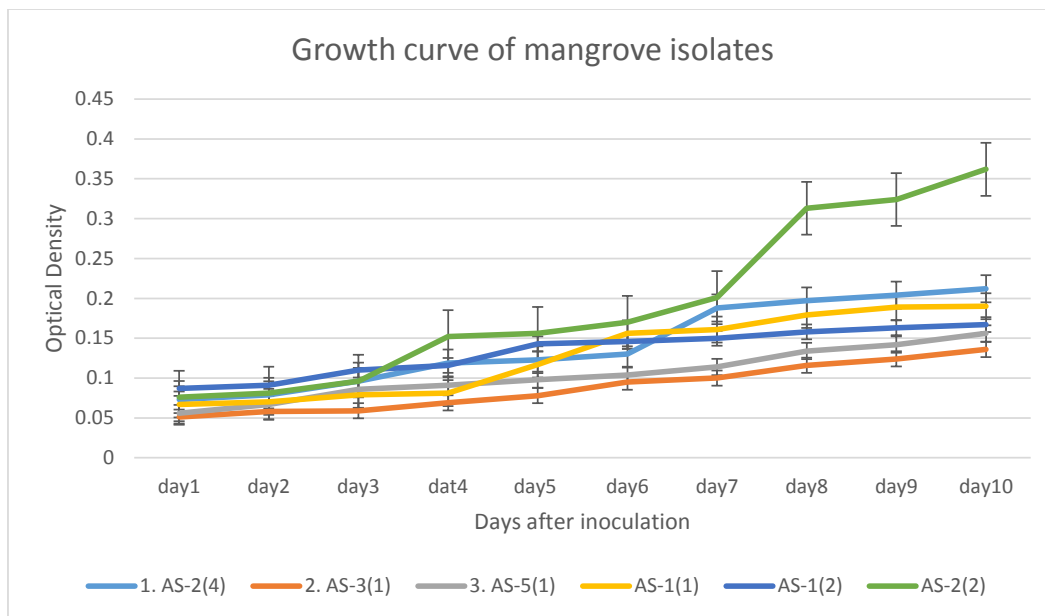


Fig. 1: Growth curve of the six cyanobacteria (Mangrove isolates), the growth was recorded every day for up to 10 days. Growth was measured as optical density at 663 nm.

**RF value of lipids in two mobile phases:** In two mobile phase systems AS1-(1) showed the largest number of the lipid spots with 9 separate spots developing with iodine followed by AS2-(2) (Plates 1-3 and 7-9)). AS4-(2)(Plate 10), and AS5-(1) (Plates, 12) both showed the development

of seven lipid spots the least number were observed in AS3-(1) (plate-11). AS1-(2) showed the development of only six spots (plates 4-6). This result clearly indicates the much better separation of lipids spots of the cyanobacteria with the two solvent systems.

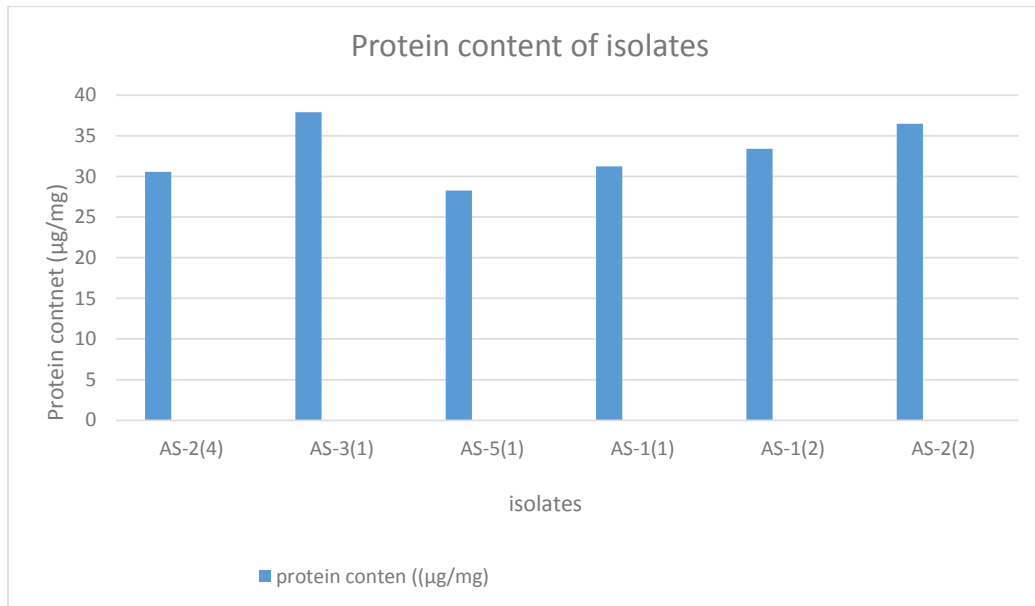


Fig. 2: Protein content of test cyanobacterial isolates (AS-2(4), AS-3(1), AS-5(1), AS-1(1), AS-1(2), and AS-2(2)) was tested for 15 DOI cultures of equal biomass (as a Chl *a* measure).

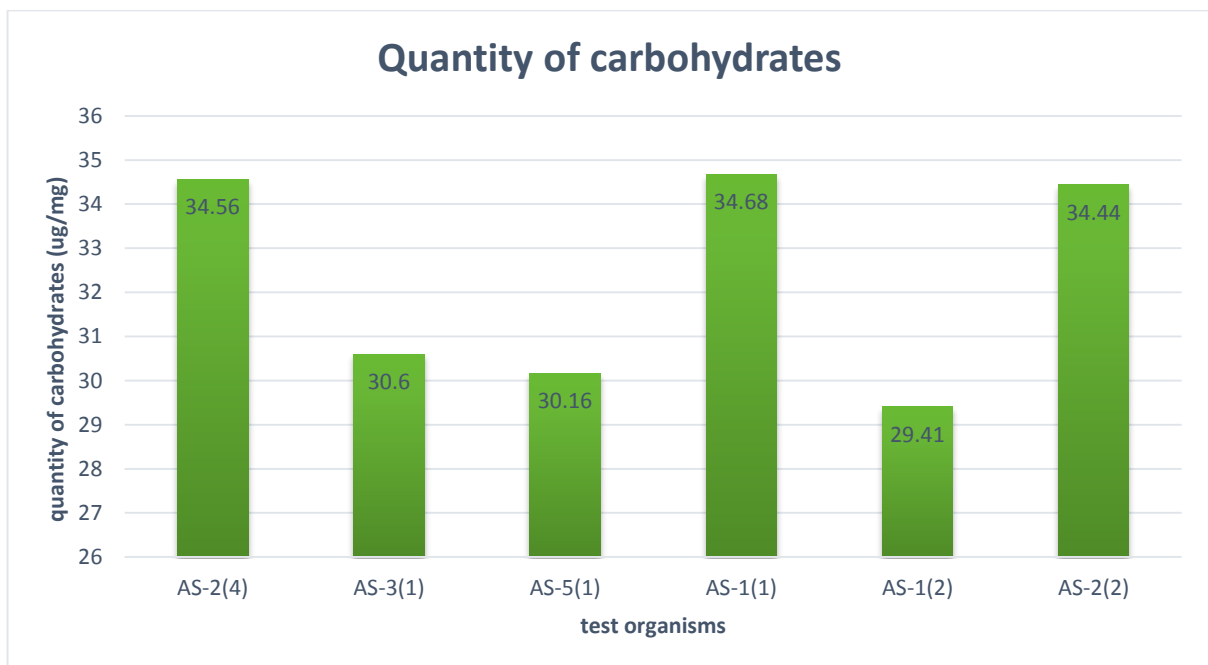


Fig 3. Quantification of carbohydrates in cyanobacterial isolates by anthrone method. Equal biomass (as a measure of OD at 663nm) of all five isolates was used to test for total carbohydrate.

**RF value of lipids in the 2D development system**

1. AS1-(1)

**Step-1**

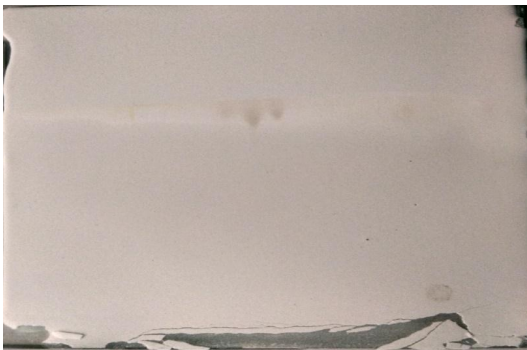
chloroform : methanol: water



**Plate 1:** Lipid separation using Two mobile phases; Step 1, of 2D separation of isolate AS1-(1)

**Step-2**

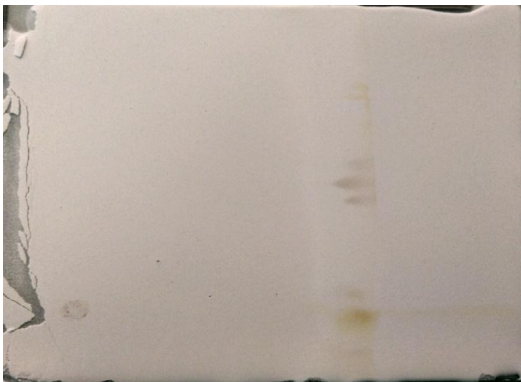
Hexane: Diethyl ether: Acetic acid



**Plate 2:** Lipid separation using Two mobile phases; Step 2, lipids after separation of isolate AS1-(1)

**Step-3**

Treating with iodine

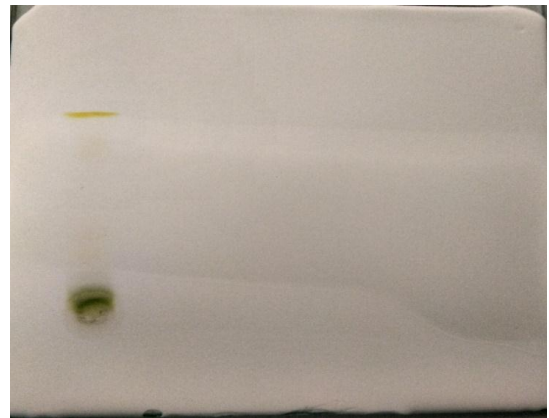


**Plate 3:** Lipid separation using Two mobile phases; Step 3, lipids stained with Iodine vapors of isolate AS1-(1)

2. AS1-(2)

**Step-1**

chloroform: methanol: water



**Plate 4:** lipid separation using Two mobile phases; Step 1, of 2D separation of isolate AS1-(2)

**Step-2**

Hexane: Diethyl ether: Acetic acid



**Plate 5:** Lipid separation using Two mobile phases; Step 2, lipids after separation of isolate AS1-(2)

**Step-3**

Treating with iodine

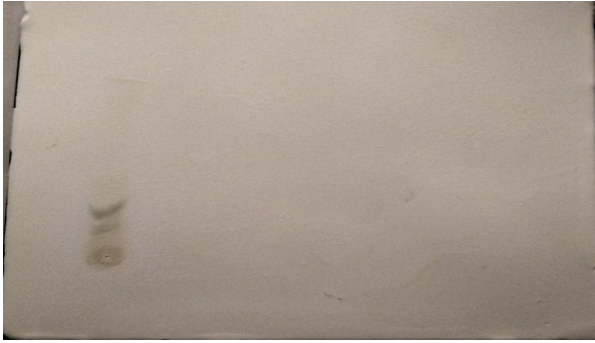


**Plate 6:** Lipid separation using Two mobile phases; Step 3, lipids stained with Iodine vapors of isolate AS1-(2)

## 3. AS2-(2)

**Step-1**

chloroform : methanol : water



**Plate 7:** Lipid separation using Two mobile phases; Step 1, of 2D separation of isolate AS2-(2)

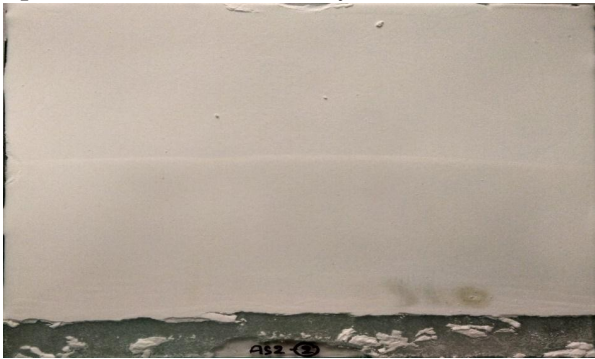
## 4. AS4-(2)



**Plate 10:** Lipid separation using Two mobile phases AS4-(2)

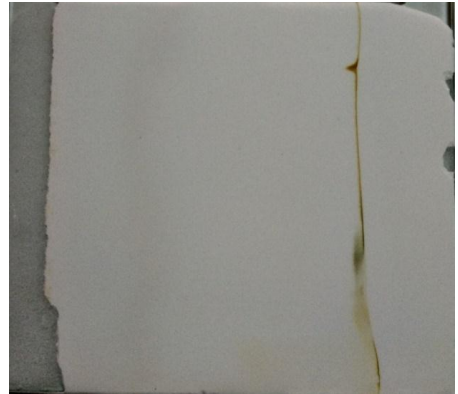
**Step-2**

Hexane : Diethyl ether : Acetic acid

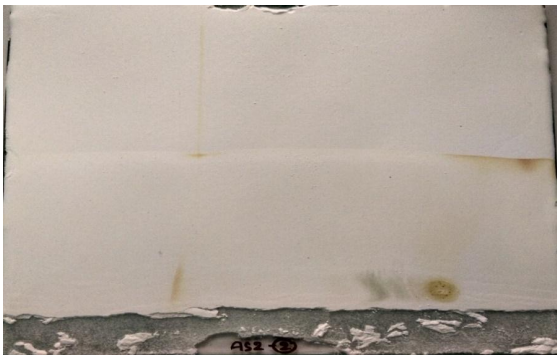


**Plate 8:** Lipid separation using Two mobile phases; Step 2, of 2D separation of isolate AS2-(2)

## 5. AS3-(1)

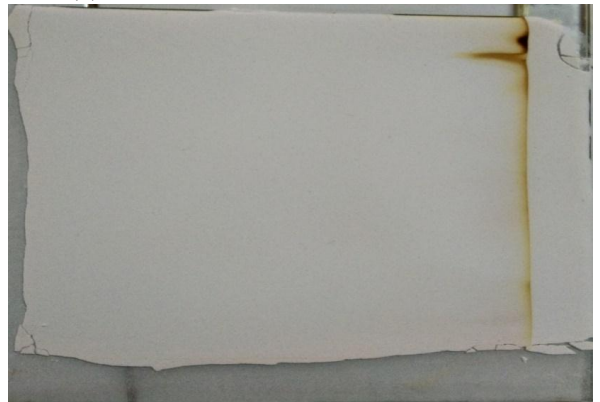


**Plate 11:** Lipid separation using Two mobile phases AS3-(1)

**Step-3** Treating with iodine

**Plate 9:** Lipid separation using Two mobile phases; Step 3, of 2D separation of isolate AS2-(2)

## 6. AS5-(1)

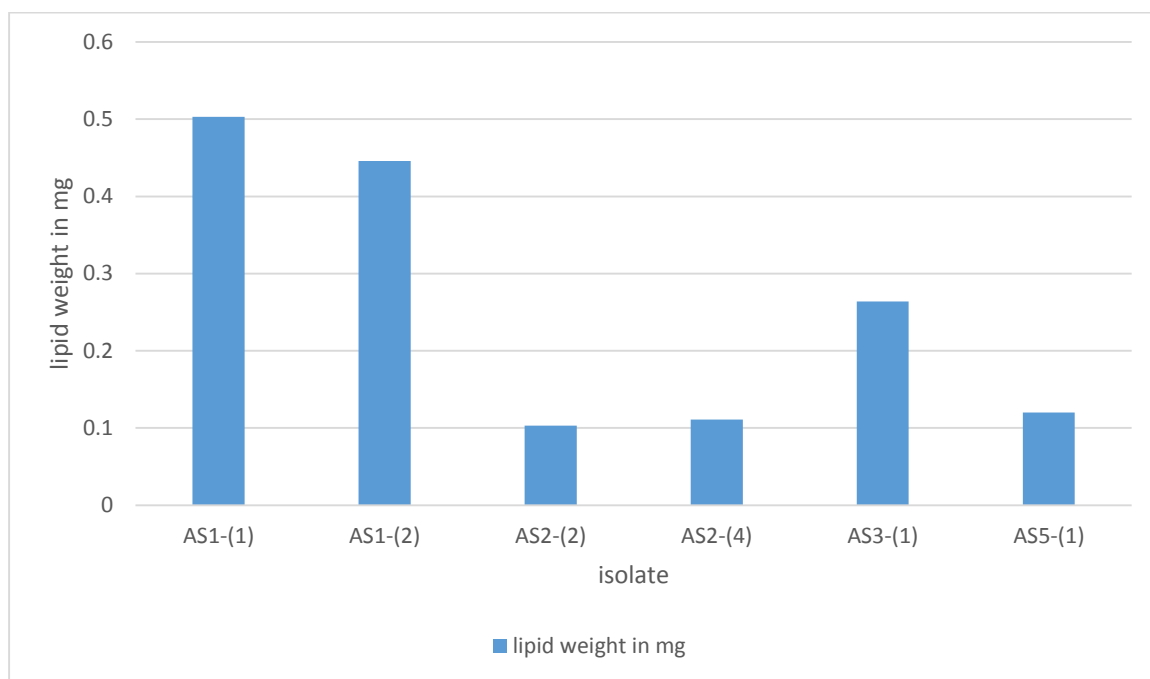


**Plate 12:** Lipid separation using Two mobile phases AS5-(1)





**Plate 13:** Cultures growing under the light.



**Fig. 4:** Lipid weight (Lipid isolated from the same amount of culture).

The third type of lipid separation used was the 2D system, however, we were unable to find good development of spots, which may have been likely due to the need for standardization of the solvent system.

The lipid weight of the lipid isolated from the same amount of culture is shown in Fig. 4. Just as observed with

the number of lipid spots developed in the two solvent systems, the lipid quantity was highest in AS1-1, however, AS1-(2) which showed production of 0.446 mg of the lipid exhibited only six spots.

It is well known that lipid production differs quantitatively and qualitatively depending on the need in different

cyanobacteria. It would be interesting to find out if the higher number of lipids produced would improve the lipid levels of a fish diet or the high quantity.

**Conclusion:** Our work points out that mangrove cyanobacteria can be used as a source of good protein and lipid content for fish or other aquatic animals.

The lipid content of cyanobacteria could prove a very important source for introducing lipids into fish.

## ACKNOWLEDGMENT

RGP thanks the Ministry of Earth Sciences for the Major Research project with no. 27/04/2017; MoES/36/OOIS/Extra/57/2016. BA thanks the MOEs grant for the JRF grant. The authors thank GITAM GSS for providing the facilities to work.

## REFERENCES

- Afnovandra, B., Zulkarnain, P., Arif Juliar, C., Abdi Dharma, K., Zakaria, I.J., Syafrizayanti, A.B. and Putra, M.Y. 2021. Omega-3 fatty acids of microalgae as a food supplement: A review of exogenous factors for production enhancement. *Algal Research*, 60.
- Alagawany, M., Taha, A.E., Ahmed Noreldin, K.A., El-Tarabily, M. and Abd El-Hack, E. 2021. Nutritional applications of species of *Spirulina* and *Chlorella* in farmed fish: A review, *Aquaculture*, 542.
- Ariosa, Y., Carrasco, D., Qucsedda, A. and Valentine E.F. 2003. Incorporation of different sources and light response curves of nitrogenase and photosynthesis by cyanobacterial blooms from rice fields. *Microbial Ecol.*, 51: 394-403
- Berg, G. and Smalla, K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.*, 68: 1-13.
- Carpine, R. and Sieber, S. 2021. Antibacterial and antiviral metabolites from cyanobacteria: Their application and their impact on human health. *Curr. Res Biotechnol.*, 3: 65-81.
- Castej6, N. and Se6or6ns, F.J. 2020. Enzymatic modification to produce health-promoting lipids from fish oil, algae and other new omega-3 sources: A review, *New Biotechnology*, 57: 45-54.
- Chittora, D., Mukesh, M., Tansukh, B., Prashant, S. and Sharma, K. 2020. Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochem. Biophys. Rep.*, 22: 1-8.
- Garcia-Pichel, F. 2009. Cyanobacteria. In Schmidt, T. (ed), *Encyclopedia of Microbiology*, Third Edition, Elsevier Publications, The Netherlands, pp. 107-124
- Garlapati, D., Thangavel, M., Susaimanickam, A., Tharifkhan, S.A., Devanesan, A.A. and Arivalagan, P. 2020. Applications of microalgal and cyanobacterial biomass on a way to safe, cleaner, and sustainable environment. *J. Clean. Prod.*, 11: 253.
- Gerwick, W.H., Coates, R.C., Engene, N., Gerwick, L., Grindberg, R.V., Jones, A.C. and Sorrels, C.M., 2008. Giant marine cyanobacteria produce exciting potential pharmaceuticals. *Microbe-American Society for Microbiology*, 3(6): 277.
- Hushchyna, K. and Nguyen-Quang, T., 2020. A New Index Contributing to an Early Warning System for Cyanobacterial Bloom Occurrence in Atlantic Canada Lakes. *Nat. Env. & Poll. Tech.*, 19(5): 1887-1897.
- Jovanovic, S., Dietrich, D., Becker, J., Kohlstedt, M. and Wittmann, C. 2021. Microbial production of polyunsaturated fatty acids-high-value ingredients for aquafeed, superfoods, and pharmaceuticals. *Curr. Opinion Biotechnol.*, 69: 199-211.
- Kumar, D.S., Tyagi, M.B. and Kumar, A. 2019. Applications in Biotechnology in Cyanobacteria. In Mishra, A.K., Tiwari, D.N. and Rai, A.N. (eds), *Cyanobacteria: From Basic Science to Applications*, Elsevier, The Netherlands, pp. 327-346.
- Li, R., Wilhelm, S.W., Carmichael, W.W. and Watanabe, M.M. 2008. Polyphasic characterization of water bloom-forming *Rapidiopsis* species (cyanobacteria) from central China. *Harmful Algae*, 7: 146-153.
- Liu, D., Liberton, M., Hendry, J.L., Aminian-Dehkordi, J., Maranas, C.D. and Himadri, B.P. 2021. Engineering biology approaches for food and nutrient production by cyanobacteria. *Curr. Opinion Biotechnol.*, 67: 1-6.
- Lowry, O., Rosebrough, N., Farr, A.L. and Randall, R. 1951. Protein measurement with the Folin phenol reagent. *J. Bio. Chem.*, 193(1): 265-275.
- Mahajan, G.B. and Balachandra, L. 2017 Sources of antibiotics: Hot springs. *Biochem. Pharmacol.*, 134: 35-41.
- Nayak, S., Prasanna, R., Prasanna, B.M and Sahoo, D.B. 2007. Analyzing diversity among Indian isolates of *Anabaena* (Nostocales, Cyanophyta) using morphological physiological and biochemical characters. *World J. Microbiol. Biotechnol.*, 23: 1575-1584.
- Oliveira, D.T., Vasconcelos, C.T., Feitosa, A.M.T., Aboim, J.B., Oliveira, A.B, Luciana P.X., Santos, A.S., Goncalves, E.C., Filho, G.N.D.R. and Nascimento, L.A.S.D. 2018. Lipid profile analysis of three new Amazonian cyanobacteria as potential sources of biodiesel. *Fuel*, 234: 785-788.
- Prasanna, R., Jaiswal, P., Nayak, S., Sood, A. and Kaushik, B.D. 2009. Cyanobacterial diversity in the rhizosphere of rice and its ecological significance. *Indian J. Microbiol.*, 49: 89-97.
- Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Roger, Y.S. 1979. Generic assignments, strain histories, and properties of pure cultures of Cyanobacteria. *J. General Microbiol.*, 111: 1-61.
- Semanti, P.R.S., Robin, R.P. and Purvaja, R.R. 2021. Fatty acid signatures of sediment microbial community in the chronically polluted mangrove ecosystem, *Marine Pollut. Bull.*, 17: 172.
- Sompong, U., Hawkins, P.R., Besley, C. and Peerapornpisal, Y. 2005. The distribution of cyanobacteria across physical and chemical gradients in hot springs in northern Thailand. *FEMS Microbiol. Ecol.*, 52(3): 365-376.
- Spiro, R.G. 1966 Analysis of sugars found in glycoproteins. *Methods Enzymol.*, 8: 3-26.
- Stanier, R., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol Rev.*, 35:171-205.
- Uma, V.S., Gnanasekaran, D., Lakshmanan, U. and Dharmar, P. 2020. Survey and isolation of marine cyanobacteria from the eastern coast of India as a biodiesel feedstock. *Biocatal. Agric. Biotechnol.*, 24: 1-8.
- Varshney, P., Mikulic, P., Vonshak, A., Beardall, J. and Wangikar, P.P. 2015. Extremophilic micro-algae and their potential contribution to biotechnology. *Bioresour. Technol.*, 184: 363-372.
- Ward, O.P. and Singh, A. 2005. Omega-3/6 fatty acids: Alternative sources of production. *Process Biochem.*, 40(12): 3627-3652.
- Watson, S.B., Brian, A.W., Scott, N.H., Hans, W., Paerl, B.W. and Brooks, W. 2015. Harmful Algal Blooms, In John, W., Sheath, J. and Patrick, K. (eds.), *In Aquatic Ecology Freshwater Algae of North America*, Second Edition, Elsevier, The Netherlands, pp. 873-920.
- Yamamoto, Y. 2009. Effect of temperature on recruitment of cyanobacteria from the sediment and bloom formation in a shallow pond. *Plank. Benthos*, 4(3): 95-103.