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RESEARCH ARTICLE

The Isolation, Identification, and Analysis of Nutritional Potential of Microalga *Spirulina subsalsa* and its Cultivation in a Low-Cost Medium

Kirisan A.*, Bowange R.W.T.M.R.T.K., Thadshadini S., Gnanavelrajah N., Ratnayake R.R.

Highlights

- *Spirulina subsalsa* isolated from local water sources and identified via molecular techniques.
- Low-cost growth media from waste materials effectively support the growth of *Spirulina*, yielding biomass comparable to standard media
- *Spirulina* biomass contains significant amounts of protein (52.1%), lipids (12.0%), carbohydrates (7.3%), and sugars (arabinose, galactose, and mannose)
- Biomass is rich in essential minerals including Potassium (950.0 g/kg), Magnesium (434.9 g/kg), and Iron (293.3 g/kg)].
- Cost-effective cultivation methods for *Spirulina subsalsa* support sustainable biomass production

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The Isolation, Identification, and Analysis of Nutritional Potential of Microalga *Spirulina subsalsa* and its Cultivation in a Low-Cost Medium

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Abstract: *Spirulina,* a multicellular filamentous blue-green alga, exhibits a wide range of biological activities and is nutritionally significant. This study aimed to isolate, identify, mass-cultivate, perform nutrient analysis, and formulate a cost-effective medium using waste materials for a less-exploited *Spirulina* strain in Sri Lanka. The organism was isolated from a pond in Jaffna, Sri Lanka. Zarrouk's culture medium was used for the isolation and subculturing of *Spirulina* sp. Mass culturing was carried out in a 15 L tank with half-strength medium concentration under greenhouse conditions. Carbohydrate, lipid, protein, mineral content, and sugars were measured by standard methods. Total Mycrocystin and Cylindrospermopsin content was measured by the ELISA technique. Various media formulations containing cow dung ash, paddy husk ash, and banana pseudo-stem extract were tested for their cultivation. The strain was identified as *Spirulina subsalsa* at the molecular level using 16S rRNA sequencing. Among the media tested, paddy husk ash medium yielded comparable biomass to that of Zarrouk's medium. The total carbohydrate, protein, and lipid content of Spirulina biomass were 7.26%, 52.13%, and 12.03%, respectively. Arabinose, Galactose, and Mannose were the main sugars found in the strain. Of the 14 minerals tested, the strain was rich in K (949.99 g kg-1), Mg (434.88 g kg-1), and Fe (293.33 g kg-1). This study identifies a low-cost medium for cultivating *Spirulina subsalsa*, highlighting its potential as an alternative nutrient source and biofertilizer.

***Keywords*:** Mass Culturing; Low-cost cultivation; Nutrient Composition; *Spirulina;* Waste-derived media

INTRODUCTION

Spirulina is a spiral-shaped, filamentous microalgae that grows naturally in the wild warm environment and is responsible for liberating oxygen to the atmosphere (Kulasooriya & Magana-Arachchi, 2016; Lafarga et al., 2020). The novelty of this study lies in the isolation of locally adapted *Spirulina* strains from Sri Lankan freshwater bodies, their mass cultivation, and the analysis of their nutrient composition, which could differ from commercially available strains (Bowange et al., 2023). Additionally, the study investigates the formulation of a cost-effective cultivation medium using agricultural waste, addressing the high costs associated with standard cultivation media and promoting sustainability (Hemamalini et al., 2023). Furthermore, the study explores the bioremediation potential of *Spirulina*, specifically its ability to remove pollutants from agro-industrial wastewater, thus contributing to environmental sustainability and enhancing the applicability of *Spirulina* for both food and feed industries (Karthik et al., 2020; Thurairajah et al., 2018). Analysis of the nutritional properties of *Spirulina* has shown an exceptionally high protein content, having up to 60 –70 percent of its dry weight (Grosshagauer et al., 2020; Ramírez-Rodrigues et al., 2021), therefore it is called as world's first superfood, and one of the most nutrient-rich foods among all other blue-green algal communities (Amin et al., 2024). It has been extensively studied in food and medical industries, as well as animal feed (AlFadhly et al., 2022; Altmann & Rosenau, 2022; Khannapho et al., 2021). Currently, for human consumption, more than one thousand metric tons of *Spirulina platensis* are produced annually as total world production (Chong et al., 2024; Iwamoto et al., 2024; Sahu & Sridhar, 2024).

Spirulina is a low-cost dietary supplement that has been shown to have no significant adverse effects on humans and animals. Additionally, *Spirulina* cultivation has the potential to mitigate environmental impacts, as it absorbs significant amounts of carbon dioxide during its growth, helping to counteract the effects of global warming caused by increased atmospheric carbon dioxide concentration. Cultivation of *Spirulina platensis* for bioremediation of agro-industrial wastewater, removing pollutants and biomass for animal feed, has been reported (Karthik et al., 2020). The recognized health benefits of *Spirulina* have encouraged several companies to commercialize it through diverse formulations and proprietary brand labels (Abomohra & Ende, 2023; Kathuria et al., 2024; Ramírez-Mérida, 2023). A sustainable alternative source of bioactive lipids for the manufacture of supplements for cardio-protective health exists in *Spirulina subsalsa* (Shiels et al., 2022). Few studies have looked into the possible biotechnological use of *S. subsalsa*. They were primarily concerned with using this Cyanobacterium as a bioremediation agent to remove pollutants (Behl et al., 2024; Jeyakumar et al., 2023; Senthamilselvi et al., 2024; Sun et al., 2024).

Spirulina subsalsa has also been used to reduce the cholesterol content (Maddiboyina et al., 2023; Rahnama et

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al., 2023). Because of *Spirulina*'s global market potential, low space requirement to grow, and increasing interest is being shown in *Spirulina* sp. There are a few studies reported on the presence of *Spirulina* species in Sri Lankan water bodies (Bowange et al., 2023; Perera et al., 2023). However, *Spirulina* species adapted to the local environment will exhibit rapid growth under prevailing conditions, resulting in a biomass rich in nutrients. Further, Zarrouk's medium is the standard control medium for *Spirulina* cultivation, and the high cost of the medium limits mass cultivation and productivity (Thadshadini et al., 2024). Our objectives include isolating promising *Spirulina* strains from Sri Lankan water bodies, mass culturing them, and analyzing the nutrient composition of the isolated strain. This study also explored the possibility of formulating a cost-effective medium using different agricultural waste to cultivate the locally isolated *Spirulina* strain.

MATERIALS AND METHODS

Isolation of *Spirulina*

A water sample was collected from Ariyakulum pond, Jaffna, Sri Lanka (5º 54' N - 9º 52' N and 79º 39' E - 81º 53' E). The water sample was filtered through a 20 µm mesh-size planktonic net. The remaining was transferred into the 50 ml tube and made up to 25 ml. Ten ml sample was transferred in to 40 ml of Zarrouk's medium ($NaHCO_3$ (16.8 g), $NaNO_3$ (2.5 g), NaCl (1.0 g), K_2SO_4 (1.0 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), $FeSO_4.7H_2O$ (0.01 g), $CaCl_2.2H_2O$ (0.04 g) and EDTA (0.08 g) at pH 7.4) in 50 ml conical flask for laboratory culturing. Cultures were incubated for 14 days at 28 ± 2°C, under a 16:8-hour light-dark photoperiod, with a light intensity of approximately 2000 lux provided by cool white fluorescent lamps. Hundred µl of samples was sub cultured onto agar plates containing the same medium solidified with 2% (w/v) bacteriological agar under aseptic conditions by spread plate techniques.

Morphological identification

The morphology of the strain was observed under a compound microscope (Euromex BioBlue.Lab BB. 1153-PLi) equipped with an image stem (Hossain et al., 2020).

Mass culturing in standard medium

To obtain an adequate amount of biomass, mass culturing of *Spirulina* was carried out in a 15 L glass tank containing half-strength Zarrouk's medium under greenhouse conditions, with natural light exposure (~2000–2500 lux) and a temperature maintained between 28–30 °C (Hossain et al., 2020). The growth performance of *Spirulina* was assessed by measuring optical density (OD) at 680 nm, pH, electrical conductivity (EC), and temperature in various culture media. Manual shaking was performed three times a day, at 9:00 AM, 1:00 PM, and 5:00 PM, to ensure uniform agitation and prevent sedimentation (Solanki et al., 2024). The readings were recorded at two-day intervals up to harvesting (30 days). Picture 3.4 shows the growth parameters of *Spirulina* culture. Algal biomass was harvested by filtering through a planktonic net. Then the biomass was dried at 50 °C in an oven overnight until a constant weight was obtained.

Nutrient analysis methods of fresh biomass of *Spirulina*

The total protein content of the fresh dried *Spirulina* biomass was determined using the Lowry method. For this analysis, 50 mg of the dried biomass was used for protein extraction (Lowry et al., 1951). The Dubois method was used to measure the total carbohydrate content of the fresh dry biomass of *Spirulina* (DuBois et al., 1956). Vitamin C content was determined by the method described by (Brandon et al., 2014). High Performance Liquid Chromatography (HPLC – Agilent 1260 Infinity 2) was used to determine the presence of different sugar types (Monosaccharides) in the fresh biomass using Laboratory Analytical Procedure (LAP) (Van Wychen & Laurens, 2013). The Soxhlet extraction method was used to determine the total lipid content in the biomass. The HPLC condition was used as a 50 µl injection volume with the mobile phase as milliQ water at a flow rate of 0.6 $mlmin^{-1}$, column temperature as 55 °C, detector temperature as 45 °C, with a refractive index detector, and the run time was 15 minutes for data collection plus 5 minutes for post-run with HiPlex- H column.

Analysis of micronutrients

Dried *Spirulina* biomass (0.1 g) was measured and placed in the microwave digester, and 3 ml of 69% HNO_3 was added and digested for 25 min. After cooling, the digested volume was transferred to 10 mL in volumetric flasks with ultra-pure water. Then the diluted volume was filtered using 0.45 µm filter papers. The resulting filtrate was again filtered using 0.45 µm cellulose acetate filters, and the final filtrate was used for the determination of micro nutrients using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP – OES). ICP-OES with radial and axial torch equipped with high high-purity (99.996%) argon saturation assembly was used for the determination of metals. High-purity argon was used as a cool, auxiliary, and nebulizer gas.

Cyanotoxin analysis

Total Mycrocystin-LR and Cylindrospermopsin were analyzed by the ELISA method. Specifically, Microcystin-LR and Cylindrospermopsin were examined and measured.

DNA extraction, amplification, and molecular identification of the *Spirulina* strain

Total genomic DNA was extracted by the method described by Smoker & Barnum (1988) with slight modification. The DNA pellet was dried in aseptic conditions for up to 10 minutes to evaporate residual ethanol, and the DNA pellet was re-suspended in sterile MilliQ water and stored at -20 °C. The aqueous phase containing DNA was directly used for further analysis. DNA concentration was estimated using the spectrophotometric method by reading the absorbance at 260 nm, 280 nm, and 320 nm. PCR amplification of the 16S rRNA gene region was performed for the purified DNA using CYA106F (5′-CGG ACG GGT GAG TAA CGC GTG A-3′) as forward primer and CYA781Ra (5′-GAC TAC TGG GGT ATC TAA TCC CAT T -3′) as reverse primer (Nübel et al., 1997). The cyclic profile of the Polymerase

Chain Reaction for the 16S gene region included an initial denaturation of template DNA at 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, annealing at 60 °C for 1 min, elongation at 72 °C for 1 min and a final elongation at 72 °C for 15 min. Amplification of the PCR product was confirmed by agarose gel electrophoresis visualization.

DNA sequencing

Further purification of the PCR products and sequencing of the 16S rRNA gene were carried out at Macrogen, South Korea, using ABI 3730XL sequencers with respective forward and reverse primers.

BLAST analysis of the 16S rRNA gene sequence and molecular identification

The DNA sequences obtained for forward and reverse primers of the cyanobacteria strain were aligned using ClustalW multiple alignment in BioEdit software version 7.2.5 to obtain the consensus sequence, and further required minor edits were carried out using the BioEdit software. The final aligned DNA sequences were compared with the existing nucleotide sequences for cyanobacteria in the National Center for Biotechnology Information database using the Nucleotide Blast option, and the cyanobacteria strain was identified at the molecular level (NCBI, n.d.). The 16S rRNA gene sequence determined for the strain was deposited in the GenBank database via the BankIt submission tool under the accession number of OQ241235.1.

Construction of the phylogenetic tree

A phylogenetic tree was constructed based on the *Spirulina* family using Molecular Evolutionary Genetics Analysis (MEGA) version 11.0.13. The most similar gene sequences of the strain from the same family were identified using the NCBI-BLASTN search tool. The gene sequences were then multiple-aligned using ClustalW Multiple Sequence Alignment. The phylogenetic tree was constructed with the multiple aligned gene sequences using the neighbour-joining method. *Gloeobacter violaceus* was used as the outgroup of the constructed phylogenetic tree (Wanigatunge et al., 2014).

Formulation of low-cost media

For formulating a new and cost-effective medium, in the first step, individuals based on the physico-chemical properties of constituents (Paddy husk ash (PH), Cow dung ash (CD), and Banana pseudo stem extract (BE) different combinations of culture media were formulated. The pH was initially measured. Eleven treatments and two replicates were used in the experiment under a complete randomised design. The treatments were, control 1 (100% SM), Control 2 (50% SM), T1 (10% Cow dung Ash (Cow dung Ash - CDA), T2 (10% Paddy Husk Ash (Paddy Husk Ash - PHA), T3 (10% Cow Dung Ash + 10% Paddy Husk Ash), T4 (25% Cow Dung Ash), T5 (25% Paddy Husk Ash), T6 (25% Cow Dung Ash + 25% Paddy Husk Ash), T7 (5% BCH (Banana pseudostem extract- B + CDA - C + PHA), T8 (10% BCH), T9 (25% BCH), T10 (50% BCH) and T11 (60% BCH).

Statistical analysis

The data recorded in triplicate for the parameters in various strains were subjected to ANOVA (analysis of variance), using the SAS 9.1.3package.

RESULTS

Morphological identification of the strain

A preliminary microscopic investigation of the culture before purification revealed the dominance of a single morphologically distinct filamentous and multicellular organism. This strain was tentatively identified as *Spirulina* sp. based on its characteristic spiral-shaped filaments, as observed under an inverted microscope at 40× magnification (Figure 1). While only one morphological type was apparent, it is important to note that morphological identification alone cannot conclusively confirm the presence of a single species, as some microorganisms may exhibit similar structural features. Further molecular or biochemical analyses would be necessary to confirm the strain's purity and taxonomic identity.

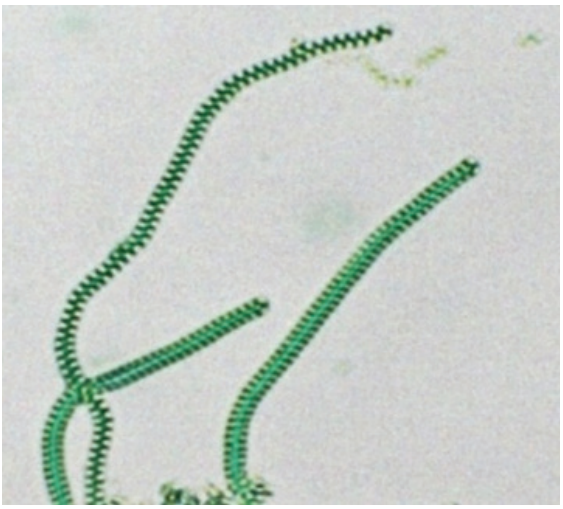

Figure 1: Microscopic identification of Spirulina under an inverted microscope at 40× magnification.

Molecular identification and phylogenetic analysis of *Spirulina sp.*

The strain was identified at the molecular level using the Blast online software in the NCBI/Genbank database. The strain shared a maximum percentage sequence similarity of 96.94% with the reference sequences from GenBank for *Spirulina subsalsa.* By using the BankIt submission tool, the 16S rRNA gene sequences discovered for *Spirulina subsulsa* were deposited in the GenBank database under the accession number OQ241235.1. In the constructed phylogenetic tree, three monophyletic groups could be identified (Figure 2).

A phylogenetic tree was constructed using the Neighbor-Joining method implemented in MEGA version 11.0.13, with evolutionary distances computed using the Kimura 2-parameter model. The isolate obtained in this study is indicated by a black triangle (▲), while closely related reference sequences retrieved from the NCBI GenBank database are labeled with "Seq." Bootstrap values (based on 100 replicates) are shown next to the corresponding branches to indicate the level of confidence in each clade. The scale bar represents the number of nucleotide substitutions per site.

Biomass production in Zarrouk's medium

Spirulina culture was inoculated to give the initial OD of 0.443. Thereafter, growth increased continuously during 21 days of culturing (Fig. 2 (b)). The biomass yielded after 21 days of cultivation was 0.8 gl^{-1}. The pH of the growth medium increased with time (Figure 2-a).

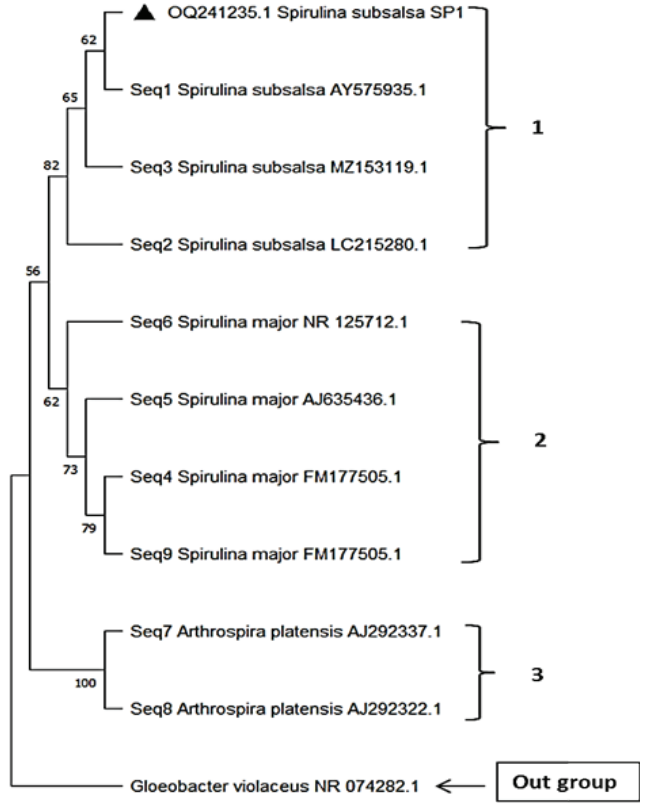

Figure 2: Phylogenetic tree of members of the family *Spirulinaceae* based on 16S rRNA gene sequences

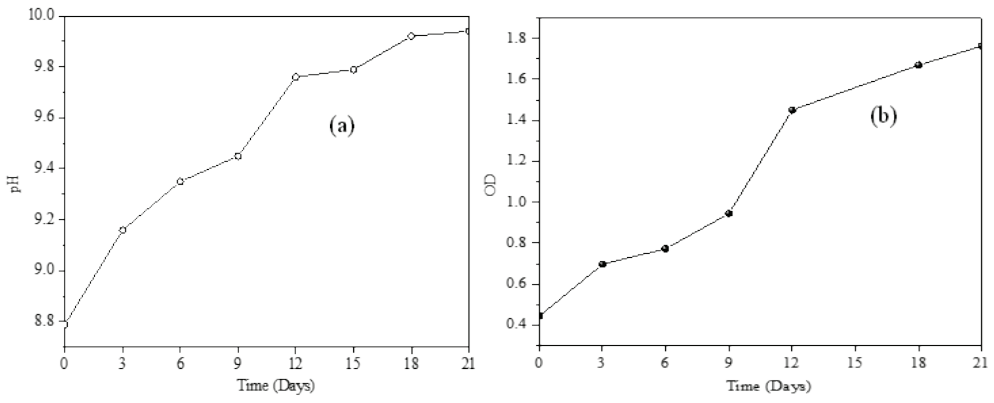

Figure 2: pH **(a)** and Optical Density **(b)** variation of *Spirulina subsalsa* culture with time

Major nutrient profile of *Spirulina* biomass

The results showed that the total carbohydrate content of *Spirulina subsalsa* was 7.26 % while total protein content was high, with a mean of 52.13% and total lipid content was 12.03 %. HPLC analysis showed that Arabinose, Galactose, and Mannose were the main sugar types present in the biomass.

The biomass of *Spirulina subsalsa* contains a variety of micro minerals in different concentrations. Among these, P is the most abundant, with the concentration of 94,999 mg kg-1, followed by Mg at 43,488 mg kg-1 and Iron at 29,333 mg kg-1. Mn and Ca are also present in significant amounts, at 282.507 mg kg-1 and 2,400 mg kg-1, respectively. In contrast, Cd and As are found in much smaller quantities, at 0.111 $mgKg^{-1}$and 0.215 $mgKg^{-1}$, respectively. Other notable minerals include Zn (87.191 $mgKg^{-1}$), Cu (70.905 $mgKg^{-1}$), Cr (44.255 $mgKg^{-1}$), Sr (55.072 $mgKg^{-1}$), Ni (6.019 $mgKg^{-1}$), Pb (5.505 $mgKg^{-1}$), and Co (3.646 $mgKg^{-1}$).

Characteristics of Raw Materials and Ash of Raw Materials

The physical and chemical properties of cow dung, paddy husk, and banana pseudostem are presented in Tables 1 and 2.

The banana pseudostem had a higher moisture content (91.41%) compared to cow dung (76.47%), while paddy husk had the lowest moisture content, ranging from 10.42% to 12.84%. Paddy husk also contained the highest percentage of total organic carbon (35.53%). In terms of ash content, cow dung had the highest percentage (49.56%), followed by paddy husk (14.48%) and banana pseudostem (6.98%).

The highest total nitrogen content was observed in paddy husk ash (0.98%), with comparable values recorded in the present study. Paddy husk ash also exhibited the highest total phosphorus content (0.62%), with no statistically significant difference between cow dung ash and paddy husk ash. Furthermore, paddy husk ash contained the highest concentration of bicarbonate (0.51%). Regarding physicochemical properties, paddy husk demonstrated a higher pH (7.96) and electrical conductivity (3.39 mS) in comparison to cow dung (2.28 mS) and banana pseudo stem (1.43 mS).

It is important to recognize that the chemical composition of rice husk can vary considerably due to factors such as geographical origin, varietal differences, climatic conditions, soil characteristics, and fertilizer application. This inherent variability poses a challenge to quality control and product consistency, particularly in applications requiring standardized nutrient profiles. To mitigate this, standardization strategies including pre-treatment of raw materials, controlled blending, and routine compositional analysis are essential. Implementing these measures can enhance reproducibility and ensure that the final product meets defined quality specifications.

Cyanotoxin content in dried *Spirulina* biomass

The total Microcystin-LR content detected in the dried *Spirulina* biomass was 0.15 mg/kg, while Cylindrospermopsin was present at 0.29 mg/kg. Both compounds are recognized cyanotoxins produced by certain cyanobacterial species, and their presence in Spirulina biomass intended for human consumption raises significant food safety concerns.

Risk Assessment and Comparison with Safety Standards

According to the World Health Organization (WHO), the provisional tolerable daily intake (TDI) for Microcystin-LR is 0.04 µg/kg body weight/day. For an average adult weighing 60 kg, this corresponds to a maximum intake of 2.4 µg/day. At a concentration of 0.15 mg/kg (150 µg/kg), a 10 g serving of the *Spirulina* product would yield approximately 1.5 µg of Microcystin-LR, which remains below the WHO's daily limit for an average adult.

In contrast, the TDI for Cylindrospermopsin is 0.03 µg/kg body weight/day, equivalent to 1.8 µg/day for a 60 kg adult. Based on the measured concentration of 0.29 mg/kg (290 µg/kg), a 10 g dose would deliver approximately 2.9 µg, which exceeds the established safe limit.

These findings indicate that while the level of Microcystin-LR may fall within acceptable limits for occasional intake,

Table 1: Physicochemical characteristics of raw materials used in the preparation of culture media

Properties	Cow dung	Paddy husk	Banana pseudo stem
Moisture %	76.47^{b}	10.46^{c}	91.41^{a}
Ash %	49.56^{a}	14.48^{b}	6.98^{c}
Total organic carbon %	26.8^{c}	38.1^{a}	30.8^{b}

Table 2: Chemical properties of ash and extracts of cow dung, paddy husk, and banana pseudo stem

Chemical Properties	Cow dung extract	Paddy husk extract	Banana pseudo stem extract
Total Nitrogen	0.7^{b}	0.98^{a}	0.14^{c}
Total Potassium	2.26^{b}	2.60^{a}	1.52^{c}
Total Phosphorus	0.59^{a}	0.62^{a}	0.25^{b}
Carbonate	0.36^{a}	0.11^{b}	0.0018^{c}
Bicarbonate	0.24^{b}	0.52^{a}	0.0061^{c}
pH	7.15^{b}	7.98^{a}	6.3^{c}
EC (mS)	2.28^{b}	3.39^{a}	1.43^{c}

the presence of Cylindrospermopsin at the reported concentration could pose a potential health risk, particularly with regular consumption or among vulnerable populations (e.g., children, pregnant individuals, and those with hepatic conditions).

Biomass production and nutritional composition of *Spirulina* grown in low-cost media

Table 3 shows the total dry biomass yield of *Spirulina* under different culture media. After 30 days of cultivation, T5 (25% PH) recorded the highest total dry biomass yield at 3.12 g/L, followed by Control 1 (100% ZM) with 3.05 g/L. No significant difference was observed between Control 1 and T5. The highest total nitrogen percentage was also found in T5 (25% PH), at 6.65%; however, this was not significantly different from Control 1 (6.58%) and Control 2 (6.51%).

The lowest total nitrogen (N) content was recorded in T11 (60% BCH) at approximately 3.50%. In contrast, T5 (25% paddy husk ash, PH) exhibited the highest total phosphorus (P) content at 0.82%, which was significantly higher than all other treatments. Similarly, T5 also recorded the highest total potassium (K) content at 7.60%, showing a significant difference compared to all other treatments. The second-highest potassium content was observed in Control 2 (50% Zarrouk's medium) at 7.31%.

T6 (25% CD + 25% PH) had the highest total organic carbon (TOC) percentage at 38.31%, followed by T5 (25% PH) at 34.32%. However, there was no significant difference between T5 and T6. Control 1 (100% ZM) had the highest total chlorophyll content at 18.78 mgL^{-1}, with no significant difference observed between Control 2 (16.05 mgL^{-1}) and T5 (14.38 mgL^{-1}).

DISCUSSION

The microscopic examination of the isolated strain revealed the characteristic filamentous and spiral morphology typical of the genus *Spirulina*. However, morphological features alone are not sufficient for conclusive species identification due to phenotypic plasticity under different environmental conditions (Whitton & Potts, 2007). To confirm its identity, molecular analysis using 16S rRNA sequencing was conducted. Phylogenetic analysis showed that the strain clustered with reference sequences of *Spirulina subsalsa* in a well-supported monophyletic group (bootstrap >50%), confirming the classification.

Despite the successful initial cultivation of *S. subsalsa*, the medium became unsustainable after four cycles due to contamination and reduced growth. These challenges underline the importance of maintaining optimal physicochemical conditions such as pH, nutrient availability, and light intensity, which are known to significantly affect cyanobacterial productivity and metabolite synthesis (Madkour & Nasr, 2012; Shahid et al., 2021). The gradual increase in pH during cultivation is attributed to the photosynthetic uptake of CO_2 and the dissociation of bicarbonate (HCO_3^-), leading to OH^- ion accumulation (Zhang et al., 2023). Such alkaline shifts are common in closed systems with bicarbonate-buffered media (Kim & Lee, 2018).

The growth performance of the strain, measured via optical density (OD), and subsequent biochemical analysis confirmed the strain's high nutritional potential. The protein content (52.13%) was substantial, although slightly lower than that of *Spirulina platensis* (~63%) (Aljobair et al., 2021), but superior to other commercially cultivated microalgae such as *Chlorella vulgaris* (47.82%) and *Isochrysis galbana* (26.99%) (Tokuşoglu & üUnal, 2003). Notably, the lipid content in *S. subsalsa* (12.03%) was higher than in *S. platensis* (7.53%), suggesting that *S. subsalsa* may serve as a better candidate for lipid-based applications, including biofuel and nutraceutical production (Kumar et al., 2013; Shiels et al., 2022).

In contrast, the carbohydrate content (7.26%) was lower than the 17.63% reported for *S. platensis* (Bensehaila et al., 2015), potentially limiting its utility in carbohydrate-rich

Table 3: Total Dry Biomass and Nutrient Content of *Spirulina* in Different Culture Media

Treatments	Total dry biomass	N%	P%	K%	TOC%	Total chlorophyll content
Control 1	3.05^{a}	6.58^{ab}	0.55^{c}	6.19^{d}	26.34^{b}	18.78^{a}
Control 2	2.73^{b}	6.51^{ab}	0.52^{cd}	7.31^{b}	21.55^{c}	16.05^{b}
T1	2.00^{c}	4.62^{g}	0.47^{de}	4.13^{b}	8.78^{e}	8.52^{cd}
T2	2.06^{c}	6.30^{b}	0.66^{b}	5.46^{e}	21.55^{c}	8.92^{c}
T3	1.51^{d}	5.53^{d}	0.65^{b}	3.99^{g}	23.22^{bc}	6.72^{def}
T4	1.94^{c}	4.13^{h}	0.43^{ef}	6.68^{c}	14.37^{d}	7.9^{cde}
T5	3.12^{a}	6.65^{a}	0.82^{a}	7.60^{a}	34.32^{a}	14.38^{b}
T6	1.41^{de}	5.18^{e}	0.51^{cd}	6.52^{c}	38.31^{a}	4.31^{g}
T7	0.73^{f}	5.95^{c}	0.22^{h}	3.40^{h}	9.58^{e}	7.91^{cde}
T8	0.62^{f}	4.76^{fg}	0.20^{h}	3.93^{g}	15.96^{d}	4.23^{g}
T9	0.62^{f}	4.55^{g}	0.29^{g}	4.79^{f}	15.96^{d}	5.47^{g}
T10	1.16^{e}	5.04^{ef}	0.51^{cd}	6.11^{d}	23.14^{bc}	6.17^{efg}
T11	1.25^{de}	3.5^{i}	0.40^{f}	5.62^{e}	25.54^{bc}	5.08^{fg}

feed or energy-dense dietary supplements. Nonetheless, HPLC analysis revealed the presence of arabinose, galactose, and mannose-monosaccharides with known prebiotic and immunomodulatory properties. Further quantitative sugar profiling is necessary to fully elucidate their concentrations and health implications.

Mineral analysis revealed high concentrations of Potassium (949.99 mg/g), Magnesium (434.88 mg/g), and Iron (293.33 mg/g). These values exceed those typically observed in other *Spirulina* strains (Campanella et al., 1998; Janda-Milczarek et al., 2023). Just 8 g of dried *S. subsalsa* biomass can meet the daily recommended magnesium intake for adults (350 mg/day), while 1.5 g suffices for iron (45 mg/day). This positions *S. subsalsa* as a highly valuable micronutrient supplement, particularly in malnourished or anemic populations.

Cyanotoxin analysis confirmed that Microcystin-LR and Cylindrospermopsin levels were within or marginally above the WHO and EPA thresholds (Falconer, 2005). Although microcystin levels (0.15 mg/kg) remained within tolerable daily intake limits, cylindrospermopsin (0.29 mg/kg) slightly exceeded safe limits, especially with regular or high-volume consumption. As Cyanotoxin production can be influenced by environmental stressors, such as nitrogen limitation or light intensity (Jiang et al., 2015), ongoing monitoring and batch testing are recommended for any biomass intended for food or feed applications.

Cultivation trials in alternative low-cost media revealed that T5 (25% paddy husk ash) yielded biomass (3.12 g/L) statistically comparable to that of the standard Zarrouk's medium (3.05 g/L). Moreover, T5 outperformed the control in total phosphorus, potassium, and organic carbon content, supporting its potential as an economical and nutrient-rich medium. Similar findings have been reported by (Madkour & Nasr, 2012), who has demonstrated the feasibility of using agro-industrial waste materials to replace costly synthetic components in *Spirulina* cultivation.

Heavy metal concentrations, including Cd, Pb, and As, were within acceptable safety limits, and the presence of essential trace elements like Zn, Cu, and Mn further supports the biomass's applicability in agriculture. Numerous studies have documented the use of microalgae as biofertilizers and soil conditioners, enhancing nutrient availability and promoting sustainable agriculture (Alobwede et al., 2019; de Souza et al., 2019; Pirushanthi et al., 2021; Renuka et al., 2017; Rumana et al., 2022). Specifically, microalgal amendments have been shown to increase soil inorganic nitrogen concentrations and improve microbial activity (Chu et al., 2020; Marks et al., 2017).

Overall, the findings affirm *Spirulina subsalsa*'s potential as a locally cultivable, nutrient-rich strain suitable for diverse applications ranging from functional foods and dietary supplements to soil enrichment and bioremediation. However, variability in the composition of agricultural inputs like paddy husk ash due to factors such as soil type and crop variety must be accounted for by implementing standardized pretreatment and quality control protocols (Marciano et al., 2024).

CONCLUSIONS

The locally isolated *Spirulina subsalsa* shows strong promise as a sustainable protein source, with 52.1% protein content in its dry biomass and safe toxin levels according to WHO standards. HPLC analysis identified Arabinose, Galactose, and Mannose as key sugars. Among the tested media, the T5 formulation (25% paddy husk) produced the highest biomass yield and superior nutrient content (Phosphorus, Potassium, and Carbon), comparable to the standard Zarrouk's medium. The newly identified *Spirulina subsalsa* shows potential for applications in the food industry, pharmaceuticals, soil improvement, and as a nutrient source. Using agricultural waste, such as ash medium, as a cultivation substrate for Spirulina platensis offers a cost-effective alternative without compromising its growth performance. This sustainable approach not only lowers medium expenses but also promotes waste recycling and environmental conservation. A study needs to be carried out to determine the toxic effect of *Spirulina subsalsa* in different conditions to introduce it as a protein supplement and biofertilizer. More research is needed to quantify the sugars present in the biomass and study the fatty acid profile towards biofuel production.

DECLARATION OF CONFLICT

The authors have no conflict of interest

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AUTHOR CONTRIBUTION

Conceptualization, funding acquisition: **N.G., R.R., A.K.;** Data collection: **A.K., T.B., T.S.;** Statistical analysis: **A.K., T.S.;** Data interpretation: **A.K., N.G., R.R., T.B., T.S.;** Writing the first draft: **A.K.;** Manuscript editing and reviewing: **A.K., N.G., R.R.**

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