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Exploration of microalgae for novel functional food ingredients through isolation, cultivation, and metabolite enhancement

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ABSTRACT: Microalgae have been explored as a remarkable and vast natural source of unique health-promoting foods ingredients. Because of the continuously increasing demand for functional foods, it is essential to identify new, suitable ingredients for use in the food industry. The aim of this research is to isolate and optimize the cultivation conditions of microalgae and cyanobacteria from diverse aquatic environments to enhance the yield of valuable bioactive compounds for use as food additives. Thirty samples were collected from various water sources, including dams, rivers, lakes, the sea, and the Lonar lake which is one of the rare basaltic meteorite impact craters in the world. A total of nine strains were isolated, including four strains of *Chlorella*, two of *Chlorococcum*, and one each of *Geitlerima*, *Scenedesmus* and *Arthrospira*. The selected strains were further studied for cultivation under different conditions (such as autotrophic, mixotrophic, autotrophic using LEDs, and mixotrophic using LEDs) and for the extraction of metabolites like phycocyanin and β-carotene. The highest protein yield (47.9%) and lipid content (4.20%) were observed in *Arthrospira sp*. The results show that *Arthrospira sp.* contains sufficient biomass, protein, and lipids to be a valuable resource. As a tropical country, India boasts a rich diversity of cyanobacterial resources with significant potential for commercial exploration in natural product development.

KEYWORDS: *Arthrospira sp*., β-carotene, Cultivation, Microalgae, Phycocyanin.

INTRODUCTION

Microalgae and cyanobacteria serve as precious ecological assets for producing diverse metabolites with applications in pharmaceuticals, nutraceuticals, cosmetics, food additives, and biodiesel [1]. Their vast biodiversity offers potential for novel products. Rich in proteins, lipids, carbohydrates, essential vitamins, and pigments such as chlorophyll, carotenoids, and phycobiliproteins, microalgae are highly versatile [2-3].

Cyanobacteria, producers of phycobiliproteins, are a key component of marine picophytoplankton, significantly contributing to oceanic carbon biomass and primary productivity [4]. Phycobiliproteins from cyanobacteria hold commercial value, serving as natural dyes, food colorants, and fluorescent labels in sensitive fluorescence techniques [5-6]. β-carotene and other carotenoids are natural pigments with significant nutritional and biological properties. Known for their provitamin A activity and antioxidant properties, β-carotene helps scavenge harmful radicals linked to cancer. It is widely used in food, pharmaceuticals, and cosmetics [7-8], recent studies focus on optimizing cultivation for phycocyanin and β-carotene extraction [9], driven by growing demand and the need for higher biomass production. *Spirulina*, a blue-green cyanobacterium consumable by humans and animals, contains bioactive pigments like chlorophyll a, lutein, β-carotene, phycocyanin, and allophycocyanin [10]. Current research focuses on biotechnological approaches to cultivate microalgae and utilize their metabolites [11]. Marine microalgae serve as a natural reservoir for diverse compounds, including carotenoids, used in pharmaceuticals, food, and cosmetics [12]. Microalgae with high biomass and secondary metabolite production are anticipated to enhance their use in the pharmaceutical and nutraceutical sectors [13].

Microalgae have been utilized as nutritional ingredients, but their active cultivation began only in the past three decades. Production faces challenges like low growth rates, limited product yields, strain improvement, and downstream processing. Addressing these issues requires a systematic approach to screening, isolating, cultivating, and extracting microalgae. The objectives of this research are to isolate and identify promising strains of microalgae and cyanobacteria from various freshwater and hypersaline environments, including the unique Lonar lake; to evaluate their growth under different cultivation conditions—autotrophic, mixotrophic, and Light Emitting Diode (LED) supported regimes; and to quantify the production of key metabolites, such as phycocyanin and β-carotene, to assess their potential for application in the food industry.

MATERIALS AND METHODS

Specimen acquisition

To conduct the screening and morphological identification of microalgae, approximately 30 water samples were obtained from various locations in *Maharashtra*, *India*, including twenty freshwater samples (labeled FS) and ten marine water samples (labeled MS). These were sourced from diverse environments like dams, rivers, lakes, and the sea (Table 1). Additionally, a sample was taken from the *Lonar* Crater Lake, one of the few basaltic meteoritic craters in the world. Located in the Deccan Traps at 19°59′ N and 76°31′ E in Buldhana district, the lake features a highly alkaline and hypersaline environment, with water pH ranging from 9 to 14. This environment supports alkalophilic and salt-tolerant organisms, including various microorganisms, predominantly microalgae and cyanobacteria like *Arthrospira, Euglena, Oscillatoria, Chlorella,* and *Eudorina* [14].

Microalgae were cultured under laboratory conditions. A significant part of the species failed to grow or produced less biomass. Out of a pool of thirty water samples, a total of nine species were isolated, of which four belonged to *Chlorella sp*., two to *Chlorococcum sp*., and one each to *Geitlerima sp*., *Scenedesmus sp*., and *Arthrospira sp*. The microalgal isolates were cultivated in liquid BG-11, artificial seawater, algae culture broth, and Zarrouk's medium using the serial dilution method for three weeks in the laboratory. The isolate tubes were kept under light of 1500–2000 lux irradiance for 24 h. The preserved isolates were observed under the microscope to confirm the identity of the algal flora. The pH, conductivity, and salinity of all the water samples were recorded.

Arthrospira species were grown through the use of alternative cultivation conditions like autotrophic, mixotrophic (using 0.1, 0.5 and 1% glucose/sodium acetate) in the presence of fluorescent light sources. Such conditions were also modeled with Light Emitting Diodes (LEDs) in various colors, including blue, green, red, white, and yellow.

Table 1. Samples from different kinds of water bodies

Sample Number	Location	Source Of Water	pH	Conductivity (mS/cm)	Salinity (%)
FS-01	Khadakwasla, Pune	Dam	7.30	0.15	0.1
FS-02	Terekhole, Sindhudurg	River	6.80	0.12	0.1
FS-03	Vani, Nasik	River	7.20	0.16	0.1
FS-04	Bhimashankar, Pune	River	7.40	0.20	0.2
FS- 05	IndrayaniAlandi, Pune	River	7.30	0.19	0.2
MS-06	Alibagh	Sea	8.80	5.68	3.5
MS-07	Devghad, Sindhudurg	Sea	9.20	5.20	3.5
FS-08	OsergaonSindhudurg	Lake	7.90	0.38	0.2
FS-09	Gad, Kankavali, Sindhudurg	River	7.70	0.14	0.2
FS-10	Mulshi, Pune	Dam	6.80	0.12	0.2
FS-11	Indrayani, Dehu, Pune	River	6.61	0.15	0.1
FS-12	Panshet, Pune	Dam	7.00	0.14	0.2
MS-13	Kihim, Alibhag	Sea	8.40	5.80	3.5
MS-14	Nagaon, Alibhag	Sea	8.30	5.70	3.5
MS-15	Akshi, Alibhag	Sea	8.45	5.50	3.5
MS-16	Main Beach, Alibhag	Sea	7.14	5.60	3.5
FS-17	Mehrun, Jalgaon	Lake	6.85	0.12	0.1
FS-18	Padmalaya, Jalgaon	Lake	7.10	0.10	0.1
MS-19	Baga Beach, Goa	Sea	7.90	5.43	3.5
MS-20	Diveagar Beach, Kokan	Sea	8.36	5.45	3.5
MS-21	Nariman Point, Mumbai	Sea	7.70	5.56	3.5
FS-22	Chandrabhaga, Pandharpur	River	7.15	0.12	0.1
FS-23	Rankala, Kolhapur	Lake	7.90	0.13	0.1
FS-24	Pawana, Pune	Dam	7.60	0.11	0.1
FS-25	Pashan, Pune	Lake	8.10	0.10	0.1
FS-26	Titur,Patna, Chalisgaon	River	7.20	0.15	0.1
FS-27	Shrivardhan, Kokan	River	7.50	0.21	0.3
FS-28	Varasgaon (Panshet), Pune	Dam	7.20	0.13	0.1
FS-29	Khadakwasla (Back), Pune	Dam	7.10	0.11	0.1
MS-30	Lonar, Buldhana	Lake	9.8	12.4	7

Medium and culture conditions

Of the thirty water samples collected, eight microalgae strains capable of growing on BG-11 medium were cultured in both liquid and solidified media (using 1.5% agar agar). Isolates from the alkaline lake were grown in modified Zarrouk's medium and further streaked onto solidified media for identification and the isolation of uni-algal cultures. The cultured flasks and plates were maintained at room temperature under 1500-2000 lux light intensity for 24 hours. Freshwater isolates were specifically cultivated in BG-11 medium.

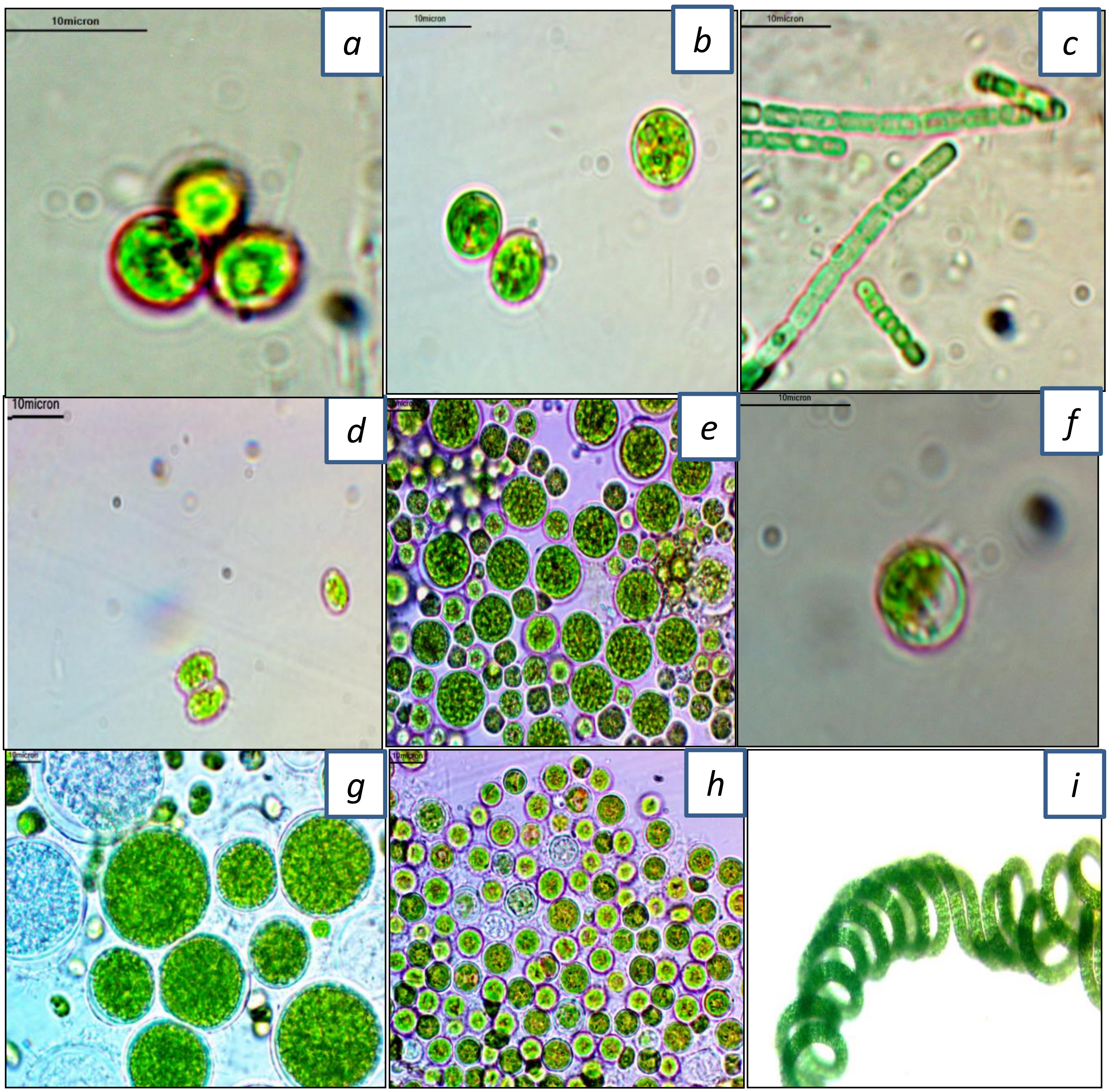

Figure 1. Microscopic observation under 100X; (a) FS-1 *Chlorella sp.*(b) FS-3 *Chlorella sp.* (c) FS-4 *Geitlerima sp.*(d) FS-18 *Scenedesmus sp*. (e) FS-22 *Chlorococcum sp.* (f) FS-23 *Chlorella sp.*(g) FS-24 *Chlorococcum sp.* (h) FS-27 *Chlorella sp.* (i) MS-30 *Arthrospira sp.*

Isolation and Morphological Identification of Isolates:

The microalgal colonies began to multiply after two weeks and were observed under a microscope. To record the morphological characteristics of the species, individual colonies were carefully transferred from the agar plate to a drop of BG-11 medium on a glass slide. A cover slip was placed gently on the slide, and a drop of cedarwood oil was applied to the coverslip for observation under an oil immersion lens at 100X magnification to perform morphological identification of the cultures (Figure 1).

After characterization, the growth of the species of interest was transferred to 5 mL of the respective medium, while non-targeted colonies and other species were discarded. After three weeks, 5 mL of the microalgal culture was seeded into 100 mL of sterile BG-11, artificial seawater, algae culture broth, Zarrouk's medium, and modified Zarrouk's medium. The cultures were kept at room temperature under 1500–2000 lux irradiance for 15 days. Subsequently, they were grown under continuous fluorescent light (2500-3000 lux) with regular microscopic monitoring to ensure the purity of the species.

Biomass analysis and dry weight collection

The biomass used for analysis was dried using a lyophilizer (Operon, Model FDU 7003). Isolates were centrifuged at 10°C for 10 minutes at 5000 rpm, and The the separated biomass was rinsed with sterile distilled water to remove salt impurities. All microalgae samples were labeled, stored at -80°C for 2 hours, and then lyophilized for 7-8 hours. The weight of the wet biomass was recorded using an analytical balance, and the weight of the freeze-dried biomass was calculated by the weight difference.

Biochemical Analysis

Carbohydrate quantification was done on the isolated samples, with a standard glucose curve plotted. The relationship between glucose concentration and optical density was established using a regression equation.

Total protein extraction from the isolated microalgae Sample assessment was conducted using the Lowry method. Protein concentration was established by referencing a standard Bovine Serum Albumin (BSA) curve (10 mg/ml).

Total lipid extraction from the isolated microalgae samples was performed using a combination of the Bligh and Dyer method and the Folch method. The lipid content was determined using the following equation:

$$Weight\ of\ lipid = \frac{Amount\ of\ lipid\ extracted}{Weight\ of\ original\ sample} \times 100 \qquad (1)$$

Identification of isolate by 16S rDNA

The isolated sample from Lonar lake, (Sample ID: MS-30) was identified in collaboration with Aavanira Biotech, Pune. Genomic DNA of the microalgae was isolated and confirmed on a 1% agarose gel. Amplification was performed using the Paq 5000TM DNA polymerase kit (Agilent, Cat no- 600680), with a primer amplicon size of 392bp. The amplified PCR product was analyzed via 1% agarose gel electrophoresis. Sequencing was done using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Cat no-4337455) on a 3500 Genetic Analyzer, with analysis through Mega 5.1 software. The sequence was refined and evaluated against the NCBI database using the BLAST program. Phylogenetic analysis performed with MEGA6, identified the isolates as *Arthrospira sp*. of the class Cyanophyceae.

Cultivation of *Arthrospira*

Triplicates of cultivation experiments were executed under autotrophic and mixotrophic conditions. Autotrophic studies used modified Zarrouk's medium and fluorescent lamps (3000 lx, 24 h). For mixotrophic conditions, organic substrates (0.1, 0.5, and 1% glucose/sodium acetate) were added to Zarrouk's medium. Cultivation continued for 15 days, with LED strips in blue, green, red, white, and yellow as light sources, replacing fluorescent lamps. The flasks were agitated 2-3 times daily for proper mixing and aeration.

Phycocyanin and β-carotene content

Phycocyanin extraction was performed by an author using probe ultrasonicator on lyophilized biomass of *Spirulina*. Experimental trials were performed for different levels of amplitude such as 20, 40, 60, 80, and 100 meters with time intervals of 2, 4, 6, 8, 10, and 12 min and operating at 33%, 53%, and 66% of the duty cycle sequentially performed. Optimum conditions that resulted in higher yield of phycocyanin were Utilized, and the remaining biomass was subsequently used for the extraction of β-carotene and *Phycocyanin* from *Arthrospira sp.*

RESULTS AND DISCUSSION

Morphology Identification

The nine algal isolates were examined microscopically, confirming their purity and allowing for preliminary identification. The isolates were then classified up to the genus level based on their distinct morphological characteristics, as outlined in Table 2. Morphological identification was conducted using an inverted microscope at 100x magnification, as illustrated in Figure 1. Key morphological characteristics considered for identification included cell or filament shape, the type of cell division, in addition to the presence of a sheath or envelope surrounding the cells [16-18].

Biochemical Characteristics

Biomass analysis of all isolated samples was performed on the 15th day post-inoculation under continuous white fluorescent light (2500-3000 lux). Dry weight measured biomass, while carbohydrate, protein, and lipid profiles were assessed for the nine isolates. Table 3 summarizes biomass yield (g/L) and percentages of carbohydrate, protein, and lipid content. MS-30 showed the highest biomass yield, protein, and lipid content but had the lowest carbohydrate levels. The study emphasized phycocyanin and β-carotene, key compounds: phycobiliproteins, light-harvesting proteins in cyanobacteria and some algae, and carotenoids, lipid-soluble pigments in plants and algae [19].

Table 2. Morphological observation of isolates

Sample ID	Colony characteristics	Microscopic Observation at 100X magnification	Genus
FS-1	Green colonies with a circular shape, convex surface, and smooth edges, (Size- 10^3µm).	Cells are unicellular, circular, pyrenoids present, cup shaped chloroplast present, mucilaginous sheath absent.	*Chlorella* sp. (Selvarajan, 2015)
FS-3	Circular green colonies with a convex structure and smooth, unbroken margin (Size- 10^3µm).	Cells are unicellular, circular, pyrenoids present, cup shaped chloroplast present, mucilaginous sheath absent.	*Chlorella* sp. (Selvarajan 2015)
FS-4	Colonies with green mat like growth with mucoid origin	Trichomes are single, thin, straight, and not constricted at cross walls. Cells are longer than wide, with erect, non-attenuated ends and a convex, thick membrane. The cells exhibit high motility and intensive gliding movement..	*Geitlerima sp*. (Desikacharya, 1959)
FS-18	Green colonies presenting a circular form, convex profile, and entire edge (Size- 10^3µm).	Unicellular or 2-3 celled coenobium forms, arranged linearly or laterally in 1-2 rows, often surrounded by mucilage. Cells are elongated, cylindrical, or ovoid, with rounded apices.	*Scenedesmus* sp. (Hindakova , Hindak, 1998)
FS-22	Colonies with a green circular shape, convex nature, and uninterrupted margin, (Size- 10^3µm).	Cells are spherical and vary in size, with thin, smooth walls that thicken as they grow. The chloroplasts are located at the periphery, with or without a peripheral opening, and contain a single pyrenoid.	*Chlorococcum sp*.(Feng et al. 2016)
FS-23	Green colonies characterized by a round shape, convex surface, and complete margin, (Size- 10^3µm).	Unicellular cells, cup- to girdle shaped chloroplast seen cell, pyrenoids present	*Chlorella sp.* (Selvarajan, 2015)
FS-24	Green circular colonies with a convex shape and intact margin, (Size- 10^3µm).	Cells are spherical and vary in size, with thin, smooth walls that thicken as they grow. The chloroplasts are parietal, with or without a peripheral opening, and contain a single pyrenoid.	*Chlorococcum* sp. (Feng *et al.*, 2016)
FS-27	Green colonies with a round form, convex structure, and smooth margin, (Size- 10^3µm).	Cells are unicellular, circular, pyrenoids present, cup shaped chloroplast present, mucilagenous sheath absent	*Chlorella* sp. (Selvarajan 2015)
MS-30	Colonies with green mat like growth with mucoid origin	Trichomes are multicellular, cylindrical, coiled without a sheath, and slightly narrower at the ends. Morphologically resembling Spirulina, but with distinct cross-walls; differentiation verified through both molecular and morphological analysis.	*Arthrospira sp.* (Desikacharya, 1959)

Table 3. Biochemical characters of isolated microalgae

S N	Sample No	Biomass (g/L)	Carbohydrate (%)	Protein (%)	Lipid (%)
1	FS-01	0.41±0.05	7.4±0.07	27.3±0.06	1.68±0.13
2	FS-03	0.81±0.09	7.0±0.06	35.8±0.07	1.80±0.17
3	FS-04	0.93±0.05	4.0±0.09	35.4±0.09	1.93±0.22
4	FS-18	0.65±0.15	5.1±0.12	37.4±0.10	1.83±0.24
5	FS-22	0.99±0.12	6.3±0.19	35.9±0.11	1.63±0.20
6	FS-23	0.63±0.06	6.2±0.12	37.2±0.17	1.61±0.26
7	FS-24	0.27±0.08	6.3±0.08	34.7±0.10	1.20±0.14
8	FS-27	0.12±0.13	5.5±0.05	38.7±0.12	0.90±0.13
9	MS-30	5.50±0.20	2.5±0.20	47.9±0.10	4.2±0.14

Identification by 16 S R-DNA polymorphism

The MS-30 isolate, obtained from Lonar Lake, Maharashtra, was identified in collaboration with Aavanira Biotech, Pune. The morphological identification of *Arthrospira* was carried out, followed by 16S rDNA polymorphism analysis for verification. Cyanobacterial genomic DNA was extracted and examined on a 1% agarose gel. PCR amplification was conducted using the Paq 5000TM DNA polymerase kit, and the PCR product, along with a 100 bp DNA marker, was loaded on a 1% agarose gel, revealing a 392 bp band. DNA sequencing of the full 16S rDNA of MS-30 (1286 base pairs) confirmed the species as *Arthrospira sp*. of the class Cyanophyceae. Phylogenetic analysis, based on the BOOTSTRAP method (500 replicates), was used to infer the evolutionary history, with evolutionary distances shown on the phylogenetic tree. Table 4 illustrates the phylogenetic relationships of the taxa.

Sequencing of microalgae DNA

The sequencing results from cycle S3E2 were analyzed using the Genetic Analyzer 5.2 and software sequencing analysis, following the instrument's manual. The sequence was further analyzed to meet the specified criteria. Table 4 and Figure 2 present the evolutionary divergence estimates between sequences. The analysis, based on the Maximum Composite Likelihood model, included 6 nucleotide sequences with codon positions 1st, 2nd, 3rd, and noncoding. Gaps and missing data were removed, leaving 1286 positions in the final dataset. The phylogenetic tree was constructed MEGA 6, and bootstrap analysis was performed with 1000 replicates. Bootstrap support values are indicated at the respective nodes. The analysis revealed that sequences KX840360.1 and KU606509.1 are the most closely related, with a bootstrap support of 18%, suggesting moderate confidence. Similarly, KX279415.1 and KT779290.1 are grouped together with a bootstrap support of 19%. Our isolate (AB 06 17-18 CRS-26) is positioned on a separate branch, indicating evolutionary divergence from the compared sequences. The 99% similarity values in the table refer to the sequence similarity between the DNA sequences of the samples and known sequences in the Gene Bank database (typically from 16S rRNA gene sequencing, which is commonly used for bacterial identification).

Effect of cultivation on biomass of *Arthrospira*

Cultivation of *Arthrospira sp.* was conducted under autotrophic (using fluorescent lamps), mixotrophic (using fluorescent lamps and organic carbon sources), and heterotrophic (in the absence of light) conditions to enhance biomass yield in a shorter period.

Figure 3 compares the growth of *Arthrospira sp.* under autotrophic, mixotrophic, and heterotrophic conditions. The results showed that autotrophic conditions led to better growth than mixotrophic conditions. Maximum growth occurred under autotrophic conditions, followed by 0.1% glucose in mixotrophic conditions. Biomass was reduced by 36% with 0.1% glucose and by 56% with 0.1% sodium acetate compared to autotrophic conditions (control). Increasing organic carbon concentration from 0.1% to 0.5% or 1% led to further biomass reduction. Photosynthesis uses light energy to produce ATP and NADPH, enabling carbon fixation into organic compounds within chloroplasts. Light is captured by chlorophyll-carotenoid complexes and directed to reaction centers to generate electrons. Insufficient light causes etiolation. Cool-white fluorescent light supports controlled plant growth, simulating natural conditions. In mixotrophic growth, *Arthrospira* combines photosynthesis and respiration; excess organic carbon (e.g., glucose, acetate) under high light (3000 lx) may inhibit biomass production [20-24].

Table 4. Comparative Analysis of *Arthrospira* Species with percentage Similarity

Sample	Gene bank Entry	% similarity	Kingdom	Phylum	Class	Order	Family	Genus	Species
AB/06/17-18/CRS-26	KX840360.1	99%	Bacteria	Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	*Arthrospira*	PMC737.11
	KX279418.1	99%	Bacteria	Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	*Arthrospira*	*platensis*
	KX279415.1	99%	Bacteria	Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	*Arthrospira*	*platensis*
	KU605609.1	99%	Bacteria	Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	*Arthrospira*	--
	KT779290.1	99%	Bacteria	Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	*Arthrospira*	--

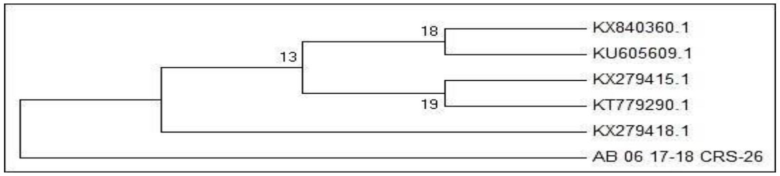

Figure 2. Phylogenetic relationship with first five BLAST results from NCBI GenBank Database

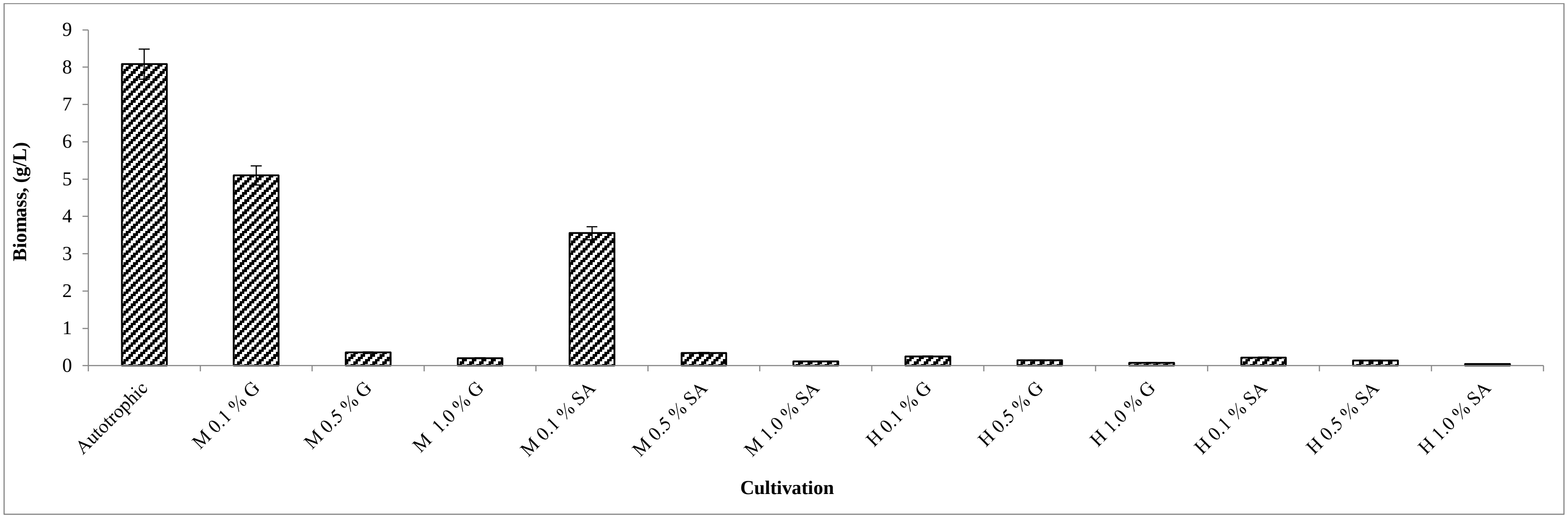

Figure 3 Biomass of *Arthrospira sp.* under various trophic conditions with fluorescent light

Effect of autotrophic conditions using LEDs on biomass of *Arthrospira*

Arthrospira was cultivated autotrophically with light-emitting diodes (LEDs) to evaluate the impact of different wavelengths on microalgal growth (Figure 4). Five LED colors (blue, green, red, white, and yellow) were tested, with fluorescent light as the control. Among the different LEDs, yellow LED produced the highest biomass yield (10.5 g/L), while blue LED resulted in the lowest biomass (4 g/L). Red LED produced 8.8 g/L, which was higher than the control (6.8 g/L). Green and Blue LEDs yielded less biomass than the control. Yellow LED resulted in a 35% higher biomass yield compared to the control. The ranking of LED colors for biomass growth efficiency was: Yellow > Red > White > Green > Blue. Figure 4 illustrates biomass yield under different LED colors. Comparable results were reported by Wang, who found that red LED promoted the highest biomass accumulation in Spirulina platensis [25]. This aligns with the fact that microalgae absorb red light through chlorophyll, boosting photosynthesis [26]. Although red light is typically associated with enhanced photosynthesis due to strong chlorophyll absorption, our results showed higher biomass under yellow LED. Yellow light, positioned between red and green in the spectrum, may activate both chlorophyll and accessory pigments such as carotenoids, improving overall light absorption and energy transfer. It may also cause less photo-oxidative stress than red light, thereby supporting better growth. In contrast, other LED colors are only partially absorbed, contributing less to photosynthesis.

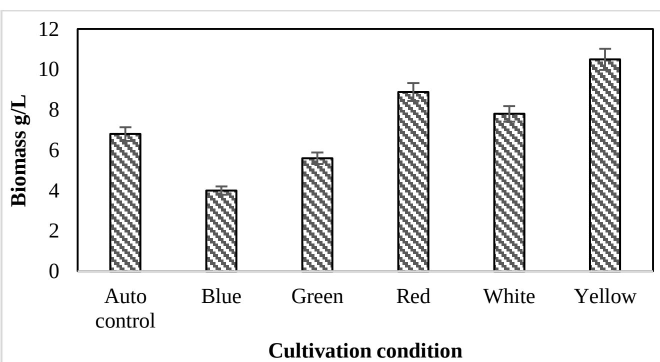

Figure 4 Biomass of *Arthrospira* under autotrophic growth with different LED colors

The LED data sheet shows that yellow LED's emission spectrum significantly overlaps with red light, while green LED's overlap is minimal. This likely explains why *Arthrospira* grew faster under yellow LED compared to green LED. In contrast, blue LED, with no overlap with red light, was less effective in promoting growth. White LED, combining red light with less effective spectra, produced moderate biomass, indicating a mixed effect from the entire light spectrum. Similar studies [26, 27] found red light yielded the highest biomass, while blue light led to the lowest during Spirulina cultivation under various LEDs. *Arthrospira* sp. yielded results that differed from those of Wang. Akimoto's findings on light-harvesting system adaptation in *Arthrospira sp.* under varying light conditions also support this, as analyzed through time-resolved fluorescence spectroscopy. Akimoto reported that under yellow light, energy transfer within the phycobilisomes occurred rapidly since only the phycobilisomes absorbed the light [28].

Effect of mixotrophic condition using LEDs on biomass of *Arthrospira*

Initial experiments showed that mixotrophic conditions significantly increased biomass production, with LED lighting further enhancing this effect. Based on these results, a combination of mixotrophic conditions and LED lighting was chosen for further optimization of biomass yield. Glucose (0.1%) was used as the carbon source to achieve maximum biomass production. Cultivating *Arthrospira* under *mixotrophic* conditions (0.1% glucose) with LEDs as the light source resulted in varied biomass production, with no significant increase compared to the control under fluorescent light (Figure 5). Zhang's mathematical model for Spirulina platensis suggested that the optimal glucose concentration decreases with increasing light intensity, likely due to reduced aerobic respiration at higher intensity of light, which limits the effect of high glucose concentrations on growth [24]. In contrast, at lower light intensity, higher glucose concentration is needed to support respiration. Given that LEDs provide high light intensity, the addition of organic carbon, such as glucose, was unnecessary for optimal *Arthrospira* growth, making 0.1% glucose ineffective for improving biomass production.

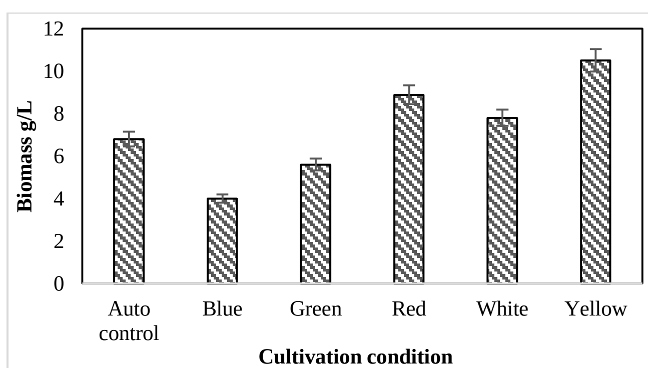

Figure 5 Biomass of *Arthrospira* under mixotrophic (0.1% glucose) growth with different LED colors

Phycocyanin and β- carotene content

Phycocyanin extraction through ultrasonication requires ultracentrifugation to separate the pigment from cellular debris [29]. In a study using lyophilized *Spirulina platensis* biomass, Taguchi's experimental design was applied to optimize ultrasonication for phycocyanin extraction and observed increased yield with higher amplitude due to improved cavitation effects [30]. As amplitude increased, the ultrasonic horn delivered more power, improving cavitation, mixing, and emulsification.

The highest phycocyanin yield (62 mg/g) from *Arthrospira sp*. was achieved at 80% amplitude, 4-minute sonication time, and 33% duty cycle. The maximum yield of β-carotene (6.73 mg/g) from *Arthrospira* lyophilized biomass occurred at 66% amplitude, 10-minute sonication time, and 33% duty cycle. The phycocyanin yield of 62 mg/g obtained in this study surpasses several previously reported values. Moraes et al. [31] reported yields around 45–50 mg/g under optimized ultrasonication conditions, while Tavanandi et al. [32] documented even lower yields using conventional extraction methods. The use of high amplitude (80%) and optimized sonication parameters in the present work likely enhanced cavitation, resulting in superior pigment release. Similarly, the β-carotene yield of 6.73 mg/g is notable, as earlier studies such as those by Hemlata et al. [20], and Dufossé et al. [33] reported yields ranging from 3 to 5 mg/g under comparable or less intensive extraction conditions. These findings indicate that the applied ultrasonication protocol particularly with lyophilized biomass and precise control of amplitude and duration can significantly improve pigment recovery from *Arthrospira species*.

CONCLUSION

In this study, thirty algae samples from different water sources were screened, resulting in the isolation and identification of nine strains. *Arthrospira sp*. was identified through molecular characterization and found to contain sufficient biomass, protein, and lipids. It exhibited the highest protein yield (47.9%) and lipid content (4.2%) among the isolated strains, highlighting its potential for metabolite production. Cultivation under various conditions, including LED-based setups, revealed that light spectra significantly influence biomass yield. Yellow LEDs, due to their overlap with red light spectra, produced the highest biomass, while mixotrophic conditions with glucose inhibited growth. *Arthrospira sp*., particularly from Lonar Lake, proved to be a cost-effective feedstock for extracting phycocyanin and β-carotene. Furthermore, Lonar Lake's unique saline ecosystem offers opportunities for exploring diverse algal species for future research.

To enhance industrial applicability, pilot-scale testing of *Arthrospira sp*. under optimized conditions is recommended. This would assess scalability and economic feasibility for commercial production of high-value metabolites and biofuels, paving the way for sustainable algal biotechnology solutions across various industries.

DECLARATION

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Authorship Contributions

Concept and Writing: Dr. Manisha Bachchhav; Data Collection and Interpretation: Dr. Manisha Bachchhav; Dr. Bhanudas Bachchhav: Methodology: Dr. Manisha Bachchhav; Dr. Bhanudas Bachchhav; Review & Editing: Dr. Manisha Bachchhav; Dr. Bhanudas Bachchhav

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Competing interests

The authors declared that there is no conflict of interest.

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