Research Article

Microscopic and Biochemical Identification of *Spirulina* spp. for its Biomass Cultivation by Using Different Types of Photobioreactors at Lab and Pilot Scale

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Abstract: This study enlightens the methods used for the identification as well as the growth of *Spirulina* species in Pakistan. *Spirulina* was identified based on morphology using microscopic technique along with its biochemical identification using SDS PAGE. After the endorsement of spirulina species via microscopy and SDS PAGE, *Spirulina* was subjected to growth in Zarrouk’s media. Efficacy of growth was observed using three different methods Tubular photobioreactors, Fed-batch photobioreactors, and Attached cultivation which boosts the yield of *Spirulina* biomass via improving its biochemical composition. Hence, it concludes that production of the *Spirulina* using tubular photobioreactors on pilot-scale yields cost-effective benefits and provides us with an enhanced biochemical content ratio of *Spirulina* and can easily be identified by using several approaches including biochemical, molecular, and microscopic methods.

Keywords: *Spirulina* Spp.; Biochemical identification; microscopic identification; SDS PAGE; Tubular Photobioreactors; Fed-batch photobioreactors; Attached cultivation

1. Introduction

*Spirulina* is commonly known as blue-green algae. *Spirulina* is a renowned phytoplanktonic photosynthetic cyanobacterium comprised of the filamentous body that formulates a massive population in tropical in addition to subtropical water bodies having high levels of carbonates [4]. It is a primeval species that originated about 3.5 billion years ago. *Spirulina* is a multicellular cyanobacterium having phycocyanin as photosynthetic pigment whereas it also comprises chlorophyll a and carotenoids. Morphologically *Spirulina* is composed of cylindrical trichome of about 50 – 500 μm in length and 3 – 4 μm in width [14]. In terms of Genetic Makeup, *Spirulina* is a uni-chromosomal organism with 6.8 million Bases, 6,631 circular genes, and almost 44.3% of GC content. *Spirulina* comprises 2.2%-3.5% of RNA and 0.6 %-1% of DNA which depicts the component of the nucleic acid is less than 5% of its dry weight [7]. Spirulina can readily be grown in alkaline water, especially in back rush waters with high levels of carbonates and bicarbonates. It has
gained worldwide attention because of its unusual protein content of 60–70% in comparison to other organisms. It can grow at the optimum pH range of 9 – 12 & Temperature range of 30 - 40 C. Its composition mainly varies with the variation in the nutrient content of the media in which it is cultivated [14]. *Spirulina* biomass mainly comprises biopeptides, vital fatty acids, biopolymers, sterols, carbohydrates, minerals, and oligo-elements [11]. It has been approved as a superfood by Food and Agriculture Organization in 2008. It is beneficial as it depicts several health properties like anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal, anti-cancerous, and many more as it aids in weight loss manages diabetes, reduces blood pressure as well as different cardiovascular diseases by lowering the cholesterol level in the blood [5]. As *Spirulina* can endure high temperatures and pH, they are highly resistant to contamination and can encounter unfavorable environmental conditions easily through the production of protective compounds. So, it is not easy to use genetic makeup tools developed for the algal characterization on it for genetic-based characterization. Several works have been done on it depicting that it may foretell the advancement of atherosclerosis alongside coronary heart disease by lowering the level of saturated fatty acid and triglycerides present in the blood plasma. *Spirulina* has been coping up high attention because of its ability to crop a diverse variability of chemicals along with biological active complexes, such as vitamins, proteins, lipids, carotenoid pigment, and polysaccharides [16]. To analyze the potential benefits of *Spirulina* it needed to be cultivated at the commercial level. Researchers are undertaking strenuous exertions to yield cyanobacteria on a commercial scale [1,2].

2. Materials and Methods

**Identification methods**

Identification of spirulina species has been done by different kinds of methods including biochemical and microscopic methods.

**Sample collection**

A total of twenty-six samples were collected from thirteen (two samples from each site) different specifically selected sites in Punjab that corresponds to the district Dera Ghazi Khan and Lahore. Lahore latitude and longitude are 31°31’50.3”N 74°19’34.0” E respectively. Dera Ghazi Khan’s latitude and longitude are 30°03’11.2”N 70°37’46.3” E respectively. While collecting the samples, temperature and pH were kept into consideration. The collected samples were preserved in plastic bottles. Samples were isolated using the streak plate method as well as the serial dilution method.

**Microscopic method**

Samples have been studied microscopically under different objective lenses including 4x, 10x, 40x, and 100x. *Spirulina* has been identified under the 10x and 40x lenses by their spiral structure.

**Biochemical Method**

Entire protein extraction has been done using the Livne [10] method, and then attained protein of each sample was subjected to the Sodium dodecyl sulfate (SDS PAGE) method [9].
Growth and cultivation methods

Different cultivation methods were applied for the estimation of growth and cultivation of spirulina species at the lab scale.

Tubular Photobioreactors

The inoculum was grown in a sodium nitrate culture medium for 6-8 days at a pH of approx. 9.5 and temperature nearly at 29°C with a continuous supply of CO₂ on daily basis in tubular photobioreactors. The temperature was maintained using a thermal chamber while sodium nitrate was used as a nitrogen source resulting in better production of *Spirulina* in tubular photobioreactors [3,8].

Fed-batch Process

In the fed-batch process, Erlenmeyer flasks were used to grow spirulina in Zarrouk’s media. The first 0.1g/L of inoculum was added. Every time after the assimilation of all the substrate the new inoculum was added. That’s why it is known as the fed-batch process because of the continuous supply of substrate. This process yields 2.67 times higher production if urea is used as a nitrogen source instead of sodium nitrate of Zarrouk’s media [6,12].

Attached Cultivation

*Spirulina* was subjected to growth in the Zarrouk’s medium. In the middle of the chamber that was used for attached cultivation inoculum containing a glass strip attenuated with the cellulose and covered by a slip has been placed. The *Spirulina* species grows using the light from white fluorescence lamps. Biomass productivity of spirulina attained during this process was estimated i.e 60 g m⁻² d⁻¹ [15].

3. Results

3.1. Collection and Isolation of samples

Samples have been collected from 13 sites in Dera Ghazi Khan and Lahore. Then they are morphologically isolated by using the microscopic technique.

3.2. Identification Methods

3.2.1 Microscopic identification

All the species have been viewed under the 10x and 40x lenses of a microscope and spirulina species were identified based on their spiral structure morphology. Images obtained from 10X magnification have been presented in figure 1:

![Microscopic images of spirulina platensis](image-url)
3.2.2. Biochemical Identification

The study of different bands attained via SDS PAGE using specific strains helps in the differentiation and identification of different species of *Spirulina*. UTEX 552 and AC-GEF sp3 strains have been used for *Spirulina maxima* and UTEX 1926, ACGEB sp2 and ACGEB sp1 strains have been used for *Spirulina platensis* as shown in Table 1. The highest value of the similarity index (0.94) was observed between two strains (ACGEB sp1 and ACGEB sp2) whereas the lowest value (0.53) recorded was between the local *S. platensis* strain ACGEB SP1 and the standard *S. maxima* UTEX 552 strains. Figure 2 illustrates the banding pattern of SDS PAGE divides four strains into two groups i-e one local *Spirulina* platensis strain (three sub-groups S1, S2 & S3) and one standard strain of *S. maximum* (UTEX 552).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Species</th>
<th>Sample 4</th>
<th>Sample 3</th>
<th>Sample 2</th>
<th>Sample 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTEX 1926</td>
<td><em>S. platensis</em></td>
<td>0.75</td>
<td>0.75</td>
<td>0.61</td>
<td>0.67</td>
</tr>
<tr>
<td>ACGEB sp1</td>
<td><em>S. platensis</em></td>
<td>0</td>
<td>0.94</td>
<td>0.51</td>
<td>0.65</td>
</tr>
<tr>
<td>ACGEB sp2</td>
<td><em>S. platensis</em></td>
<td>0</td>
<td>0</td>
<td>0.56</td>
<td>0.64</td>
</tr>
<tr>
<td>UTEX 552</td>
<td><em>S. maxima</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table 1: Similarity coefficient between different Spirulina samples by using SDS PAGE.

![SDS PAGE](image)

Figure 2: SDS PAGE for different species of *Spirulina*

3.3. Cultivation of Spirulina Species

3.3.1. Tubular Photobioreactor

The oxygen concentration has been increased by the cultural movement toward the tubular circuit that enhances productivity. Lengths of photobioreactors possess a different
impact on the growth of *Spirulina* species due to the greater exposure to light as described in Table 2:

<table>
<thead>
<tr>
<th>Biochemical composition</th>
<th>Length of photobioreactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>260m</td>
</tr>
<tr>
<td>Crude protein</td>
<td>62.1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>19.0</td>
</tr>
<tr>
<td>Total lipids</td>
<td>13.6</td>
</tr>
<tr>
<td>Ash</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Table 2: Influence of the length of photobioreactors on the biochemical composition of *Spirulina*

Figure 3: Influence of length of photobioreactors on the biochemical composition of *Spirulina*

3.3.2. Fed-batch photobioreactors

Growth of biomass has been observed concerning the duration of 50 hours.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Biomass (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-50</td>
<td>0.2</td>
</tr>
<tr>
<td>50-100</td>
<td>0.3</td>
</tr>
<tr>
<td>100-150</td>
<td>0.35</td>
</tr>
<tr>
<td>150-200</td>
<td>0.4</td>
</tr>
<tr>
<td>200-250</td>
<td>0.55</td>
</tr>
<tr>
<td>250-300</td>
<td>0.8</td>
</tr>
<tr>
<td>300-350</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 3: Change in Biomass with reference to time
3.3.3. Attached photobioreactor

A high rate of cultivation biomass has been observed i.e. 59.9 gm²d⁻¹. Table 4 shows a high yield of percentage of the dry weight of carbohydrates; lipid and protein have been calculated.

<table>
<thead>
<tr>
<th>Cultivation method</th>
<th>Culture time duration (d)</th>
<th>Crude Protein (%age dry weight)</th>
<th>Lipid (%age dry weight)</th>
<th>Carbohydrate (%age dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attached Cultivation</td>
<td>1</td>
<td>45.87±0.61</td>
<td>10.18±0.61</td>
<td>12.31±0.60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>48.56±0.67</td>
<td>10.24±0.60</td>
<td>12.34±0.61</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.91±0.21</td>
<td>10.74±0.23</td>
<td>12.67±0.64</td>
</tr>
</tbody>
</table>

Table 4: Percentage of dry weight Protein, Lipid, and Carbohydrates of Spirulina spp. with attached cultivation.

3.3.4. Comparative study of Spirulina production by different methods:

Spirulina exhibits different biomass concentrations when subjected to diverse methods of cultivation. Spirulina biomass cultivation in tubular photobioreactors, fed-batch process and Attached cultivation process is 64.3gm⁻²d⁻¹, 52.6 gm⁻²d⁻¹ and 59.9 gm⁻²d⁻¹. The comparative study between all three above-mentioned methods shows that spirulina yields high biomass productivity within tubular photobioreactors due to its great exposure to light as shown in figure 5 below:
4. Discussion & Conclusion

In this study, sampling was done from 13 different localities of Lahore and the Dera Ghazi Khan district. Amongst all the attained species, *Spirulina* has been identified based on morphology under microscope 40X (as shown in Figure 1) whereas endorsement of *Spirulina* species was done by verifying its biochemical composition using SDS PAGE [9,10]. This study tends to be very useful in the identification and growth of *Spirulina* on a distinct scale. However, it also dictates the improved methods for the agronomy of *Spirulina* species in the lab as well as on a pilot scale by exploiting three different types of photobioreactors including tabular photobioreactors [3,8], fed-batch photobioreactors [6,12], and attached photobioreactors [15] that boost the production of *Spirulina* biomass by enhancing its biochemical composition (as illustrated in Table 2 & 4). Whereas, the comparison between all three methods shows that the tubular photobioreactors yield the highest biomass productivity due to effective light intensity (as shown in Figure 5). Hence, it achieves that cultivation of the *Spirulina* on a pilot scale produces cost-effective benefits and offers us a heightened biochemical content ratio of *Spirulina*.

5. Future Perspectives

As far as the future perspectives are concerned, we will tend to imply all this work for the conversion of the cultivation process from pilot to industrial scale in Pakistan. So that in the future, *Spirulina* species can easily be cultivated on an industrial scale more effectively and conveniently. Due to the inevitable benefits of *Spirulina* on nutritional as well as pharmaceutical levels, it can be utilized in many fields as a more efficient or nutri-
tional feed and medicine. Whereas, in third world countries like Pakistan facing malnutrition, production of *Spirulina* on large scales or industrial scale may appear as a better food alternative because of its nutritional properties and ease of cultivation. On other hand, this also tends to be useful economically.

**Author Contributions:**

Author individual contributions include Conceptualization, Muhammad Asjad Khan.; methodology, Musaba Zaheer and Maria Hafeez.; software, Muhammad Asjad Khan; validation, Muhammad Asjad Khan; formal analysis, Muhammad Asjad Khan, Musaba Zaheer and Maria Hafeez.; investigation, Muhammad Asjad Khan, Maria Hafeez, and Musaba Zaheer; resources, Muhammad Asjad Khan, Musaba Zaheer and Maria Hafeez.; data curation, Musaba Zaheer and Maria Hafeez; writing—original draft preparation, Maria Hafeez and Musaba Zaheer; writing—review and editing, Dr. Uzma Hameed.; visualization, Muhammad Asjad Khan, Maria Hafeez, and Musaba Zaheer; supervision, Muhammad Asjad Khan; project administration, Muhammad Asjad Khan; funding acquisition, Dr. Uzma Hameed. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:**

The authors declare no conflict of interest.

**References**


