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STUDYING THE GROWTH OF SPIRULINA PLATENSIS IN NOVEL ORGANIC MEDIA

Biotechnology

Rahul Anand* PhD Scholar, RKDF University and Research Associate, Bunshi Bioscience
*Corresponding Author

Deepak Kumar Director, Bunshi Bioscience.

ABSTRACT

Spirulina platensis is one of the most popular and common food supplements used in many parts of the world in ages. It is rich source of protein, and other nutrients like Vitamins and minerals. It is cultivated and marketed as a supplementary source of nutrition. The objective of this study is to achieve an efficient media composition and optimized growth conditions for cultivation of Spirulina platensis in the climatic conditions of Ranchi, Jharkhand.

KEYWORDS

Spirulina Platensis, Malnutrition, Cyanobacteria, Dietary Supplement, Single Cell Protein.

INTRODUCTION:

Spirulina platensis, commonly known as Spirulina is a photoautotrophic, filamentous, multicellular organisms that belong to the *Oscillatoriaceae* family of *Cyanophyceae* class. It consists of filamentous cyanobacteria that have a characteristic spiral-shaped chain of cells enclosed in a four layered (Eykelenburg C 1977) thin sheath. Spirulina is generally found in marshes, freshwater lakes and ponds, brackish water, marine waters, and even in thermal springs. It has a very long history of being used a source of food by the people of Aztec Civilization of Mexico (Dillon et al., 1995).

It is a well-known source of nutrition since centuries and still used as a popular food supplement. Spirulina is not only high in protein i.e., up to 70% content (Phang *et al*., 2000); it is also rich in vitamins and minerals. *Spirulina platensis* are a type of cyanobacteria that due to its high nutritional value and health benefits have been recorded to be consumed for centuries in many parts of the world. Due to high protein content, it is also called as Single Cell Protein. It can also be applied in sustainable processes with co-product streams (Belay *et al*., 1993).

Spirulina platensis is a rich source of biologically active compounds, crucial for feed purposes, especially β-carotene, chlorophyll, lutein, phycobiliprotein, astaxanthin, amino acids, polyunsaturated fatty acids and vitamins (Lum *et al*., 2013; Yaakob *et al*., 2014; Farag *et al*., 2016; Madeira *et al*., 2017; Nawrocka *et al*., 2017). Extracts of *A. platensis have been reported to be suppressive against common bacterial and fungal pathogens like Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia, Candida tropicalis*, *Candida albicans*, *Candida glabrata*, *Aspergillus flavus*, *Aspergillus fumigates* and *Aspergillus niger* (Abdel-Moneim *et al*., 2022). *It can also be used for the development and production value added consumer products like cookies (*Donato *et al*., 2019*), soft cheese, ice cream (*Agustini *et al*., 2016) *and biscuits (*Setyaningsih *et al*., 2019*).*

Apart from the nutraceutical properties Spirulina has also been investigated for clinical applications. It contains energy providing polysaccharides like Glycogen and Rhamnose that can be used to get rid of fatigue; and essential fatty acid like Gamma Linolenic Acid that the body cannot synthesize. These can be easily absorbed by the human body and help in energy release. It also acts as a probiotic that encourage the lactobacillus in the intestine that in turn produces Vitamin B6 which is also promotes energy release (Baicus C and Baicus A, 2007).

Spirulina has recently been investigated for anti-inflammatory properties. It has been established that feeding Spirulina can significantly reduce the Interleukin-4 (IL-4) levels (Mao *et al*., 2005). Although, there no *in vivo* study to claim the effect of Spirulina in treating the viral infections, but *in vitro* studies provide strong evidence for the anti-viral properties of Spirulina. Water extract of *S. platensis* has active component Calcium-spirulan (Ca-Sp) that in *in vitro* conditions inhibits the replication of many enveloped viruses including Herpes simplex type I, human cytomegalovirus, measles and mumps virus, influenza A virus and human immunodeficiency virus-1 virus (HIV-1) (Hayashi *et al*., 1997). However, more studies in clinical conditions needs to be done to use Spirulina as a source of antiviral drugs.

In a first human study, it has been found that Spirulina intake can significantly reduce the LDL-cholesterol (Low Density Lipoprotein – cholesterol) in Type – 2 Diabetes patients (Nakaya *et al*., 1988; Rostami *et al*., 2022). It has been long argued that the combined immune modulation and antioxidant characteristics of *Spirulina platensis* might have a possible mechanism of tumor destruction and hence play a role in preventing cancer development.

Effect of Spirulina intake has been investigated in oral carcinogenesis (Mathew *et al*., 2009). Antiproliferative properties of water extracts of *A. platensis* against the breast cancer adenocarcinoma cell line (MCF-7) and mice intestine carcinoma cell line (L20B) suggest that new promising anticancer natural products from blue-green algae can be a reality.

Spirulina platensis is one of few natural sources that can cater to multiple nutritional challenges; from malnutrition to cancer prevention, controlling diabetes and many more. However, despite of the many benefits and demand of Spirulina, very few commercial Spirulina farms are operational in India. The current study aims to optimized the growth conditions and devise an efficient media composition for Arthrospira sp., suitable for the climatic conditions of Ranchi, Jharkhand

MATERIALS AND METHODS:

Water samples were collected from lakes, water reservoirs and ponds (Ranchi Lake- 23°21'57.8"N 85°19'09.1"E; Dhurwa Dam - 23°17'21.6"N 85°15'29.6"E) and in Ranchi city of Jharkhand state of India. Water samples from different sources were brought to laboratory and serially diluted in the process to obtain pure culture of cyanobacteria species.

Growth media: The serially diluted, crude water samples were initially inoculated on BG-11 media (pH – 7.5). Spiral shaped, filamentous cells were detected and visualized by a compound microscope and were inoculated in different concentration of organic extract media (Table – 2) developed in lab, combined with CFTRI media (Table – 1). pH was maintained at 10. Light intensity was continuously maintained at 2,300 lumens and temperature at 25°C.

On every 15th day, dry weight per ml was recorded. Regular microscopic observations were taken to ensure that the cultures had no contamination.

Elemental composition of the organic extract media developed in the lab used for growth of Spirulina has been mentioned in Table-7. pH was adjusted to 10 with NaOH.

Preparation of Organic extract: Organic waste materials were sun dried. The dried organic material was grinded into fine powder. It was soaked into distilled water for overnight and filtered with Whatman filter paper No. – 1. The filtrate was then used in different media combinations.

Table 1: CFTRI Media composition (Jainab et al., 2014; Salunke et al., 2016)

Composition	Concentration (g/L)
Sodium Bicarbonate	4.5
Dipotassium hydrogen phosphate	0.05
Sodium Nitrate	1.5
Potassium Sulphate	1.0
Sodium Chloride	1.0
Magnesium Sulphate	0.2
Calcium Chloride	0.04
Iron Sulphate	0.015

Combinations of media used for investigating the optimum growth:

Combination of Organic extract with CFTRI media:

Combination 1: 50% Organic Extract + 50% CFTRI media
Combination 2: 75% Organic Extract + 25% CFTRI media
Combination 3: 100% Organic Extract

Combination With Distilled Water:

Combination 4: 25% Organic Extract diluted with distilled water
Combination 5: 50% Organic Extract diluted with distilled water
Combination 6: 75% Organic Extract diluted with distilled water
pH of all the combinations was maintained at 10 using NaOH.

RESULTS:

Crude environmental sample from Ranchi Lake was screened by serial dilution. Spirulina was successfully grown in different combinations of media with slightly differed performance. Microscopic observation revealed the pure culture of spirulina in different media combinations (Figure – 1). Our study demonstrates that spirulina can be cultured and cultivated in an inexpensive organic media effectively. In our study, 6 different media combinations were tested for spirulina growth. The media combinations were a mix of organic extract and CFTRI media in different proportions. Among different media combinations, most efficient was found to be organic extract diluted with distilled water in 1:1 ratio. However, spirulina can still be grown in other combinations but for maximum productivity, combination 5 was found to be best. Combination 1 initially showed good response but in long run its productivity decreased. 100% extract did not yield proper growth may be due to excessive concentration of nutrients that may hamper with the growth of spirulina.

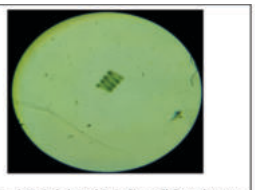

Figure – 1: Spiral shaped Spirulina cell from the pure culture.

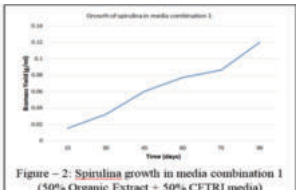

Figure – 2: Spirulina growth in media combination 1 (50% Organic Extract + 50% CFTRI media)

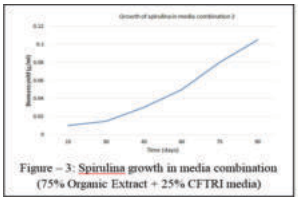

Figure – 3: Spirulina growth in media combination (75% Organic Extract + 25% CFTRI media)

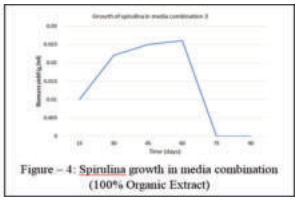

Figure – 4: Spirulina growth in media combination (100% Organic Extract)

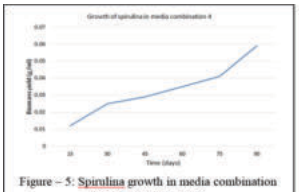

Figure – 5: Spirulina growth in media combination (25% Organic Extract)

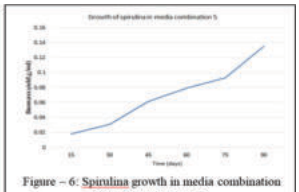

Figure – 6: Spirulina growth in media combination (50% Organic Extract)

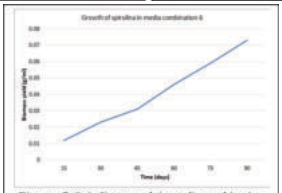

Figure – 7: Spirulina growth in media combination (75% Organic Extract)

CONCLUSION:

Our study concludes that spirulina can be grown in growth media prepared using extract of organic wastes. The highly alkaline (pH – 10) organic extract diluted to 50% strength with distilled water works best for spirulina culture at laboratory scale.

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