J. Indian bot. Soc. (2023) Vol. 103 (4): 290-293
https://doi: 10.61289/jibs2023.01.25.1143
e-ISSN:2455-7218, ISSN:0019 - 4468
www.indianbotsoc.org

RESEARCH PAPER

Optimization of nutrient media for enhanced production of bioactive compounds in *Spirulina fusiformis* Voronichin

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Abstract

Spirulina fusiformis is an economically important cyanobacterium well known for diverse biological activities and nutritional significance due to high concentration of natural products. In recent years, they have gained more importance because of their tremendous potential in the field of biotechnology, biofuel, functional food, nutraceuticals and pharmaceutical industries. The present study was focused on screening of low cost synthetic medium combination along with animal waste that would enhance the growth of *Spirulina fusiformis*. The biomass was evaluated on the basis of its photosynthetic pigments. The results obtained from the study indicated that SM-1 synthetic medium is most suitable nutrient media for the growth of *S. fusiformis*.

Key words: Batch culturing, Bio-active compounds, Cyanobacteria, Medium

Introduction

Cyanobacteria (Blue-green Algae) are autotrophic micro-organisms and occur in various environments. In recent days, they have gained much attention as a rich source of bioactive compounds and considered as the most promising group of organisms. They are the most prominent micro-organism because of its high protein, minerals, vitamins and enzymes. At present many species of *Spirulina* are available, but among them, *Spirulina maxima* and *S. platensis* are used for health advantages worldwide (Belay *et al*. 1993). The commercial production of *Spirulina* has gained worldwide attention for their use as human food supplement, animal feed and pharmaceuticals. It is the richest algal source of Gamma-linolenic acid (GLA) and many other biologically active compounds. The growth of *Spirulina* is influenced by many factors *viz.* nutrient, temperature and light.

The present study is focused on optimization of cheap synthetic nutrient media in combination along with animal waste for the maximum synthesis of chlorophyll, carotenoids, phycobiliproteins, proteins, carbohydrates and lipids.

Materials and Methods

Experimental Organism

The experimental organism used in the present study was *Spirulina fusiformis* obtained from Algal Biotech Laboratory, Botany Department, Chaudhary Charan Singh University, Meerut (U.P.), India.

Exponential culturing of *S. fusiformis*

Exponential culture of *S. fusiformis* was developed in conical flasks (Borosil-150ml) containing 100ml of BG-11 medium (Stainer *et al*., 1971) supplemented with 16g/L Sodium bicarbonate ($NaHCO_3$). All the culture flasks were kept under controlled conditions (Temp. 28 ± 2°C, Light 4-6 Klux and 14:10 hours Light: Dark regime) for ten days and were shaken continuously by magnetic stirrer (LABQUEST-MHPS15P).

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How to cite this article: Sarma, K., Tyagi, A., Doli, Gupta, D., Gauri, Kumar, N., Sharma, M., Kant, R. 2023. Optimization of nutrient media for enhanced production of bioactive compounds in *Spirulina fusiformis* Voronichin. J. Indian bot. Soc., 103 (4): 290-293. Doi: 10.61289/jibs2023.01.25.1143

Source of support: Nil

Conflict of interest: None.

Received: 09/04/2023 **Accepted:** 15/06/2023 **Published online**: 22/12/2023

Formulation of synthetic nutrient media

For experiments total three different synthetic nutrient's media *viz*. SM1, SM2 and SM3 were formulated by using 6.25% and 12.5% cow's urine along with modified BG-11 medium (SM1) and their chemical constituents are given in Table 1. The culture flask containing SM1 was used as control while SM2 and SM3 were used as variants.

Experimental design and batch culturing

Exponentially grown culture of *S. fusiformis* was batch cultured in three different synthetic media (SM1, SM2 and SM3) and was harvested for the estimation of growth, chlorophyll-*a*, carotenoids, phycobilins, carbohydrates, lipids and proteins. The experiment was conducted in a complete randomized design and in triplicate.

Estimation of Chlorophyll-a

Chlorophyll-*a* estimation was done by the method given by Lichtenthaler and Wellburn (1983).

Estimation of Carotenoid

Estimation of carotenoids was done by the method followed by Jensen (1978).

Estimation of Total Protein

Total protein was estimated by Lowry method (Lowry *et al*., 1951) using Bovine Serum Albumin (BSA) as standard.

Estimation Phycobilins

Phycobilins estimation was done by the method of Bennett and Bogorad (1973).

Estimation of Total Carbohydrate

The carbohydrate content was determined by the method of Hedge and Hofreiter (1962).

Statistical analysis of the data

The statistical analysis was carried out in Microsoft Office excel 2007 (Mondal and Mondal, 2016).

Results

Chlorophyll-a content in *S. fusiformis* was calculated every 10th day till third harvesting under the influence of different synthetic medium. The content Chl-a was observed maximum (10.19µg/ml) in SM1 medium while declining in Chl-a content was observed in SM2 medium after 10 days. But Chl-a content was negligible in SM3 medium as it could be toxic while the content of Chl-a was minimum (0.02µg/ml) in the SM3 after 30 days. Detailed result on synthesis of Chlorophyll-a by *S. fusiformis* is given in Figure 1(A).

Table 1: Nutrient media used for *S. fusiformis* culturing

Synthetic media	*Nutrient constituents medium*
SM1	Modified BG-11 nutrient medium
SM2	6.25% Cow's Urine + 93.75% SM1
SM3	12.50% Cow's Urine + 87.5% SM1

Carotenoids are photoprotective pigments found in cyanobacteria. Synthesis of total carotenoid pigment by *S. fusiformis* in different synthetic media was observed. *S. fusiformis* when cultured in SM1 medium showed steady increase in carotenoid synthesis on 1st harvesting, while after that, decline in carotenoid was observed till 3rd harvesting. *S. fusiformis* when cultured in SM2 medium showed a slow but steady growth till 3rd harvesting. Maximum carotenoid content (0.09µg/ml) was observed in SM1 after 10 days while it was minimum (0.001µg/ml) in SM3 after 30 days. Detailed result of total carotenoid synthesis by *S. fusiformis* is given in Figure 1(B).

Carbohydrates are the storage compounds of every cell. Maximum carbohydrate content (6387.2µg/ml) was observed in the SM1 medium after 20 days while it was minimum (2434.6µg/ml) in the SM3 medium after 30 days. Detailed result on synthesis of total carbohydrate in all the different media is given in Figure 1(C).

C-phycocyanin is a pigment protein complex from the light harvesting phycobiliprotein family. Phycocyanin is a characteristic light blue colour pigment absorbing orange and red light. *S. fusiformis* cultured in SM1 medium showed gradual increase in the synthesis of C-phycocyanin after 2nd harvesting while decline in C-phycocyanin content was observed after 3rd harvesting. *S. fusiformis* when cultured in SM2 medium initially showed a decline in the content of C-phycocyanin after 2nd harvesting but during 3rd harvesting gradual increase in C-phycocyanin content was observed. But when culturing *S. fusiformis* in SM3 synthetic medium, a gradual decline in the C-phycocyanin synthesis was observed. Maximum C-phycocyanin content (0.04µg/ml) was observed in SM1 synthetic medium after 20 days while minimum C-phycocyanin content (0.005µg/ml) was observed in SM3 synthetic medium over a period of 30 days. A detailed result on the synthesis of C-phycocyanin pigment is given in Figure 1(D).

Synthesis of C-allophycocyanin by *S. fusiformis* was observed for 30 days. *S. fusiformis* when cultured under the influence of SM1 synthetic medium showed a sigmoid curve with initial increase till 2nd harvesting. After that concentration of C-allophycocyanin was started declining. But when it cultured in SM1 medium a gradual increase in the content of C-allophycocyanin was observed till 10 days later only decline in C-allophycocyanin content was observed till 30 days. Maximum synthesis of C-allophycocyanin (0.044µg/ml) was observed in SM2 medium over a period of 20 days while C-allophycocyanin was minimum (0.003µg/ml) in SM2 medium over a period of 30 days. A detailed result on synthesis of C-allophycocyanin is given in Figure 1(E).

Phycoerythrin is accessory and red protein pigment complex of phycobiliprotein family. Synthesis of C-phycoerythrin from *S. fusiformis* was observed in all the three different media for period of 30 days. An increase

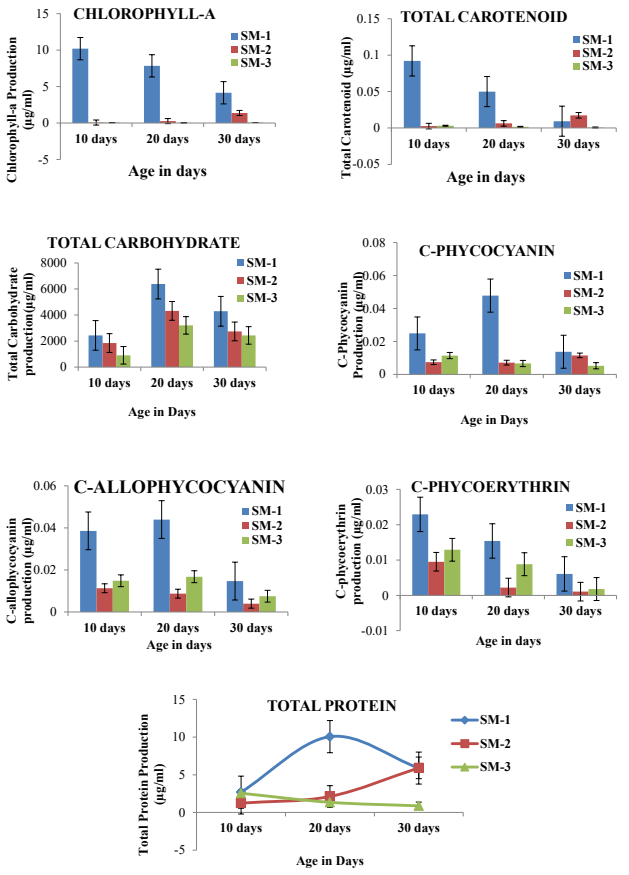

Figure 1(A-G): Showing the effect of different synthetic nutrient media in synthesis of (A) Chlorophyll-a, (B) Total carotenoids, (C) Total Carbohydrates, (D) C-Phycocyanin, (E) C-allophycocyanin, (F) C-phycoerythrin, and (G) Total Protein in *S. fusiformis* grown by batch culturing for 30 days

in C-phycoerythrin was observed in all the culture flasks supplemented with all the three different synthetic media (SM1, SM2 and SM3) till 10 days while after that a gradual declining was observed in synthesis of C-phycoerythrin pigment in all the flasks supplemented with three different media on 2nd and 3rd harvesting. A detailed result on synthesis of C-phycoerythrin in different synthetic media over a period of 30 days is given in Figure 1(F).

Spirulina is commercially cultivated worldwide as a food because of its high protein content than all other food crops. Maximum total protein content (10.06µg/ml) in the cells was observed in SM1 medium over a period of 20 days. While after 30 days total protein content was observed minimum (0.877µg/ml) in SM3 medium. A detailed result on the synthesis of total protein by *S. fusiformis* in three different media is given in Figure 1(G).

Discussion

Cyanobacteria have gained much attention in recent years because of their tremendous potential for exploitation in the field of biotechnology, phycoremediation, food, nutraceuticals and pharmaceutical industries (Kant *et al*., 2006). The present work analyzes the *Spirulina* growth by growing in different concentrations of animal waste, each containing different nitrogen concentration Raoof *et al*. (2006) formulated a synthetic medium (RM6) for the growth of *Spirulina* by stepwise substitution of essential nutrients of Zarrouk's medium using locally available agricultural fertilizers, such as SSP and MOP and concluded that RM6 medium contains single super phosphate (1.25 g l^{-1}), sodium nitrate (2.50 g l^{-1}), muriate of potash (0.98 g l^{-1}), sodium chloride (0.5 g l^{-1}), magnesium sulphate (0.15 g l^{-1}), calcium chloride (0.04 g l^{-1}), and commercial grade sodium bicarbonate (8 g l^{-1}) is five times cheaper than Zarrouk's medium (Zarrouk, 1966).

Dineshkumar *et al*. (2021) used baking soda in place of Zarrouk medium for the cultivation of *Spirulina* and revealed that Zarrouk medium produced approximately ten times higher biomass than that of baking soda media. *S. subsalsa* when cultured with in seawater supplemented with monosodium glutamate residue (MSGR) than in freshwater medium (modified Zarrouk medium) ensured this cyanobacterium obtained high lipid productivity (120 mg/L/day) and suffered limited bacterial infections during growth. From our study, it was observed that, SM1 synthetic medium could be used to maximize synthesis of Chl-a, carotenoids, phycobilins, total carbohydrates and proteins in *S. fusiformis*.

Conclusion

From the present work it could be concluded that different combinations of synthetic nutrient medium along with animal waste could proliferate the growth of *S. fusiformis* and synthesis of Chl-a, carotenoids, phycobilins, total carbohydrates and proteins. Further from the study on screening of new combinations of synthetic medium, it is also concluded that SM1 medium is most suitable nutrient supplement for the growth of *S. fusiformis*.

Acknowledgement

Authors are thankful to Head, Botany Department, Chaudhary Charan Singh University, Meerut, India for providing necessary facilities. Authors (RK and KS) are thankful to the U.P Govt., Lucknow for financial support (F.No.70/2022/1543/ Sattar-4-2022/ 001-70-4099-7-2022).

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