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Research Article

MEDIA OPTIMIZATION FOR THE ENHANCED GROWTH AND PHYCOCYANIN YIELD OF TWO SPECIES OF SPIRULINA VIZ, *SPIRULINA PLATENSIS* AND *SPIRULINA PLATENSIS* VAR *LONAR* USING MULTIVITAMINS SUPPLEMENTS

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ABSTRACT

The present study was carried out to obtain enhanced yield of Spirulina biomass and phycocyanin by media optimization with two species of Spirulina i.e... *S.platensis* (filamentous type) and *S.platensis* var *lonar*. Modified Zarrouk's medium was used as the basal medium (it also served as control), in which different concentration of multivitamins was supplemented i.e., 250mg, 300mg, 350mg, 400mg, 450mg and 500mg/l was added. The cultivation was carried out in a 1000ml Erlenmeyer flask's for a period of 15 days at room temperature with artificial aeration and lighting. The growth was monitored by Direct Microscopic Count (DMC), Optical Density (OD), and Dry biomass on daily basis till 15th day. On comparison of two species the *S.platensis* var *lonar* showed the highest biomass than the *S.platensis* in this study. The highest yield of *S.platenisis* was 2.56g/l in 500mg/l supplements and the *S.platensis* var *lonar* showed 3.52g/l in 250mg/l multivitamin supplements. The highest C-phycocyanin content was observed with *Spirulina platensis* in 400mg/l supplementation (1004mg/ml) and in the control it was 480mg/ml. in terms of purity both species gave increased purity in the 300mg/l and 400mg/l supplements when compared to the control.

Keywords: Multivitamin supplement, *Spirulina* species, Biomass, C-phycocyanin.

INTRODUCTION

The *Spirulina platensis*, a photoautotrophic microalga has been used as a good food supplement because of its high protein content, vitamins and essential fatty acids like Gamma Linoleniec Acid (GLA) and Docosahexaenoic Acid (DHA). *Spirulina* is cultivated for the production of valuable chemical compounds including natural pigments, dietary supplements etc., the Spirulina contains up to 74% dry weight of proteins along with high concentrations of minerals, pigments, unsaturated fatty acids and vitamins (Cohen 1997)[1], Because of which it is used as dietary supplements for both animals and humans. In Spirulina photosynthesis is the main carbon fixation route, but during the light phase of cultivation Spirulina can combine autotrophic photosynthesis and heterotrophic assimilation of organic compounds in a process known as mixotrophy (Marquez et al 1993; Villarejo et al 1995; Chen et al 1996)[2, 3, and 4]. Light plays an important role in the cultivation of photosynthetic organisms with growth decreasing when illumination levels are too low. In myxotrophs the presence of an organic substrates means that cell growth is not strictly dependent on

photosynthesis and hence light stops being and indispensable growth factor. Spirulina growth is stimulated during the light phase in media supplemented with glucose (Chojnacka 2004)[5] and there is less biomass in the dark phase (Torzillo et al 1991)[6]. The culture of Spirulina was practiced in different media especially inorganic and decomposed organic nutrients. Different types of Spirulina were cultured to evaluate growth and biochemistry under similar controlled conditions (Bhattacharya and Shivaprakash 2005)[7]. They cultured three species of Spirulina viz. *Spirulina platensis, Spirulina laxissima* and *Spirulina platensis* var *lonar*. In our previous study (Murugan &Radhamadhavan 2010)[8] the cultivation of *Spirulina platensis* alone was done with multivitamin media supplementation in a broad range. In this study narrow range supplementation was made and the growth and pigment enhancement pattern were compared with two species of Spirulina viz. *Spirulina platensis* and *Spirulina platensis* var *lonar.*

MATERIALS AND METHODS

Culture collection and Maintenance

In the present study the growth of two species of *Spirulina,* viz., *Spirulina platensis* (filamentous) and *Spirulina platensis* var *lonar* were used to cultivate on Zarrouk medium (Modified 1966)[9] supplemented with multivitamin. The *Spirulina platensis* was obtained from C.A.S Botany Department, University of Madras, Tamilnadu, India. *Spirulina platensis* var *lonar* was collected from P.G Research Institute, Kattupakkam, A Unit of Tamilnadu Veterinary and Animal Science University, Tamilnadu, India. The culture was maintained in Zarrouk medium in a 1000ml Erlenmeyer flask in the normal room temperature, with 12 hours light and 12 hours dark photo period with normal white light and the flask were aerated artificially.

Cultivation

The cultivation was carried out in Erlenmeyer flasks containing Zarrouk medium(Modified 1966)[9] supplemented with 250mg/l, 300mg/l, 350mg/l, 400mg/l, 450mg/l and 500mg/l multivitamin and the initial pH was adjusted to 8.5. The flask containing the supplemented medium was inoculated with 10% of the 15 days old culture of *Spirulina platensis*. The flasks were maintained under laboratory conditions and provided with artificial light source and the medium was continuously aerated. The growth of the culture was monitored as per the protocol of Venkataraman (1983)[10] for a period of 15 days. The generation time was calculated as per Prescott et al (2008)[11].

Phycocyanin extraction

The C- phycocyanin was extracted from fresh biomass by the following procedure (Sarada et al. 1999)[12]. Fresh biomass was homogenized with 50mM sodium phosphate buffer, the homogenate was subjected to alternate freezing and thawing(3 to 4 cycles) and centrifuged at 5000rpm for 10 minutes. The phycocyanin content was estimated by the method of Sigelman and Kycia(1978)[13].

RESULT AND DISCUSSION

The growth of the two Spirulina culture tested were monitored by means of direct microscopic count(DMC), optical density(OD_{560nm}) and dry biomass on daily basis. Following graphs(from 1-3) compared the growth of Spirulina platensis on 6 different concentrations of multivitamin supplemented medium by the above said three growth monitoring parameters and the graph(from 4-6) for Spirulina platensis var lonar. The graph-7 showed the comparison of C-phycocyanin yield and their purity in the supplemented medium by both species of Spirulina i.e. *Spirulina platensis* (filamentous) and *Spirulina platensis* var *lonar.*

The culture of Spirulina was practiced in different media especially inorganic and decomposed organic nutrients. The growth and pigment production of three micro algal species (blue green algae- *Spirulina platensis, Spirulina platensis* var *lonar* and the green alga- *Chlorella* sp.) were studied on organic media (Murugan et al.2012)[14]. Different types of Spirulina were cultured to evaluate growth and biochemistry under similar controlled conditions (Bhattacharya and shivaprakash 2005)[7]. They cultured three species of Spirulina viz. *Spirulina platensis, Spirulina laxissima* and *Spirulina platensis* var *lonar*. Of these three species, *S.platensis* showed highest growth rate, biomass and pigment concentration. In our previous study (Murugan and Radhamadhavan 2010)[8] *Spirulina platensis* was cultivated in different concentrations of multivitamins supplemented in Zarrouk medium (modified 1966)[9]. The concentrations of multivitamins were 250mg/l, 500mg/l, 750mg/l and 1000mg/l. in that cultivation the growth was promoted in first two concentration of the multivitamins supplemented Zarrouk medium. The growth was monitored by various parameters like DMC, optical density, and dry mass of the

culture. The final dry mass concentration was 2.84g/l and 2.08g/l respectively.

In the present study the concentrations of multivitamins are taken as 250, 300,350,400,450 and 500 mg/l to obtain enhanced biomaas yield. The growth was compared with two species of Spirulina viz., *S.platensis* (filamentous) and *S.platensis* var *lonar* (Graph 1-6). In the case of *S.platensis* the growth was high in the concentration of 500mg/l of multi-vitamins (2.56g/l) which was higher than the control medium (2.48g/l). The *S.platensis* var *lonar* species showed increased growth in 250mg/l supplements (3.52g/l) than the control medium (3.40g/l) and decreased to 2.28g/l in 500mg/l supplements.

The comparison of two species show that *S.platensis* var *lonar* showed the highest biomass than the *S.platensis* in this study. The highest yield of *S.platenisis* was 2.56g/l in 500mg/l supplements and the *S.platensis* var *lonar* showed 3.52g/l in 250mg/l multivitamin supplements. The highest phycocyanin content was observed with *Spirulina platensis* in 400mg supplementation (1004mg/ml) and in the control it was 480mg/ml. The purity of phycocyanin extracted from two species in different concentration was also determined (graph 7). Both species gave increased purity in the 300mg/l and 400mg/l supplements when compared to the control.

CONCLUSION

In this study the *S.platensis* var *lonar* showed the highest yield biomass and the highest C-phycocyanin content was observed with *Spirulina platensis*. In terms of purity both species gave increased purity in the 350mg/l and 400mg/l supplements when compared to the control.

From the Graph 1, based on the direct microscopic count the 500mg supplement showed higher cell count than the remaining supplements.

From the Graph 2, based on the optical density value at 560nm the 400mg supplement showed higher cell count than the remaining supplements.

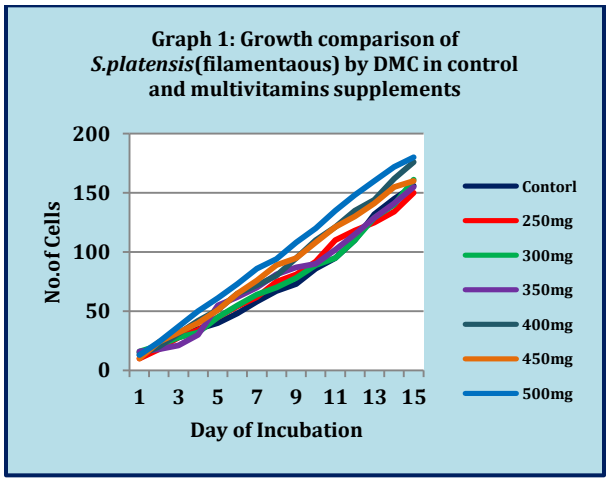
From the Graph 3, based on biomass weight the 500mg supplement showed higher biomass than the remaining supplements.

From the Graph 4, based on the direct microscopic count the 250mg supplement showed higher cell count than the remaining supplements.

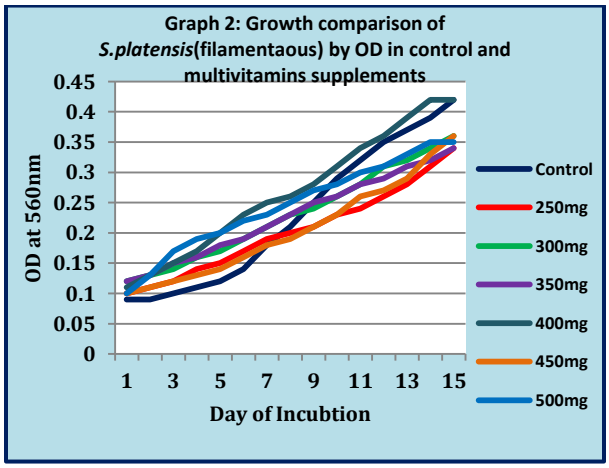
From the Graph 5, based on the optical density value at 560nm the 250mg supplement showed higher cell count than the remaining supplements.

From the Graph 6. The *S.platensis* var *lonar* yielded higher biomass at 250mg multivitamin supplementation.

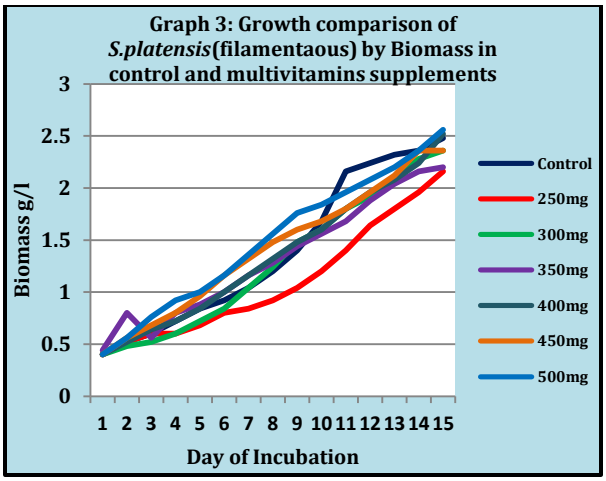
From the graph 7, the *S.platensis* yield higher C-phycocyanin in the media supplemented with 400mg multivitamin with higher purity at 300mg multivitamin supplement.



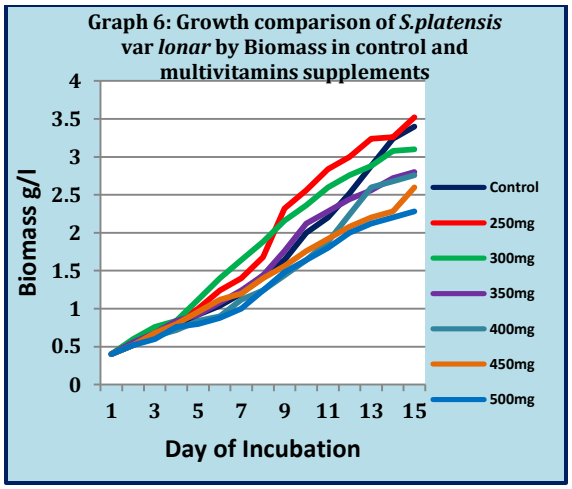
Graph 1: Direct microscopic count of *Spirulina platensis* (filamentous) on control and supplemented media



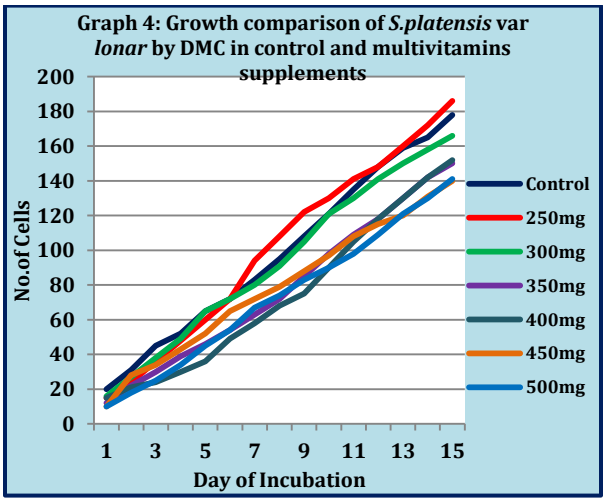
Graph 2: Optical density (at 560nm) of *Spirulina platensis* (filamentous) on control and supplemented media



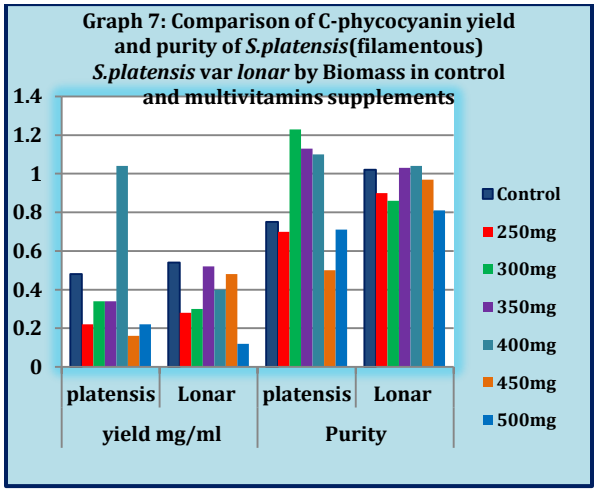
Graph 3: Biomass yield (g/l) of *Spirulina platensis* (filamentous) on control and supplemented media



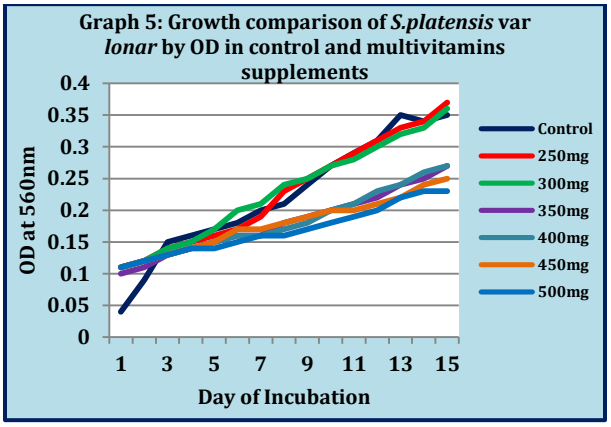
Graph 6: Biomass yield of *Spirulina platensis* var *lonar* on control and supplemented media



Graph 4: Direct microscopic count of *Spirulina platensis* var *lonar* on control and supplemented media



Graph 7: Phycocyanin yield and putiry of *Spirulina platensis* and *Spirulina platensis* var *lonar* on Control and supplemented media



Graph 5: Optical density of *Spirulina platensis var lonar* on control and supplemented media

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