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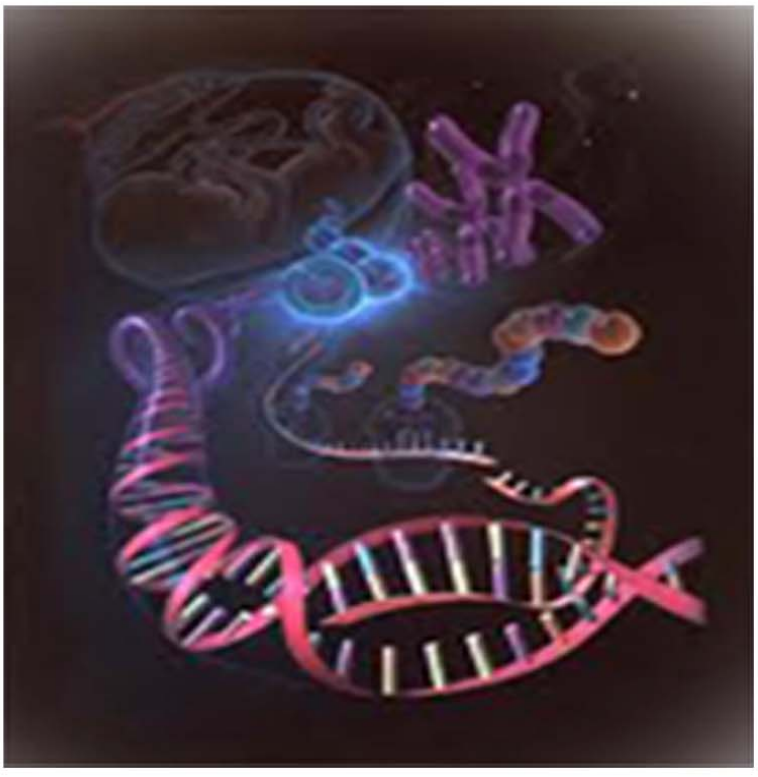

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Research Paper

OPTIMIZATION OF GROWTH OF SPIRULINA PLATENSIS LN1 FOR PRODUCTION OF CAROTENOIDS

M Thirumala

Corresponding Author: ***M Thirumala,*** ✉ *thirumala_21@yahoo.com*

Growth of Spirulina platensis LN1 was observed using eight different media consisting of different combinations of Lonar lake water, N, P and K and Zarrouk medium composition under different incubation periods. Medium II was suitable on 9th day of incubation and medium III was suitable on 15th day of incubation for its growth whereas, 12th day of incubation was shown to be suitable for its growth in remaining media. Optimization of growth of Spirulina platensis was observed at 35^0C temperature and pH 10. Carotenoids of Spirulina platensis were also analysed.

Keywords: *Spirulina platensis* LN1, Lonar lake, Carotenoids, Zarrouk medium

INTRODUCTION

Spirulina platensis is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids (Aiba and Ogawa, 1977; Umesh and Sheshagiri, 1984). Microalgae now combine the traditional and new biotechnologies where algal biomass can be a source of biochemicals, lipids, polysaccharides and colorants. For centuries *Spirulina* has been used as food by the natives living near Lake Chad in Central Africa and near Lake Mexico (Borowizka, 1988b). It is capable of growing in high alkalinity with the presence of carbonate bicarbonates and inorganic nitrogen (Aiba and Ogawa, 1977) The biomass of *Spirulina platensis* has been recognized to be a "wonderful food health" since it contain high proteins (Umesh and Sheshagiri, 1984), and various bioactive compounds such as, essential fatty acids (linolenic and ã-Linolenic acid) (Borowizka, 1988b; Cohen et al., 1987), B complex vitamins (Riboflavin, cyanocobalamin, thiamine and nicotinic acid), bio-pigments (phycocyanin and chlorophyll-a) (Achmadi and Tri-Panji, 2000) and also it contains antioxidant compounds carotenoids (Cohen, 1997).

Carotenoids are natural pigments which are synthesized by plant and algae. There are several dozen Carotenoids in the food and most of these Carotenoids have antioxidant activity. The α-carotene is a member of Carotenoids, which is important for good health, pharmaceutical, cosmetics industries and food industries and is

[1] Department of Biochemistry, Mahatma Gandhi University, Nalgonda - 508254, A.P., India.

thus potential to be produced in large scale (Borowizka, 1988a). Natural α carotene is chemically and physically different from the synthetic form and although there is evidence that the body absorbs natural α carotene ten times more easily than it absorbs the synthetic form (George 1992; Schwartz et al., 1988). Anti oxidants in particular carotenoids, help to prevent the free radicals damage associated with the aging process itself. There is a strong evidence that α carotene also enhances many aspects of immune function (Ben-Amotz et al., 1989; Adrianne,1988), stimulates immunocompetence in healthy individuals and enhances immune function in people who have tested HIV positive (Garewal et al., 1992).

Carotenoids are natural colorants—two types of colours are used in food industries natural and synthetic. Synthetic ones are mainly coal tar derivatives made from chemicals which are by products of coal distillation and they are banned in many countries because of the health risks. Thus there is an increasing demand for natural colours represent an apparently more sustainable source of colorants than synthetic counterpart which is derived from non renewable sources.

Synthetic colours are in more use compared to natural ones due to the fact that yield of natural colours from plant sources is low as compared to the yield from modern synthetic processes. A greater amount of plant biomass is needed for the extraction of natural colours which would require large scale cultivation of plant species and the disposal of large volumes of crop residue may be another problem. Besides colours from higher plant origin, carotenoids and phycobiliproteins from microalgae are good alternatives for natural colours. Production of colours from microalgae has a number of advantages such as cheaper and easy production, easier extraction, higher yields, no lack of raw materials and no seasonal variation (De Oliveira et al., 2010).

Attempts to use the effluents from secondary waste water treatment plants as an economical source of nitrogen and phosphate salts for *Spirulina maxima* have been reported (Faucher et al., 1979). Sea water was also tested as a source of trace minerals along with urea as the source of nitrogen for the cultivation of *S.maxima* (Gurpreet Kaur et al., 2009). As the production cost of the algae is high due to its huge medium cost. The present investigation deals with the optimization of the growth of *Spirulina platensis* LN1 using Lonar lake water, N, P, K and Zarrouk medium to evolve a low cost suitable medium for the growth of *Spirulina platensis* LN1.

MATERIALS AND METHODS

Microorganism

Spirulina platensis LN1 was an old isolate from the Lonar soda crater, Bhuldhana, Maharashtra. The strain was maintained in Zarrouk's medium (Zarrouks, 1966) in 500 ml Erlenmeyer flasks. Maintenance of the culture was carried out in continuous illumination at 35^0C and 1500 lux with shaking of culture manually thrice a day. The monitoring of S*pirulina* growth was measured spectrophotometrically at 640nm. This culture was used for all further experiments.

Optimization

The cells were suspended in 20ml Zarrouk medium (pH 10) containing 50ml flasks and incubated under different temperatures ranging from 25-40°C and different pH between 6 and 11 with continuous illumination of 1500 lux provided with white fluorescent lamps for two weeks. The flasks were hand shaken twice daily.

GROWTH AND PIGMENT ANALYSIS

Lonar Lake water was sterilized by autoclaving at 15 lbs for 20 min. A combination of media varies as given below:

Medium I : Distilled water 250 ml + Zarrouk medium composition

Medium II : Lonar water 250 ml + without addition

Medium III : Lonar water 250 ml + Zarrouk medium composition

Medium IV : Lonar water 250 ml + N.P.K. (17:17:17) 0.5 g/lit.

Medium V : Lonar water 250 ml + N.P.K. (17:17:17) 1 g/lit.

Medium VI : Lonar water 250 ml + N.P.K. (17:17:17) 1.5 g/lit.

Medium VII : Lonar water 250 ml + N.P.K. (17:17:17) 2 g/lit.

Medium VIII : Lonar water 250 ml + N.P.K. (17:17:17) 2.5 g/lit.

Five percent inoculum of *S.plantensis* was inoculated into 500ml sterilized autoclavable PVC conical flasks containing 250ml media and grown at room temperature 35°C and pH 10 with illumination under a fluorescent light intensity of 1500 lux for 14hrs/day. Flasks were hand shaken twice daily. Growth of the *Spirulina* isolate was observed at 3 days intervals by measuring the optical density at 640 nm (Thimmaiah, 2004). Every time 50 ml of culture was removed from the each media and used for analysis of carotenoids and growth studies.

ANALYTICAL PROCEDURE

Estimation of Total Carotenoids

a) Extraction of Carotenoids: The total Carotenoids were extracted by cell lysis (sonication) and partitioned in organic solvent (acetone) on the basis of their solubility. Carotenoids that are bound as esters were hydrolysed using aqueous 60% KOH (Anaga and Abu, 1996).

b) Estimation of Caratenoid Compounds: Total Carotenoids were estimated by spectrophototmetric method at 450 nm and α-Carotene was used as a standard compound for preparing the calibration curve (Jensen, 1978).

RESULTS AND DISCUSSION

Comparison of growth analysis of *Spirulina platensis* LN1 in eight different media containing Lonar water with addition of other nutrients i.e., N P K was shown in Table 1. The optical densities were measured at three days interval at 640 nm till 15th day. According to Table 1, comparatively high growth was observed in Medium-VI (i.e., Lonar water 250 ml + N.P.K. (17:17:17)1.5g/l and less growth in medium II (Lonar water 250 ml + without addition). Medium II was suitable for its growth on 9th day of incubation whereas, medium III was suitable on 15th day of incubation. But for other media 12th day of incubation was suitable.

Growth was observed for *Spirulina platensis* LN1under different temperature conditions. *Spirulina platensis* LN1 showed highest growth under 35^{0}C when compared to other temperatures (Table 2).

When *S. plantensis* was grown under different pH, under pH 10 it showed good results when compared to other pH conditions. After pH 10 the same growth was observed even if pH was increased to 11(Table 3).

Table 1: Growth Analysis of *Spirulina platensis* LN1 in Eight Different Media

Media	3rd day O.D. 640 nm	6th day O.D. 640 nm	9th day O.D. 640 nm	12th day O.D. 640 nm	15th day O.D. 640nm
M – I	0.05	0.071	0.136	0.772	0.712
M – II	0.155	0.216	0.56	0.53	0.534
M – III	0.11	0.206	0.332	0.64	0.695
M – IV	0.103	0.213	0.224	0.612	0.524
M – V	0.101	0.223	0.213	0.578	0.566
M – VI	0.138	0.251	0.25	0.797	0.635
M – VII	0.127	0.312	0.253	0.683	0.529
M – VIII	0.153	0.281	0.297	0.72	0.642

Table 2: Optimization of the Growth of *Spirulina platensis* LN1 Under Different Temperatures

Temperature	25	30	35	40
Optical density at 640 nm	0.43	0.58	0.75	0.63

Table 3: Optimization of the Growth of *Spirulina platensis* LN1 Under Different pH

pH	6	7	8	9	10	11
Optical density at 640nm	0.32	0.51	0.74	0.83	0.89	0.89

Estimation of total carotenoids of *Spirulina platensis* LN1 in eight different media was clearly shown in Table- IV. The absorbance was calculated at 450 nm. The total amount of carotenoids was calculated at three days interval till 15 days of incubation. High concentration of total carotenoids obtained was 0.0998 µg/ml at 15th day in medium VII (Table 4).

Table 4: Estimation of Total Carotenoids of *Spirulina platensis* LN1 in Eight Different Media

Media	3rd day		6th day		9th day		12th day		15th day	
	O.D	C	O.D.	C	O.D	C	O.D	C	O.D	C
M-I	0.043	0.0016	0.078	0.0034	0.259	0.0104	2.126	0.0854	2.933	0.0923
M-II	0.008	0.0004	0.019	0.0009	0.089	0.0016	0.238	0.0118	1.329	0.0177
M-III	0.023	0.0013	0.029	0.0078	0.135	0.0112	1.967	0.0198	2.564	0.0871
M-IV	0.043	0.0018	0.028	0.0075	0.965	0.0056	1.896	0.0176	2.078	0.0789
M-V	0.034	0.0012	0.079	0.0036	0.873	0.0232	1.233	0.0112	1.998	0.0987
M-VI	0.039	0.0015	0.879	0.0123	1.002	0.0157	1.982	0.0882	2.083	0.0831
M-VII	0.049	0.0019	0.992	0.0101	1.089	0.0121	2.245	0.0679	2.999	0.0998
M-VIII	0.044	0.0017	0.089	0.0034	0.997	0.011	1.899	0.0178	2.344	0.0788

The present investigation deals with the growth and optimization of *Spirulina platensis* LN1 to determine the best low cost media for biomass and pigment production with Lonar water with addition of other nutrients i.e. N.P.K in Zarrouk medium. The variation in the growth of *Spirulina platensis* LN1was observed in eight different media with variations in the concentrations of N.P.K. (17:17:17) and Zarrouk medium. Based on the observation we could conclude that medium VI and medium I would be the best growth media for *Spirulina platensis* LN1. As can be seen from the data, growth was affected by different medium composition, incubation period, temperature and pH. Unlike many other photosynthetic microorganisms, it is capable of utilizing ammonia even at high pH value (Umesh and Sheshagiri, 1984).

CONCLUSION

Culturing *Spirulina platensis* in conical flask has its limitation in providing complete information related to growth, development and production of value added chemicals viz. vitamins, amino acids, fatty acids, protein and polysaccharides both in quantity and quality and disposing of carbon dioxide (Faucher et al., 1979). It was also observed that an increase of temperature caused a marked decrease in protein content, while carbohydrate synthesis was stimulated (De Oliveira et al., 2010). Nutrient deficiency, especially nitrogen (N), may affect the cultures in various ways. In N-sufficient growth mediums, protein production is supported, while carbohydrate synthesis is limited. Urea-sea water was used as culture medium to support the growth of *Spirulina maxima* (De Oliveira et al., 2010). In contrast, carbohydrate synthesis increases and protein production drops in N-deficient mediums (Jensen, 1978). As stated by many authors, the optimum temperature of *Spirulina* is in the range of 35-40 ^{0}C (Cohen et al., 1987, Ben-Amotz et al., 1989).

Further research has to be done to improve the cost effective media and to industrialize the production of *Spirulina plantensis*. The isolate *Spirulina platensis* LN1needs further investigation for optimized production of carotenoids.

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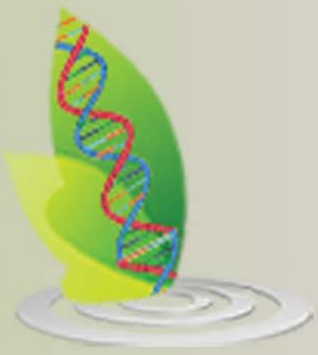

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