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Effect of Different Culture Media on Growth and Biopigments of *Dunaliella Salina* Isolated From Sambhar Lake

Chanchal Lata and Gajendra Pal Singh

Algal Biotechnology Lab, Department of Botany, University of Rajasthan, Jaipur-302004 (Rajasthan) India.

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ABSTRACT

We studied *Dunaliella salina* on different media to find out optimum culture media for algal growth and biopigment production. *D. salina* is a flagellated, halophile green microalga especially found in brackish water. *D. salina* is known for its antioxidant activity because of its ability to create large amount of carotenoid. Growth kinetics of cultures was showed on various media with significant modifications such as ASWM[1] (2M NaCl), 2ASWM[1] (medium composition same as ASWM[1] but double strength of 4M NaCl, modified D medium[2] (.01 g of $NaHCO_3$ and 2M NaCl), modified Johnson medium[3] (3M NaCl), FE medium (2m NaCl).pH was adjusted to 7.8. Maximum growth rate, cell production with maximum accumulation of chlorophyll and carotenoid were found in ASWM. Carotenoid content was found maximum in double concentration of NaCl (4-5M) in 2ASWM. Chlorophyll content was found maximum in D medium (19.4 mg/gm) and minimum FE medium (2.2mg/gm). Improvement in the carotenoid content with increase in salinity in ASWM could be a good basis for the exploitation of microalgae as a source of biopigment.

Introduction

D. salina contains the highest amount of carotenoids, including alpha-carotene, beta-carotene, zeaxanthin, lutein, cryptoxanthin and lycopene[4]. It is rich in essential minerals, vitamins, proteins, amino acids, and abundant unsaturated fatty acid, especially linolenic acid (essential fatty acids), carbohydrates, chlorophyll and other important nutrients[5].

D. salina is a type of halophile green microalgae able to tolerate varying NaCl concentrations, ranging from 0.2% to approximately 35 % [6]. Thus, *D. salina* is a hyper-halotolerant organism found in high densities in saline lakes. It produces a distinct pink and red colour often characteristic of saltern ponds[7]. So it is used in cosmetics (lipstick, eye shadow due to β-carotene production) [8] and dietary supplements. D. salina has adapted to survive in high salinity environments by accumulating glycerol to balance osmotic pressure. D. salina is also adapted to solar radiation using β-carotene to protect against ionizing energy.

D. salina can contain more than twice the chlorophyll of Spirulina, 8 times the mineral content and over 6,000 times the antioxidant content. They may help to protect against free radical cell damage responsible for premature ageing, cataracts, cardiovascular disease and other chronic diseases. Minerals like magnesium which plays a vital role in perfect cell metabolism in the absorption of calcium in the body which leads to healthier teeth and bones. It also maintains the normal rhythmic activity of the heart and maintains the blood pressure. The naturally formed beta-carotene converts into active vitamin A is good for healthy eyes and a youthful skin [9]. Algae are easy to grow and cultivate with less energy requirements and using very few of the nutrients. The ideal growth media for micro-algal cultures are strain specific. The biomass productivity depends upon many factors which includes media composition. A suitable media supports fast growth and high productivity. The identification of suitable nutrient medium is an important for achieving optimal growth of microalgae and high productivity. Inorganic constituents of the media are responsible for the growth and morphology of the various algae.

Many inorganic media have been formulated for nutritional requirements of various algae Impact of different media formulations on the growth, and pigments of different microalgae has been studied by number of workers [10-17]

In a recent study (Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae) results revealed that Media composition, light intensity and photoperiod significantly affect the algal growth and productivity and their optimization is important for the commercialization of microalgae based biofuels[18].

It was also studied Effect of different culture media formulations on growth and biodiesel production potential of *Chlorella pyrenoidosa* result shows that, a suitable media supports fast growth for cultivation of the algae and high productivity[19].

The present study was aimed at evaluating the effect of various inorganic media on growth and biopigments of *D. salina*.

Materials and Methods

The algal samples will be collected from Sambhar Lake. The isolation will be carried out of desired algae through serial dilution method. The cultures will be developed in the laboratory at the Department of Botany at the University of Rajasthan.

Tele:

E-mail address: chanchal16121988@gmail.com

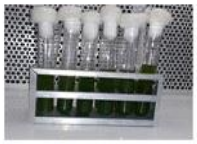

Fig:1(A)

Fig:1(B)

Fig:2

Fig:3(A)

Fig:3(B)

Fig 1.A and 1B show serial dilution for isolation of desired algae, fig.2 showing mother culture after isolation of *D. Salina*, Fig.3A and B showing effect of different culture media.

The culture of *D.salina* will be studied on different culture media with significant modifications in ASWM (2M NaCl), 2ASWM (medium composition same as ASWM but double strength of 4M NaCl), modified D medium (.01 g of $NaHCO_3$ and 2M NaCl) in modified Johnson medium (3M NaCl), FE medium (2M NaCl). pH of all medium will be adjusted 7.8 Alternate light and dark period (12:12 L/D) will be provided in culture cabinets against continuous light. Suitable temperature giving best growth will be identified by using different ranges of temperature. Growth will be followed through optical density, Dry weight and cell count (in table1, 2 and 3 respectively) the culture.

Culture grown under optimum culture conditions will be analyzed biochemically for their biopigments by using standard methods for chlorophylls and carotenoids [20-21].

Results and Observations

As compared to other inorganic media, the best growth of *D. salina* was recorded in ASWM. The optical density increased up to the end of the experiment i.e. 1.03. It was maximum OD and increased 10 times more than the initial value. The dry weight also supported optical density record. Highest cell count on ASWM was 202 $X10^4$cells/ml on III^{rd} week observation.

2ASWM was next to ASWM in supporting the growth of *D. salina*. The optical density and dry weight was continuously increased. Highest cell count on 2ASWM was found 120 $X10^4$cells/ml on IV^{th} week observation.

In Modified Johnson's medium optical density and dry weight was increased but lower than both ASWM and 2ASWM. Highest cell count on MJ medium was found 80 $X10^4$cells/ml on IV^{th} week observation.

Modified D medium was next to modified Johnson's medium in supporting and maintaining the growth of the alga. Highest cell count on D media was found 70 $X10^4$cells/ml on IV^{th} week observation.

In comparison to all the inorganic media tested, FE medium was found least effective in promoting the growth of *D. salina*. The optical density and dry weight were recorded as minimum growth i.e.0.13 and 0.120 respectively that is lowest as compare to the other media at the end of the experiment. Highest cell count on FE medium was found 29 $X10^4$cells/ml on II^{nd} week observation that is much less than highest cell count on ASWM.

Table No1. Growth measured by OD (in nm).

	initial	I^{st} week	II^{nd}	III^{rd} week	IV^{th} week	V^{th} week
ASWM	.1	.18	.33	.51	.80	1.03
D Medium	.1	.14	.24	.48	.63	.70
MJ Media	.1	.15	.20	.34	.45	.50
2ASWM	.1	.15	.22	.38	.59	.85
FE Media	.1	.16	.18	.16	.15	.13

Table No2. Growth measured by Dry Weight (gm/100ml).

	initial	I^{st} week	II^{nd}	III^{rd} week	IV^{th} week	V^{th} week
ASWM	0.080± 0.008	0.102± 0.009	0.178± 0.03	0.222± 0.04	0.320± 0.06	0.392± 0.07
D Medium	0.058± 0.006	0.098± 0.008	0.076± 0.007	0.100± 0.009	0.200± 0.05	0.305± 0.06
MJ Media	0.066± 0.007	0.122± 0.01	0.165± 0.02	0.180± 0.03	0.275± 0.05	0.286± 0.05
2ASWM	0.020± 0.003	0.095± 0.008	0.164± 0.02	0.210± 0.05	0.265± 0.05	0.320± 0.07
FE Media	0.066± 0.007	0.089± 0.008	0.152± 0.01	0.132± 0.02	0.128± 0.02	0.120± 0.01

Table No 3. Growth measured by Cell Count ($X10^4$cells/ml).

	initial	I^{st} week	II^{nd}	III^{rd} week	IV^{th} week	V^{th} week
ASWM	36 ±2.3	40 ±2.5	95 ±4.8	202 ±7.8	100 ±6.2	140 ±7.1
D Medium	30 ±1.8	29 ±1.77	41 ±2.61	50 ±3.78	70 ±5.2	30 ±1.8
MJ Media	26 ±0.9	28 ±1.6	30 ±1.8	60 ±4.1	80 ±6.31	45 ±3.2
2ASWM	26 ±0.9	18 ±0.6	40 ±2.5	70 ±5.2	120 ±6.7	72 ±6.31
FE Media	15 ±0.4	17 ±0.54	29 ±1.77	20 ±0.71	15 ±0.4	10 ±0.2

Table No4. Carotenoid (in mg /gm).

	I^{st} week	II^{nd}	III^{rd} week	IV^{th} week	V^{th} week
ASWM	0.13±0.02	0.26±0.04	0.64±0.05	0.25±0.04	.03±0.009
D Medium	0.11±0.009	0.21±0.03	0.29±0.05	0.36±0.06	0.35±0.05
MJ Media	0.14±0.02	0.19±0.03	0.32±0.06	0.34±0.06	0.58±0.04
2ASWM	1.8±0.07	1.5±0.06	2.1±0.08	1.1±0.07	0.8±0.04
FE Media	1.2±0.06	0.9±0.06	0.7±0.06	0.5±0.05	0.3±0.05

Table No.5 Chlorophyll (in mg /gm)

	I^{st} week	II^{nd}	III^{rd} week	IV^{th} week	V^{th} week
ASWM	3.4±0.26	6.8±0.57	9.2±0.62	19.2±0.98	12.2±0.81
D Medium	3.6±0.37	6.9±0.58	11.0±0.71	19.4±1.00	9.7±0.77
MJ Media	7.0±0.59	8.5±0.83	8.8±0.58	13.7±0.86	16.06±0.88
2ASWM	5.3±0.46	8.1±0.52	8.8±0.58	15.7±0.92	2.4±0.14
FE Media	4.4±0.42	3.6±0.37	2.4±0.14	2.5±0.18	2.2±0.10

Highest chlorophyll content was observed in modified D medium. ASWM was next to Modified D medium then followed by 2ASWM, Modified Johnson medium and least chlorophyll content was observed in FE medium (table no.4).
Highest carotenoid content was observed in 2ASWM. FE medium was next to 2ASWM in supporting carotenoid content then followed by modified D medium, ASWM and Modified Johnson medium (table no.5).

Discussion

In contrast to other green algae, *D. salina* do not contains rigid cell wall. As a result cell responds rapidly to the changes in osmotic pressure. Hence, **Carotenoid** content of microalgae, *D. salina* was increased when cell experiences stress in different media (stress parameters such as cell division inhibition, nutritional starvation, and high salinity and high irradiation).

In 2ASWM**,** double concentration of NaCl (4M) used as in ASWM (2M) hence carotenoid content increased with significant decrease cell growth due to osmosis under saline condition. External environment of the cell contains the hypertonic solution at high salinity i.e. higher concentration of the solute (NaCl) and lower the concentration of the water present inside the cell in such conditions, there is a net flux of water molecules leaving the cell. This results in plasmolysis because of this cell count decreases (table no.3) 2ASWM.
When a cell is depleted due to high salinity, photosynthesis also gets adversely affected. Hence the chlorophyll content was decreased with increase in salinity. However *D. salina* does not have rigid cell wall unlike other green algae, it exhibits some ability to adapt to salinity as seen from the behavior at 4 M salinity (2ASWM). In study, it was observed that cells started growing after 6 days cell count increased (table no.3) on 2ASWM. Cells adapt to the high salinity due to presence of glycerol as it has ability to balance the extra cellular osmotic stress. It has been reported that the intra cellular glycerol concentrations exceeds nearly 50% and is sufficient to develop adequate osmotic pressure necessary to balance the extracellular osmotic stress[22].

It is evident that salinity above 3M significantly decreased the cell growth rate due to cell death but increased the carotenoid content and salinity below 2M significantly decreased the cell growth due to external environment of cell has low salinity and forms a hypotonic solution. In such condition, there is a net flux of water molecules entering the cell. Thus causing swelling and eventually bursting of the cells[23].

However, In ASWM (2 M salinity) showed maximum biomass of 2.02 x 10^6 cells/ml and hence it can be said that 2M is the optimum concentration for the growth of cells.
In modified D medium Chlorophyll content (19.4mg/gm) was observed highest. It was observed Chlorophyll content was much dependent on amount of Mg added in media, Because of the strategic position magnesium occupies in the photosynthetic apparatus as the central atom of the chlorophyll molecule, all algal species have an absolute requirement of this element. Another key function of Mg is its role in aggregation of ribosomes into functional units and for the formation of catalase. MgSO4 as a source of magnesium was common in most of the media.

In FE media, cell experiences a nutritional stress of carbon and Mg deficient medium. Carbon has been known to be an essential nutrient for the production of energy and assimilation of ammonical nitrogen. Addition of inorganic carbon as $NaHCO_3$ stimulates the growth of cell [24-25].

The supply of inorganic carbon also appears to affect tolerance to high light and temperature in *Dunaliella*[26]. Because of the strategic position magnesium occupies in the photosynthetic apparatus as the central atom of the chlorophyll molecule, all algal species have an absolute requirement of this element. So in absence of Mg in FE medium, Chlorophyll content was lowest (2.2mg/gm).Due to nutritional stress carotenoid content (as compared to ASWM) significantly increased with much decrease in cell growth.

In modified Johnson medium all growth parameters showed some variations depending upon the time in weeks.

Conclusion

Maximum growth rate, cell production with maximum accumulation of chlorophyll and carotenoids were found in ASWM. Carotenoid content was found maximum in double concentration of NaCl (4-5M) in 2ASWM. Improvement in the carotenoid content with increase in salinity in ASWM could be a good basis for the exploitation of microalgae as a source of biopigment. It was concluded that biopigments varies with different media formulation and many authors[27-28] have been reported this in his study.

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