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EVALUATION OF VARIOUS INORGANIC MEDIA FOR GROWTH AND BIOPIGMENTS OF *DUNALIELLA SALINA*

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ABSTRACT

Dunaliella salina is a unicellular green microalga that is considered as one of the best commercial sources of carotenoids. Growth and pigment production of *D. salina* (isolated from Sambhar Salt Lake, Rajasthan) was studied by using various inorganic media differing in chemical compositions viz. Artificial Sea Water media, De Walne's media, Johnson's media, AS-100 and Modified Bold's Basal media. The best growth with highest pigment production was observed in the artificial sea water medium as compared to other media.

KEY WORDS: ***Dunaliella salina*, carotenoids, inorganic medium, growth.**

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INTRODUCTION

Microalgae are a major natural source for a vast array of valuable compounds, including a diversity of pigments, for which these photosynthetic microorganisms represent an almost exclusive biological resource. Increasing awareness of harmful effects of synthetic dyes and inclination of society towards the uses of natural products, such as plant/microbial based colours in food and cosmetics, has led to the exploitation of micro algae as a source of natural colours. Algal products have great commercial value as natural colourants in nutraceuticals, cosmetic and pharmaceutical industry, besides their health benefits. Since the first formal description of the green algae *Dunaliella salina,* its presence in hypersaline environments worldwide and its physiological responses to different environmental conditions have attracted a great deal of scientific interest. The halophytic properties of *Dunaliella* confer to this alga an important advantage for outdoor cultivation. *Dunaliella salina* is considered as one of the best commercial source of natural carotenoids in the world. Among eukaryotic microalgae, the relatively unique ability to accumulate carotenoids in response to stress has made this alga the "star of algal biotechnology". The unique feature of *Dunaliella* cells is that it lacks rigid cell walls, a feature distinguishing them from other unicellular green algae. Absence of cell wall makes disruption of cells much easier than other algae. These traits make *Dunaliella salina* an attractive "cell factory" for the commercial production of carotenoids. The identification of suitable nutrient medium is a prime and imperative step for achieving optimal growth of microalgae. Inorganic constituents of the media are generally said to be responsible for the growth and morphology of the various algae in nature as well as in the laboratory. It is a well established fact that for the successful growth of algae, the environment has to be conditional pertaining to as many intrinsic requirements of the organism as possible. Many inorganic media have been formulated to suit the requirements of various algae but a single medium has not been identified as the best one. Impact of different media formulations on the growth, and pigments of different microalgae has been studied by number of workers [1-8]. The present study was aimed at evaluating the impact of various inorganic media on growth and pigments of *D. salina*.

MATERIALS AND METHODS

Microalgae source

The test alga *D. salina* was isolated from the Sambhar salt lake, Rajasthan, which is about 35 km from Jaipur (Rajasthan).

Experimental set up

To evaluate inorganic requirements for the growth and biopigments of *D. salina*, five different suggested media viz. Artificial sea water media (ASWM) [9], De Walne's media (DWM) [10], Johnson's media [11-12], AS-100 [13] and Modified Bold's basal media (Modified BBM) [14] were experimented upon. All the inorganic media were sterilized in autoclave at 121°C for 20 min. before inoculation. Conical flasks of 500 ml capacity were prepared containing 250 ml media and 50 ml culture (inoculum). The cultures were incubated at 25°C in a thermo-statically controlled room, with a 12:12 h light: dark regime and at a light intensity of 2,500 lux. Observations were carried out every week over a period of five weeks after initial readings. Cultures were shaken manually thrice a day to avoid clumping and accelerate the growth process. Experiment for each medium was carried out in triplicates.

Growth Measurement

Growth was followed through optical density (OD), dry weight, and growth rate. Biomass was determined by optical density of cultures at 670 nm using Shimadzu UV/VIS

spectrophotometer. The dry weight against standard absorbance unit was followed throughout the experimental period. 75-100 mL samples of culture were filtered on Whatman GF/C filters, rinsed with distilled water, and weighed after drying for 24 h at 80 °C [15].

Growth rate was calculated on dry weight basis according to the equation given below:

$$\mu\ (\text{divisions/day}) = \frac{3.322(\log DW2 - \log DW1)}{t2 - t1}$$

Where t = time and DW = dry weight. Subscripts denote values at different times [16].

Biopigments Analysis

The chlorophyll contents of samples were estimated by Parson and Strickland method [17] and carotenoids by Jensen method [18].

Statistical Analysis

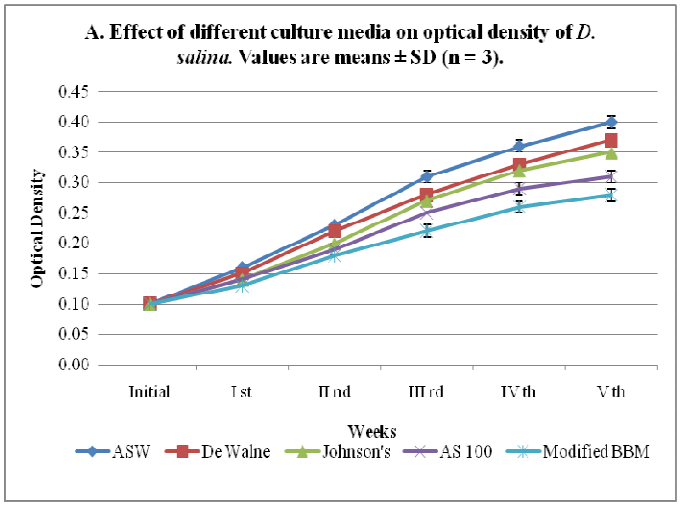
Effect of different culture media on growth and biopigment accumulation of *D. salina* were compared by one way analysis of variance (ANOVA). Statistical analysis of data was carried out using MS Office Excel analysis ToolPak. The significance between pairs of variable means was analysed using least significant difference (LSD) test at 5% level of significance [19].

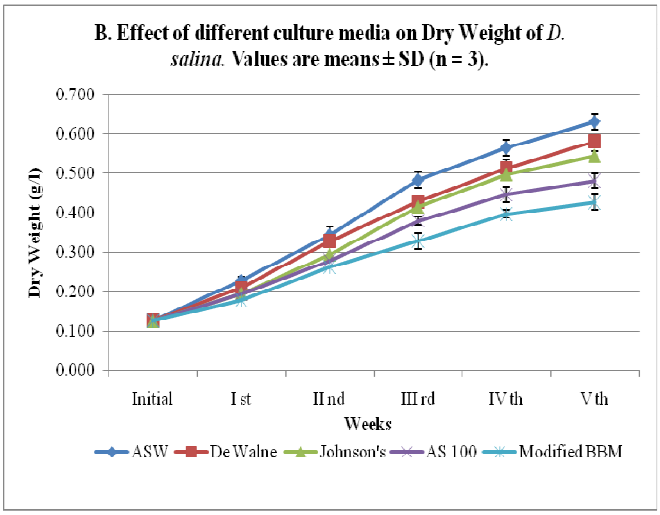
RESULTS AND OBSERVATIONS

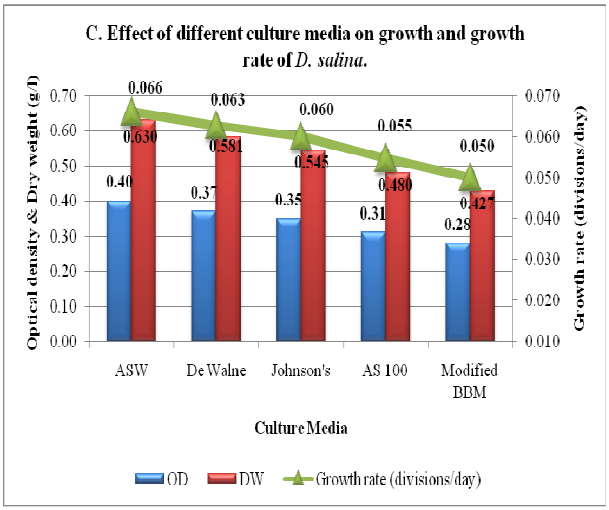
Growth analysis of *D. salina* in different inorganic media shows different growth patterns. As compared to other inorganic media, the best growth of *D. salina* was recorded in ASW medium. The optical density increased exponentially upto the end of the experiment. It was increased 4.0 times more than the initial value. The dry weight also supported optical density record and at the end of the experiment increased exponentially 4.96 times than the initial values. Highest growth rate (i. e. 0.066 divisions/day) was recorded in this medium (Graph 1). De Walne's medium was next to ASW media in supporting the growth of *D. salina*. The optical density was increased linearly, which was about 3.7 times more than its initial value at the end of V^{th} week. The optical density observation is also supported by dry weight record which showed an increase of 4.57 times than the initial one. The growth rate of cultures in this media was just next to ASW medium i.e. 0.063 divisions/day. In Johnson's medium optical density was increased 3.5 times and dry weight 4.29 times of the initial records. AS-100 media was next to Johnson's medium in supporting and maintaining the growth of the alga. At the end of the experiment, optical density increased 3.1 times and dry weight 3.78 times than its initial values. In comparision to all the inorganic media tested, Modified Bold's Basal medium was found least effective in promoting the growth of *D. salina*. The optical density and dry weight results were recorded to increase about 2.8 and 3.36 times respectively than the initial values at the end of the experiment. The least growth rate i.e. 0.050 divisions/day was observed in this medium (Graph 1).

Graph 1

Effect of different culture media on the growth and growth rate of Dunaliella salina.







The pigment contents of algae correlates with the growth of *D. salina*. The maximum pigment contents were observed in ASWM. Significantly higher amounts of chlorophyll-a (1.246 %) chlorophyll-b (0.456 %) and carotenoid contents (1.843 %) were found in cultures grown in ASWM followed by De Walne's medium, Johnson's medium, AS-100 medium and least pigment production was reported in modified bold's basal medium i.e. (1.126 % chlorophyll-a, 0.372 % chlorophyll-b and 1.245 % carotenoids) (Table 1).

Table 1

Effect of different culture media on pigment composition of D. salina. Values are means ± SD (n=3). Variable means with the same letter in the column are not significantly different (p>0.05).

Culture Media	Chlorophyll-a (%)	Chlorophyll-b (%)	Carotenoids (%)
ASW	1.246±0.01 [a]	0.456±0.004 [a]	1.843±0.020 [a]
De Walne	1.217±0.01 [b]	0.433±0.006 [b]	1.693±0.021 [b]
Johnson's	1.196±0.02 [c]	0.42±0.004 [c]	1.595±0.021 [c]
AS 100	1.156±0.01 [d]	0.391±0.003 [d]	1.394±0.019 [d]
Modified BBM	1.126±0.01 [e]	0.372±0.005 [e]	1.245±0.020 [e]

DISCUSSION

In the present experiment, a great variability in the nutritional requirements of *D. salina*, have been found. The inorganic constituents of media have been credited to be responsible for the pattern of growth and biopigment content of algal forms growing either in natural or laboratory conditions. The algal species under present investigation has been no exception to these findings. Among various inorganic media tested for *D. salina*, the best growth with maximum biopigment production was found in Artificial sea water medium followed by De Walne's, Johnson's, AS-100 media and the least growth with minimum pigment content was observed in Modified BBM. 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 *Dunaliella* as long as there is no precipitation of carbonates [20-21]. The supply of inorganic carbon also appears to affect tolerance to high light and temperature in *Dunaliella* [22]. Nitrogen is known to be an essential component required for protein synthesis as well as growth. The best source of nitrogen for *D. salina* is nitrate. Like phosphorus, sulfur is vital to all cells, because it is constituent of some essential amino acids (methionine, cysteine, cystine), vitamins etc. It is provided as inorganic sulfate in the culture 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. $MgSO_4$ as a source of magnesium was common in most of the media. *D. salina* also needs a high concentration of sulfate for maximal growth. Phosphate was most required for the synthesis of nucleic acid, lipid and in generating high energy in the cells. Phosphate (KH_2PO_4 or K_2HPO_4) as a source of phosphorus has been found to be an additional requirement for the substantial growth of the algae. Low concentration of Fe is necessary for the growth of *Dunaliella* and this element may be limiting for its growth. Iron limitation is known to affect the in vivo chl a fluorescence profile of phytoplankton and it was used successfully to assess nutritional status in laboratory culture. Iron enrichment experiments confirmed the nearly exclusive increase in the cell number of microalgae that correlates with an increase in concentration of chlorophylls per cell [23].

Manganese, zinc, cobalt and copper are also necessary for the optimal growth of *Dunaliella* [24] and all these constituents are present in adequate amount in ASW medium. Artificial Sea Water medium for *D. salina* not only rendered optimum growth but also improved the pigments content of the cultures.

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