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Growth Performance of the Green Microalgae *Dunaliella sp.* Reared on Various Culture Media and Salinity Levels

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Abstract - The study was conducted to determine the best culture medium and optimum level of salinity in the production of *Dunaliella* sp. The *Dunaliella* sp. was grown in 1-liter dextrose bottles at 15 ppt, 25 ppt, 35 ppt, and 45 ppt salinities and fertilized using various established culture media such as Walne's Conway medium, Liao and Huang's Modified TMRL medium and Guillard & Ryther's Modified F medium. The experimental treatments were replicated thrice and run until the alga *Dunaliella* sp. reached the death phase. The growth of *Dunaliella* sp. was determined daily by counting the number of cells (cells/mL) and analyzed using two-way Analysis of Variance to determine the significant difference between and among treatment means. Results showed that *Dunaliella* sp. was capable of growing at all of the salinities and culture media used. *Dunaliella* sp. attained the highest cell density at a shortest culture period (day 4) using 25 ppt salinity and fertilized with Walne's Conway medium at mean density of 1.75×10^6 cell mL^{-1}. In contrast, the lowest peak of the production of *Dunaliella* sp. was noted at salinity level of 35 ppt fertilized with Liao and Huang's Modified TMRL medium on the 11^{th} day with mean density of 7.87×10^5 cell mL^{-1}. Majority of the treatments started to decline within the 14^{th} day to 18^{th} day culture period. The study concluded that the Conway medium and salinity of 25 ppt have dominated between and among salinities and media. However, further studies are strongly suggested to develop the culture technique of the *Dunaliella* sp. for aquaculture use.

Key words: *Dunaliella*, Conway medium, Microalgae

INTRODUCTION

Microalgae or phytoplankton are indispensable food source in the commercial rearing of marine animals because they are the base of the food chain. They have been used as food for the mass production of zooplankton, which serves as initial food for early larval stages of many marine fishes and invertebrates.

One of the species of microalgae that grow in a wide range of environmental conditions is *Dunaliella* sp., a unicellular green alga. *Dunaliella* cells are ovoid, spherical, pyriform, fusiform or ellipsoid with sizes varying from 5 to 25 μm in length and from 3 to 13 μm in width [1].

The use of *Dunaliella salina* for aquaculture purposes as a source of β- carotene to provide the desired pigmentation for farmed prawns for several weeks before harvesting was demonstrated by Boonyaratpalin et al. (2001) [2]. This species of algae had also been used as potential live feed to improve the nutritional status and reproductive characteristics of *Artemia* sp. [3]. It also showed higher weight gain and survival as well as improved resistance to stress and disease in shrimp when fed with shrimp diet

with *Dunaliella* extract [4]. The carotenoid from *Dunaliella* spp. has been used in food, cosmetic and pharmaceutical products as colorant and/or antioxidant and many other commercial applications [5, 6].

Considering the future of commercial algal cultivation, the present study focused on *Dunaliella* sp. maintained at the natural food laboratory of the Bureau of Fisheries and Aquatic Resources - Regional Mariculture Technology Demonstration Center (BFAR-RMaTDeC) in Lucap, Alaminos City, Pangasinan, Philippines. The different species or strains of microalgae require different environmental conditions to obtain high beta-carotene content. However, little is known about the different environmental and nutritional requirements of *Dunaliella* sp. under local conditions. Therefore, evaluating the effects of various factors such as culture media and salinity levels on the algal production of *Dunaliella* sp. is worth investigating. Hence, the study aimed to evaluate the growth performance of a locally isolated strain of *Dunaliella* sp. on various established culture media and salinity levels.

MATERIALS AND METHODS

Location of the Study

The study was conducted at the Phycology Laboratory of the Bureau of Fisheries and Aquatic Resources – Regional Mariculture Technology Demonstration Center (BFAR–RMaTDeC), Lucap, Alaminos City, Pangasinan, Philippines.

Experimental Treatments

The experimental treatments of the study were replicated thrice (Table 1). The experiment was run until *Dunaliella sp.* reached the death phase. The various salinity levels used in the study was based on the levels used for breeding and hatchery of fish and invertebrates.

Table 1. Experimental Treatments used in the study.

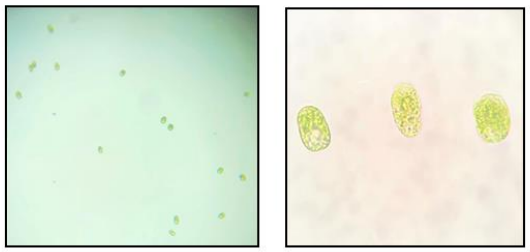
Salinity (ppt)	Control Salinity (CS)	25
	Treatment 1 (S1)	15
	Treatment 2 (S2)	35
	Treatment 3 (S3)	45
Culture Media	Control Medium (CM)	Walne'sConwy Medium
	Treatment 1 (M1)	Liao and Huang's Modified TMRL Medium
	Treatment 2 (M2)	Guillard&Ryther's Modified F medium

Preparation of Culture Media

The culture media were prepared following the established protocol used in the laboratory. Analytical grade reagents were used in the preparation of the culture media. The dry reagents were prepared individually based on the prescribed amounts and dissolved in distilled water to come-up with a stock solution for use in the experiment.

Growth Experiment

The unialgal culture of the green microalgae *Dunaliella sp*. maintained at the BFAR-RMATDEC (Figure 1) was used in the study. The various salinity levels used in the study were prepared by addition of freshwater or sodium chloride (NaCl) and measured using a refractometer.



(a) (b)

Figure 1. Microscopic view of the green microalgae *Dunaliella* sp. (a) low magnification, 100X; (b) 400X.

Previously boiled seawater at various salinity levels, the culture media at 1 mL/L was administered in accordance with treatment assignments. *Dunaliella sp*. was introduced in each 1- liter capacity dextrose bottle at an inoculum density of 1 $x10^5$ cells ml^{-1}. The inoculated bottles were aerated and illuminated at 2,000 lux using 40 watts white fluorescent tube at

21°C room temperature all throughout the experiment.

Data Gathering Procedure

Algal samples (3 mL) from each bottle were collected for growth determination using a haemacytometer. Algal cells were counted daily, and algal growth curve was determined. The total density of algal cells was computed following the formula set by the instrument.

$$\text{Growth (cells ml}^{-1}) = \frac{\text{Total number of cells counted}}{\text{Total number blocks counted}} \times 10^4$$

Data Treatment and Analysis

Two-way Analysis of Variance (ANOVA) method was employed to ascertain the significant difference that would exist between the growth performance of *Dunaliella sp*. at various culture media and salinity levels. Duncan's Multiple Range Test was used in comparing the means among treatments that will give significant result in ANOVA.

RESULTS AND DISCUSSION

Growth Performance of *Dunaliella* sp. on Various Culture Media and Salinity Levels

Dunaliella species have been reported to survive in a wide variation of salinities and culture media. The growth performance of the *Dunaliella* sp. reared on various culture media and salinity levels for the 22-day culture period showed an increasing growth trend at an initial inoculum size of 1 x 10^5 cells mL^{-1}.

The growth of *Dunaliella* sp. using Walne's Conway medium at 25 ppt attained the highest peak of production on day 4 which was the shortest culture period among the different treatments with a mean density of 1.75 X 10^6 cells mL^{-1} (Figure 1). This was a notable observation to be used for the aquaculture industry to produce microalgae at the shortest period of time. In addition, it will lessen the cost of producing microalgae and the cost of operation in the hatchery.

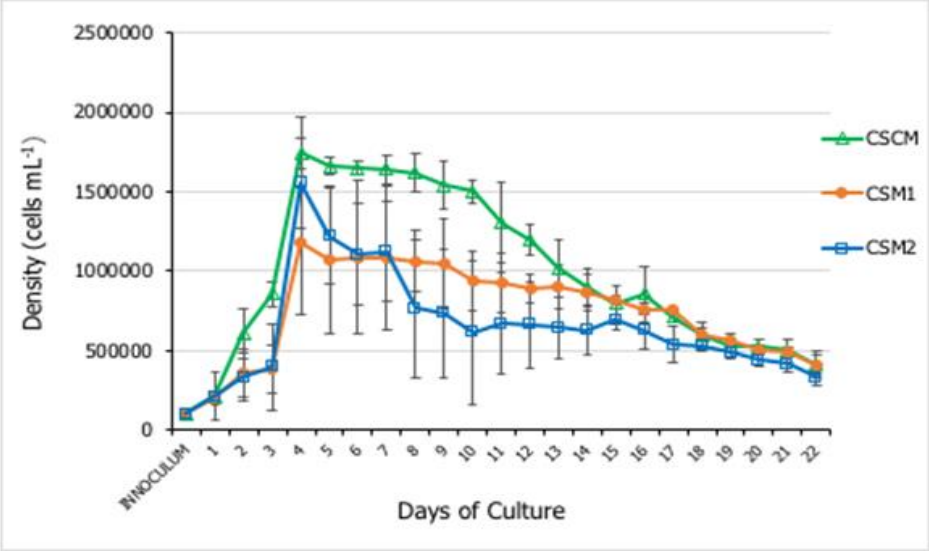


Figure 1. Growth curves of *Dunaliella* sp. reared on various culture media at 25 ppt during the 22-day culture period.

At 15 ppt, *Dunaliella* sp. cultured using Conway medium reached its highest peak on day 9 with a mean density of 1.86 X 10^6 cells mL^{-1} followed by Guillard and Ryther's Modified F medium and Liao and Huang's Modified TMRL medium with mean densities of 1.01 X 10^6 and

9.01 X 10^5 cells mL^{-1}, respectively. It can be attributed that *Dunaliella* sp. had already adapted at lower salinity since the inoculum used had been acclimatized at 25 ppt. This can be supported by the findings of Pinheiro et al. (2016) that *D. viridis* showed maximum densities of 7.33 X 10^6 cells mL^{-1} at day 8 at a salinity of 15 ppt [7].

On the other hand, the lowest peak of production of *Dunaliella* sp. was noted at a salinity level of 35 ppt fertilized with Liao and Huang's Modified TMRL medium with a mean density of 7.87 X 10^5 cells mL^{-1} on day 11.

Furthermore, it was revealed that the earliest death phase of *Dunaliella* sp. during the 22-day culture period was observed at 45 ppt with Walne's Conway medium which started at day 9 whereas the latest was at day 21 at 25 ppt with Walne's Conway medium. The majority of the treatments reached the decline phase within day 14 to 18 of the culture period.

CONCLUSIONS

In light of the findings of this study, the following conclusions were drawn.

1. The best growth of *Dunaliella sp.* was observed using the Walne's Conway medium;
2. The highest cell density (cell mL^{-1}) in the production *Dunaliella sp.* at the shortest culture period was attained using 25ppt salinity;
3. Dunaliella *sp.* attained the highest cell density at a shortest culture period (day 4) using 25ppt salinity fertilized with Walne's Conway medium; and
4. Majority of the treatments, started to decline within 14^{th} day to 18^{th} day culture period.

RECOMMENDATIONS

On the basis of the foregoing findings and conclusions, the following are being recommended:

1. There is a need to identify the isolated species of *Dunaliella* used in the study;
2. Conduct similar studies on the growth performance of *Dunaliella sp.* with additional variables like light intensity, temperature and pH;
3. Conduct similar studies on the growth performance of *Dunaliella sp.* in large volume for mass production; and
4. conduct studies on the growth performance of *Dunaliella sp.* using low-cost fertilizer.

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