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The Effect of Various Salinity Level on the Growth and Characterization of *Dunaliella* sp Isolated from Jepara Waters

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Abstrak

Dunaliella *adalah salah satu biota dengan kandungan β-carotene cukup tinggi. Upaya optimalisasi produksi b-carotene pada* Dunaliella *berhadapan dengan beberapa masalah kultivasi, untuk mendapatkan species yang paling potensial. Hal ini terkait dengan keterbatasan pengetahuan karakteritik ecophysiologi. Alga hijau* Dunaliella *diketahui dapat tumbuh pada media dengan kandungan garam yang cukup tinggi, namun karena pemahaman characteristik yang keliru dapat menyebabkan identifikasi yang salah pada satu species dalam genus* Dunaliella*. Kultur laboratoris pada media microcosms berdasarkan salinitas telah dilakukan untuk mengetahui tingkat pertumbuhan dan karakterisasi* Dunaliella *sp. dari perairan Jepara. Hasil pengamatan menunjukkan bahwa* Dunaliella *sp. dapat beradaptasi pada salinitas 0 sampai dengan 30 ‰. Berdasarkan kepada perubahan warna pigmen* Dunaliella *sp. yang tidak menjadi merah pada media pemeliharaan sampai dengan 25 ‰, maka jenis yang dijumpai di Jepara mempunyai karakter dan secara taksonomis berafiliasi dengan* Dunaliella viridis.

Kata kunci : Dunaliella *sp, Salinity, Growth, Characterization*

Abstract

Dunaliella *is one the most enriched β-carotene eucaryotic organism known. The attempt to optimize b-carotene production from* Dunaliella *has faced with several problems related to its growth management, which was suspectedly unable to meet the needs of the cultured species. This is primarily because the ecophysiological characteristic affecting growth of* Dunaliella *have not been sufficiently understood. It was known that the halophilic species of the green alga* Dunaliella *was grown in concentrated salt solutions, but based on this characterization, some misnamed of species in genus* Dunaliella *also have arisen due to wrong characterization understanding. Laboratory cultures and mixed-species microcosms were used to asses the growth and characterization of* Dunaliella *sp. from Jepara Coastal Region with special emphasis on the several factors that affecting growth of organisms including salinity. The result showed that* Dunaliella *sp. could adapted to a variety of salt concentration from as low as 0.0 % to salt saturation of about 30 ‰. Based on its pigment colour that* Dunaliella *sp. doesn't turn red in the growth on salinities up to 25 ‰, it can be characterized and affiliated taxonomically as* Dunaliella viridis.

Key words : Dunaliella *sp, Salinity, Growth, Characterization*

Introduction

Carotenoids, some of which are pro-vitamin A, have range of diverse biological function and actions, such as species spesific coloration, photoprotection, and light harvesting, and they serve as precursors of many hormones (Vershinin, 1999 *in* Lee and Schmidt-Dannert, 2002). Carotenoids are used commercially as food colorants, animal feed supplements and, more recently, as nutraceuticals for cosmetic and pharmaceutical purposes. The demand and market for carotenoids is anticipated to change drastically with the discovery that carotenoids exhibit significant anti-carcinogenic activity and play an important role in the prevention of chronic diseases (Lee and Schmidt-Dannert, 2002).

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One of availability of a considerable number of plant carotenoid genes found in the marine microalgae, *Dunaliella* sp. Algae of the genus *Dunaliella* are among the micro-algae most studied for mass culture because of its potential in producing carotenoid like b-carotene (Ben-Amotz, 1980). The study of the marine microalgae, *Dunaliella* sp. as carotenoids pigment source, was proposed an alternative source to produce b-carotene. The advantage of marine microalgae, *Dunaliella* sp is easy to culture based on genetic manipulation, simple to prepare, give a high content of b-carotene and less expensive.

The deposits of carotenoid in the *Dunaliella* sp., is part of the growth, an orderly increase of all the components of an organism. In unicellular organisms, cell multiplication is a consequence of growth. Growth leads to an increase in the number of individuals. The growth of algae is essentially dependent on several physical and chemical factors including temperature, light, nutrient composition of the medium and salinity (Ben-Amotz, 1980). Salinity is one of the environmental factors which influence the physiological and accumulation of b-carotene in the marine microalgae, *Dunaliella* sp.

The aim of the research is to investigate the effect of various salinity levels on the growth and biological characterization of the algae *Dunaliella* sp. isolated from Jepara waters.

Material and Methods

The *Dunaliella* sp. cultures used originally isolated from Jepara Ocean Indonesia. Laboratory cultures were grown in 250 ml flasks. All culture was grown in a temperature of 30 °C. The cultures were grown for tested salinity and identification in the concentration of 0 ‰, 5 ‰ (0,85 M), 10 ‰ (1,7 M), 15 ‰ (2,55 M), 20 ‰ (3,4 M); 25 ‰ (4,25 M) and 30 ‰ (5,1 M). The growth rate was expressed in terms of cell number and optical density. Optical density may also serve as a measure of growth. For induction of b-carotene synthesis, cells was grown in a sulfate-free medium ($MgCl_2$ instead of $MgSO_4$) and by applying high light intensities (600 lux). The observation were done for 240 hour.

Table 1. The Growth (density cell/ml x 10^4) of *Dunaliella* sp. cultivated at different level of salinity on 30°C

No	Hour	Salinity Level (‰)						
		0	5	10	15	20	25	30
1	24	82	60	80	65	40	50	30
		85	72	85	75	50	55	35
		70	60	90	90	60	65	40
Average		79	64	85	76	50	56	35
2	48	86	50	60	70	60	50	45
		80	55	60	75	75	60	50
		96	60	60	80	80	90	60
Average		87	55	60	75	71	66	52
3	72	95	96	60	70	65	60	55
		96	103	65	80	85	75	60
		97	110	75	90	90	85	60
Average		96	103	66	80	80	73	58
4	96	48	86	60	65	55	30	50
		50	95	60	70	60	45	55
		58	97	65	75	65	50	45
Average		52	92	61	70	60	41	50
5	120	15	85	70	70	15	30	50
		20	90	95	50	20	15	40
		25	100	100	55	35	20	45
Average		20	91	88	58	23	21	45
6	144	20	80	65	50	10	10	35
		25	85	70	50	15	15	45
		20	90	85	55	20	20	40
Average		21	85	73	51	15	15	40
7	168	20	50	60	25	5	10	30
		20	80	65	50	10	10	30
		20	85	70	50	15	15	30
Average		20	71	65	41	10	11	30

The medium artificial sea water (ASW) used was modified from Johnstons (1963) ; Quraishi and Spencer (1971) that was enrichment solution for *Dunaliella primolecta* comparing with the origin of Jepara sea water. ASW was consist of $MgCl_2.6H_2O$ 4.7 g/L, K_2HPO_4 1 g/L, $NaNO_3$ 10 g/L. $FeCl_3.6H_2O$ 1.25 mg/L, $MnCl_2.4H_2O$ 0.8 g/L, Na_2EDTA 50 mg/L, $NaHCO_3$ 0.18 g/L, distilled water. The ingredients were dissolved in 200 ml of distilled water. The solution was boiled for 10 min while adjusting the pH to 7.6 with HCl or NaOH, filtered and brought to 250 ml. Sterilization was done by autoclaving at 15 lb/in^2 (103 kPa and 120°C). The medium was used by adding 0.1 ml solution to each 10 mL of seawater.

Result and Discussion

The average higher density of *Dunaliella* sp. were reached 1.03 x 10^4 cell / ml at 72 hour in the medium salinity of NaCl 5 % in a temperature of 30 °C. The result show that the *Dunaliella* sp. growth from Jepara water was adequate in NaCl 5 - 10 % (0,85-1,7 M), with reduction of growth at 2,55 - 5,1 M.

Table 2. The Optical Density (600 nm) of *Dunaliella* sp. cultivated at different level of salinity on 30 °C

No	Hour	Salinity Level (‰)						
		0	5	10	15	20	25	30
1	24	0,335	0,307	0,306	0,325	0,294	0,292	0,306
2	48	0,371	0,352	0,331	0,365	0,304	0,296	0,289
3	72	0,398	0,39	0,368	0,358	0,338	0,325	0,317
4	96	0,391	0,373	0,367	0,349	0,334	0,323	0,309
5	120	0,388	0,378	0,376	0,361	0,342	0,33	0,304
6	144	0,384	0,365	0,366	0,363	0,334	0,33	0,302
7	168	0,384	0,365	0,372	0,368	0,333	0,328	0,31

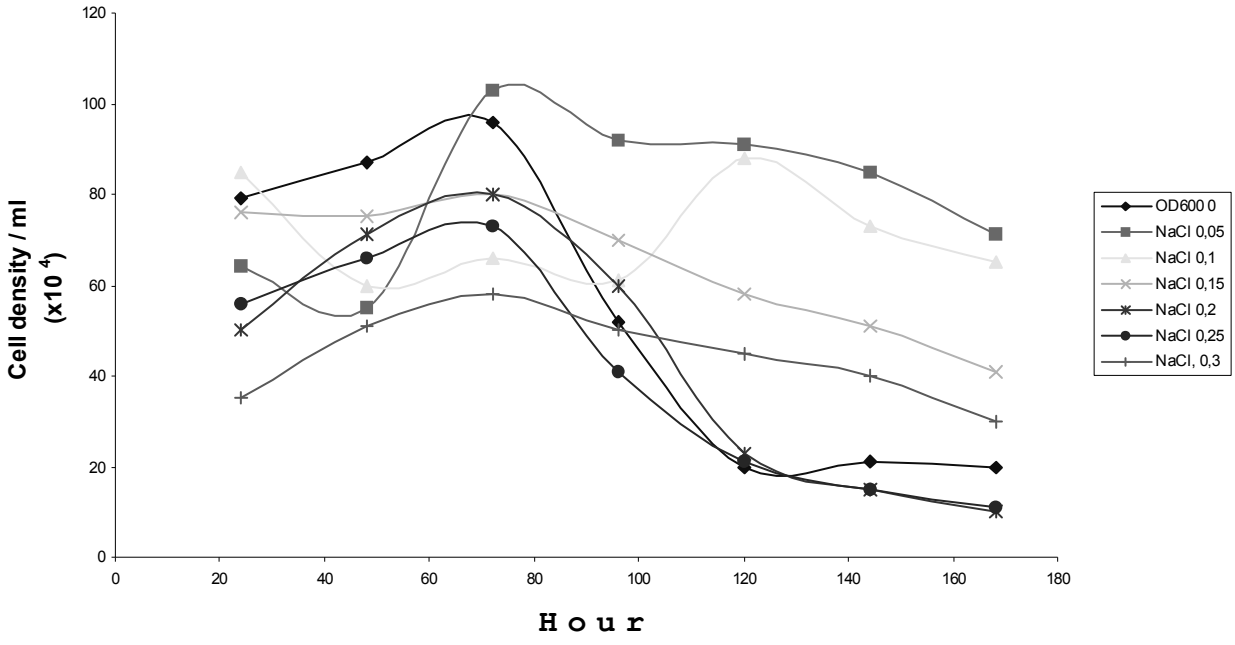

Figure 1. The effect of various salinity level to the *Dunaliella* sp. density (cell / ml x 10^4)

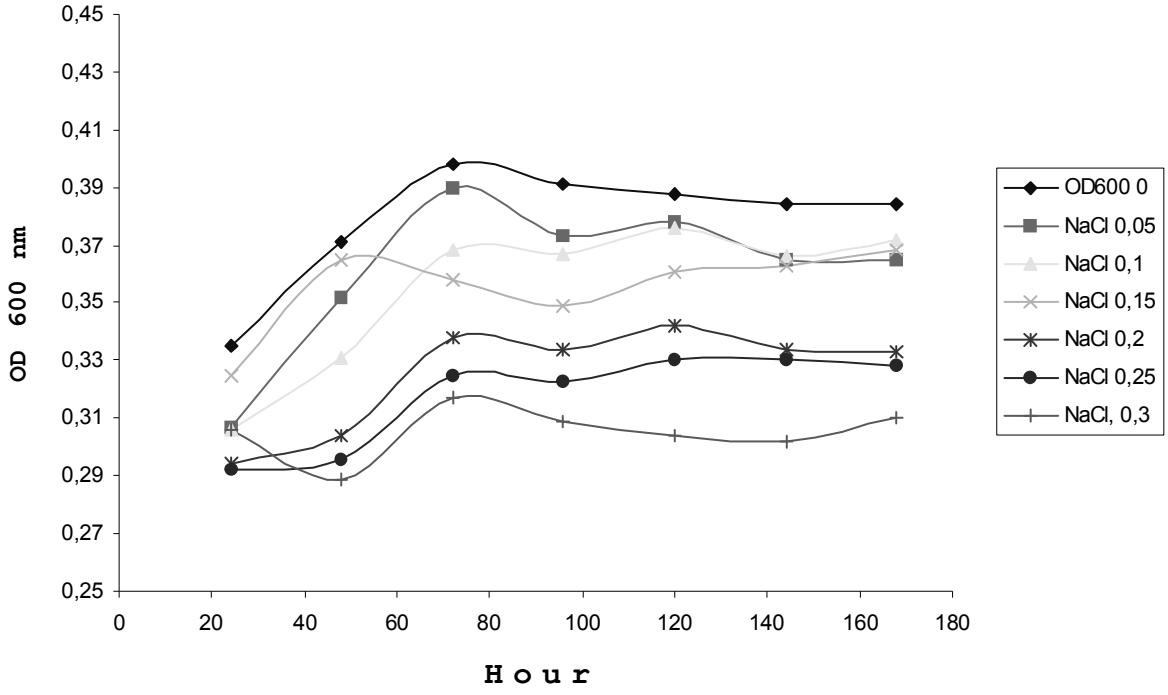

Figure 2. The effect of various salinity level to the optical density (600 nm) of *Dunaliella* sp.

This result was supported by Wong *et al.* (2000) on *D. salina* which determine that the best NaCl concentration in a range of 0,5 - 1.0 M NaCl will support its growth and produce the higher carotenoid content. These concentration will raise the balance as the impact of the responses to the extracellular osmotic pressure. The growth of *D. salina* in the media containing different salt concentration, will be the function of iso-osmotic pressure between the intracellular glycerol concentration, which is directly proportional to the extracellular salt concentration, and maintains the cell water volume and the required cellular osmotic pressure. In all concentration of NaCl, cell densities of the rapidly growing species reached maximum averagely on the third days of incubation. According to Borowitzka (1981) and Ben-Amotz (1993), *Dunaliella* is probably the most halotolerant eucaryotic organism known, showing a remarkable adaptation to a variety of salt concentration from as low as 0.2 % to salt saturation of about 3,5 %. *Dunaliella* will osmoregulated by varying the intracellular concentration of the photosynthetic glycerol. On 0,5 % NaCl, cell densities decrease slowly after three days incubation, but still maintain its number until seven day. While in the concentration of 1 % NaCl, cell could maintain itself and raised the densities on the fifth day. These conditions maybe due to accumulation of high intracellular concentration of glycerol, (Nakas *et al*, 1983). On the larger concentration of salinity (1,5 - 3 %), the growth of *Dunaliella* sp decreased significantly. The increase of salinity content will caused the larger size of cell due to lower rate of division. *Dunaliella* sp. from Jepara water showed an amazing growth on 5,1 M NaCl, although it would decrease the number and survival of the algae. In this study, salinity will influence the activities of several enzymes on CO_2 fixation of *D. viridis*. Since the fixation of CO_2 is absolutely necessary for the growth of the cell, they would maintain an internal salt concentration considerably lower than the one in the medium of high salt concentration. Stress conditions, also caused the deposit of triacylglycerol, which will induce b-carotene synthesis in *D. bardawill* (Rabbani *et al.*, 1998). The research result of *D. salina* revealed an inverse relationship between carotenoid content and salinity, which is contrarily to the result of the *D.bardawil* (Wong *et al.*, 2000). High concentration of NaCl will cause an extreme condition that can change the colour of *Dunaliella* sp. But, after samples treatment with a variety concentration of NaCl from 0 % until 30 %, there is no colour change after several days. On salinity 0.5-2.0 M, *D. salina* appeared yellow-green after less than one week of growth (Wong *et al.*, 2000). The change of color usually being

used as the method to determine the spesies of *Dunaliella* (Teodoresco, 1960 *in* Johnson *et al*, 1968 ; Loeblich, 1982 *in* Borowitz and Borowitz 1992). In addition, the color change can be used to assign the *Dunaliella* taxonomic affiliation according to Loeblich (1982), which show that *Dunaliella* sp. doesn't turn red at salinities up to 25%, it can be assigned as *Dunaliella viridis*.

The result from optical density of the *D. salina* cell under various salinity treatment also supporting the cell densities result. The determination of optical densities base on the reflection of liquid clearance, which will impact the determination of cell densities. The method will determine the total cell densities, and can't differencied between the life and the death cell.

Conclusion

1. Growth of green algae *Dunaliella* sp. isolated from Jepara Water was optimal in NaCl 5 - 10 % with reduction of growth at NaCl 15-30 %.
2. *Dunaliella* sp. isolated from Jepara Water can be assigned as *Dunaliella viridis* based on its green pigment on 30 % of NaCl.

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