MICROBIOLOGICAL AND ECOPHYSIOLOGICAL CHARACTERIZATION OF GREEN ALGAE *Dunaliella* sp. FOR IMPROVEMENT OF CAROTENOID PRODUCTION

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Abstract

An isolate of green algae *Dunaliella* sp. from BBAP Jepara is usually used as a source for carotenoid supplement for marine animal cultivation in the local area. In order to improve carotenoid production especially detection of biosynthetic pathway from the organisms investigated in this study, the main purpose of this study is characterizing *Dunaliella* sp. based on it's microbiological and ecophysiological characters.

The research was done by characterize the growth, the cell and colonies microbiologically, total pigment production, and also characterize all of the ecophysiological factors affecting the algal growth and survival. The results of this research showed that *Dunaliella* sp. posseses typical characteristic of green eucaryote alga, in their growth and ecological condition. The extreme characters which was toleration ability to high salinity environment of was used to conclude *Dunaliella* sp. as *Dunaliella salina*.

Key words : algae, *Dunaliella* sp. , microbiological, ecophysiological, characterization

Introduction

Green algae are simple photosynthetic eukaryotes which are responsible for up to 50% of the planet's atmospheric carbon fixation. The recent discoveries of health related beneficial properties attributed to algal carotenoids have spurred great interest in their production. Carotenoids, some of which are provitamin A, have range of diverse biological function and actions, such as species spesific coloration, photo protection, 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 are 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).

For many years, it was accepted that carotenoid was synthesized through the well known acetate/mevalonate pathway. However, recent studies have demonstrated photosynthetic organisms including green algae, such as *Scenedesmus obliquus, Chlorella fusca, Chlamydomonas reinhardii* use a new non-mevalonate pathway known as deoxyxylulose 5-phosphate (DXP) pathway for their carotenoid biosynthesis. The exclusive occurrence of the non-MVA pathway for the biosynthesis of plastidic isoprenoids and of sterols might represent a general feature of many green algae (Lois *et al.*, 1998; Lichtenthaler, 1999).

A local isolate of an algal species from BBAP Jepara called *Dunaliella* sp., was found potentially useful as source of carotenoids in food additives or as food supplement in fish farming. Thus, it was of great interest to know if this local isolate of algae would also follow the non-MVA pathway for carotenoid biosynthesis. This indigenous algae has been successfully cultivated. Therefore, it is important to examine species identification based on ecophysiological and morphological characteristics microbiologically, needed to support improvement of their carotenoid production.

Material and methods

1. Culture Media

The Walne medium was used for culturing *Dunaliella* sp. modified from Bidwell and Spotte (1983). The medium consist of EDTA 45 g/L, $FeCl_3.6H_2O$ 1.3 mg/L, H_3BO_3 33.6 g/L, $MnCl_2.4H_2O$ 0.36 g/L, NH_4NO_3 100 g/L, Na_2PO_4 20 g/L, B_{12} vitamin 0.001 ppm, distilled water until 1 L. Sterilization was done by autoclaving at 15 lb/in^2 (103 kPa and 120^oC). The medium was using by adding 0.5 ml solution to each 1L of seawater.

For induction of β-carotene synthesis, cells were grown in a sulfate-depleted media ($MgCl_2$ instead of $MgSO_4$), under intense illumination conditions 600 lux and with 2 – 4 ppm O_2 passing to the liquid (Rabbani *et al*., 1998)

2. Microbiological and ecophysiological Characterization

Microbiological characterization was done according to Boney (1989), Sze (1993) and Tomas (1997). Microbiological characters include cell reproduction shape, curvature, size and arrangements. Pleomorphisms, formation of daughter cell, cell division and rfeproduction, presence and arrangement of flagella,

gliding motility, presence or lack of cell walls, presence or lack of nucleus walls, presence or lack of cell sheath.

Ecophysiological characterization was conducted according to Borowitzka and Borowitzka (1988) and Ben-Amotz (1993) consist of the maximum and minimum temperatures permitting sustained growth, reproducibility, temperature tolerance, atmospheric requirements such as aeration and illumination, also salinity. Growth experiment was measured by cell count and cell density absorbancies at $OD_{600\ nm}$. Illumination was observed at 660 μEinstein.$^{m\text{-}2.sec\text{-}1}$ or 600 lux (Rabbani *et al*., 1998). Measurement of pigments concentration was done by extracting the specimen with methanol or acetone to check if residual color (blue to red) caused by the non-organic soluble phycobillins remains in the cell (Goodwin and Britton, 1988; Holt *et al*., 1994). Chlorophyl concentration were analyzed by extracting cell pellet with methanol until the pellet color is dissappeared. Concentration of chlorophyll was measured by $OD_{663\ nm}$ and $OD_{645\ nm}$, then calculated with formulas (Harborne, 1984; Goodwin and Britton, 1988) :

Total chlorophyll = $17.3\ A_{645} + 7.18\ A_{663}$ mg/ml

chlorophyll a = $12.21\ A_{663} - 2.81\ A_{645}$ mg/ml

chlorophyll b = $20.13\ A_{645} - 5.03\ A_{663}$ mg/ml

Results and Discussion

1. Microbiological and Morphological characterization

According to microscopic view as illustrated in **Fig 1**. , morphological characteristics of *Dunaliella* sp. is free-living organisms, unicellular and solitaire. Each cell has an ovoid space and is surrounded by a delicate wall. The flagella are smooth. A single large chloroplast in the shape of thick cup fills much of the volume of the cell. Cell was spherical or elongate in shape, widely oval before division and after division hemispherical. Cells of *Dunaliella* sp. swim actively by means of two anterior flagella. is non motile cells and do not have flagella. The color of the cell is bright green and turn to greenish yellow on the sixth day of growth. Cells are surrounded by narrow, fine, green colour envelopes. Cellular reproduction is by division into two morphologically equal, hemispherical daughter cells (binary fission), which reach the original globular shape before next division. Cells divide in one planes in successive generations in broth media **(Fig 2).** The envelopes around cells will split together with dividing cells. Daughter cells separate after division and grow into the original size and shape before next binary fission. Daughter cells held together by mucilaginous sheath. Reproduction of cell was sexual or asexually **(Fig 3 and Fig 4)**.

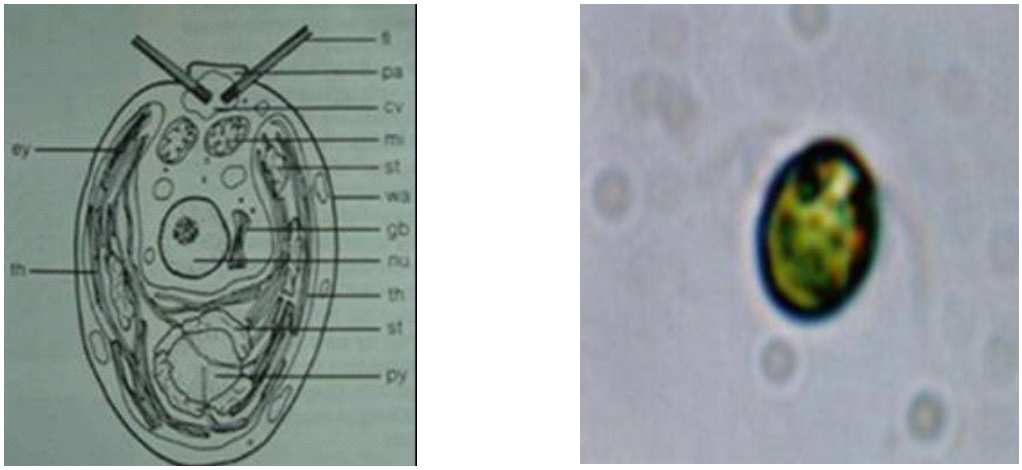

Figure 1. Microscopic View of a *Dunaliella* sp. (cv = contractile vacuole, ey = eyespot, fl = flagellum, gb = Golgi body, mi = mitochondria, nu = cell nucleus, pa = papillae, py = pyrenoid, st = starch grain, th = thylakoid, wa = wall) (Sze, 1989)

Figure 2. Cultures of *Dunaliella* sp.

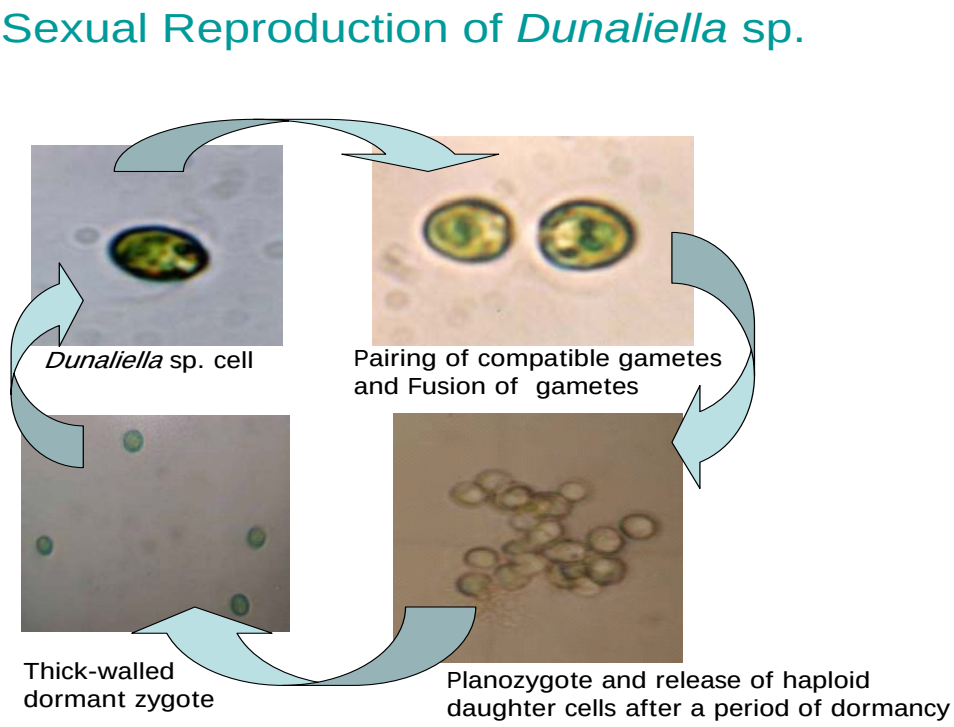

Figure 3. Sexual reproduction of *Dunaliella* sp.

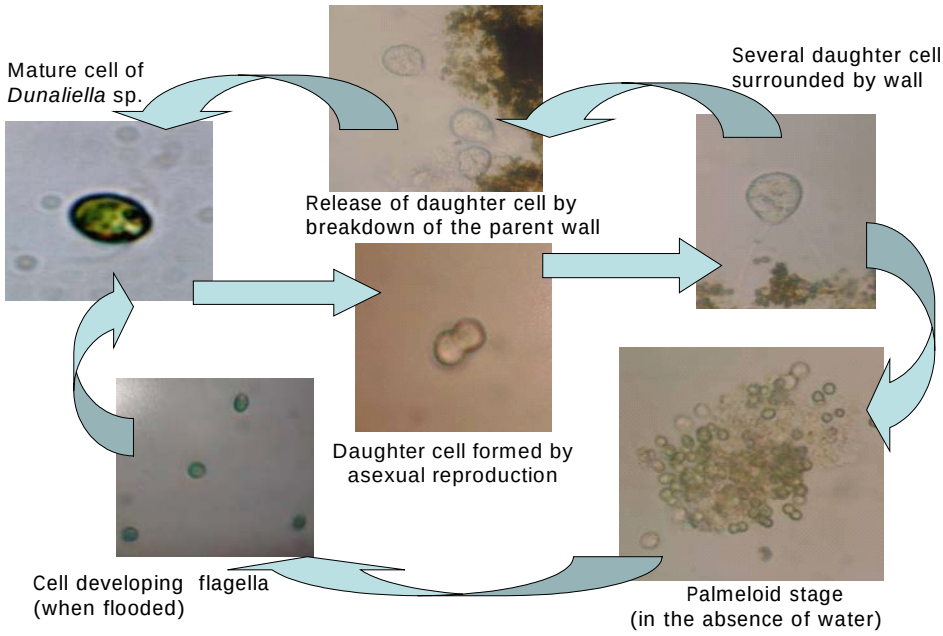

Figure 4. Asexual reproduction of *Dunaliella* sp.

Ecophysiological characterization of *Dunaliella* sp. was carried out by growth and factor influencing growth including temperature, salinity and light.

The characteristic of *Dunaliella* sp. are presented in **Table 1**. *Dunaliella* sp. usually live in sea water but also can

er but also can survive in fresh water

According to Boney (1989) *Dunaliella* sp. synthesizes glycerol which internally act as 'a compatible solvent' allowing enzyme activity to continue despite high concentrations in the surrounding medium. The glycerol is excreted when the cell return to lowered salinities.

Table 1. Microbiological and Ecophysiological Characteristics of *Dunaliella* sp. (Holt *et al.*, 1994)

Characteristic	*Dunaliella* sp.
1. Cellular organization	eucaryotic
2. Growth temperature	25°C – 30 °C
3. salinity	25– 40%
4. source of energy and carbon	Photoheterotroph, photoautotroph
5. habitat	Sea Waters
6. unicellular	+
7. coccoid or spherical	+
8. binary fission in 2 succesive planes	+
9. Extracelllular sheath	+

10. Chlorophyll a	+
11. Chlorophyll b	+
12. %GC	58.7
13. filament	-
14. thylakoid	+
15. cell diameter	5 – 6 μm
16. motility/movement	slow gliding
17. Cell	solitary
18. Colonies	Forming colonies
19. Cell color	Bright green
20. Color of sheath	bright
21. Cell division	Binary fission
22. Reproduction	solitary cells

Dunaliella sp. appeared yellow-green after less than one week of growth. It has been observed that *Dunaliella* osmoregulates by varying the intracellular concentration of the photosynthetic glycerol in respons to the extracellular osmotic pressure. On growth in media containing different salt concentration, the intracellular glycerol concentration is directly proportional to the extracellular salt concentration and maintains the cell water volume and the required cellular osmotic pressure.

4. Growth of *Dunaliella* sp.

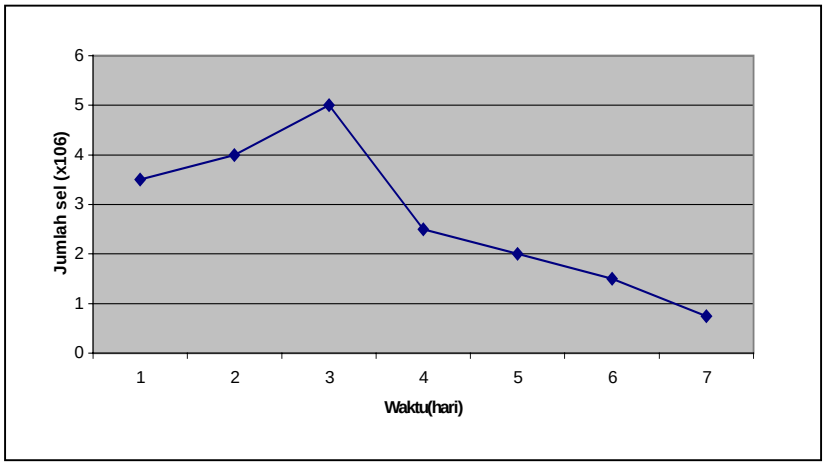

Figure 4. Growth Curve of *Dunaliella* sp. on Walne medium

The research result shows toleration ability of *Dunaliella* sp. on high salt concentration, as may occur in tide pools and lakes when evaporation concentrates salts (Sze, 1993). Some studies also display that green algae *Dunaliella* showing a remarkable adaptation to a variety of salt concentration from as low as 0,2% to salt saturation of about 35% (Borowitzka & Borowitzka, 1988; Ben-Amotz, 1993).

Some green algae will change their cell colors after several days under salinity treatment as shown by *D. salina*. Since *Dunaliella* sp. change their colour on salinity > 2.0 M and appeared yellow-green after less than one week of growth, according to Wong *et al*. (2000), it ca be concluded as *D. salina*.

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3. Pigment Production

Analysis of total pigment production on *Dunaliella* sp. exhibit an increase pigment production as illustrated in **Fig. 5.**

Highest total pigment production reaches 111,16 μg/g bks or equivalent to 3,3 – 15,56 μg/g bks β-karoten.

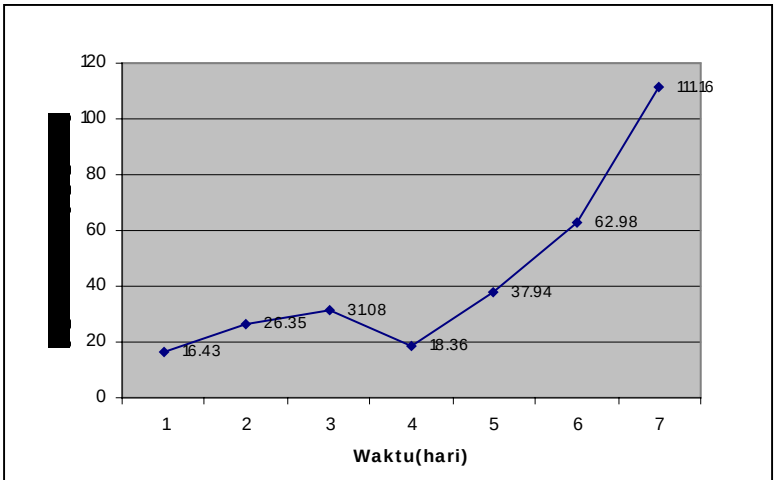

Figure 4. Total pigment production of *Dunaliella* sp.

Conclusion

Characterization of *Dunaliella* sp. based on ecophysiological, microbiological and clearly shows a common green algae characteristic. Based on the experiment results, it can concluded that the algae was similar to *Dunaliella salina* based on tolerancies in high salinity.

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