SPECIES DETERMINATION OF GREEN ALGAE ISOLATED FROM JEPARA COASTAL REGION BASED ON MICROBIOLOGICAL, ECOPHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION FOR IMPROVEMENT OF CAROTENOID PRODUCTION

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ABSTRACT

A local isolate of green algae called C1 from Jepara waters is usually used as a source for carotenoid supplement for animal fisheries in the local area. This indigenous algae has been successfully purified. Although the local isolate was known as eucaryotic green algae Dunaliella, our previous molecular study by 18S rDNA analysis to determine the species of this organisms showed negative result. In order to improve carotenoid production especially detection of biosynthetic pathway from the organisms investigated in this study, the main purpose of this study was species determination of local isolate of green algae based on microbiological, ecophysiological and molecular characterization.

The results of this research indicated that local isolate of green algae possesses Cyanobacteria characteristic, especially Synechocystis. Analyses with 16S rDNA sequence from genome of green algae isolates were also in accordance with these results, showing close similarities with Synechocystis 16S rDNA sequence. However, it should be noted that, instead of having Synechocystis dominant feature, it was also found that local isolate of green algae exhibit different characteristic in having chlorophyll a, chlorophyll b and lack of phycobilins. This character was typical for aberrant Cyanophyta, Prochlorophyta.

Key words : C1- isolate, Dunaliella, Cyanobacteria, 16S rDNA, Prochlorophyta

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INTRODUCTION

The recent discoveries of health related beneficial properties attributed to 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 such as green algae, *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; Lichtenhelter, 1999). A local isolate of an algal species from BBAP Jepara called C-1 isolate, was found potentially useful as source of carotenoids in food additives or as food supplement in fish farming. It was suspected as representing a strain of *Dunaliella*, based only on morphological identification (Sutanto-pers.comm; Kusumaningrum et al., 2004; Kusumaningrum et al., 2006). Thus, it was of great interest to know if this local isolate of algae would also follow the non-MVA pathway for carotenoid biosynthesis. Further research in detecting biosynthetic pathway from this "*Dunaliella*" by genetic engineering techniques, has faced several problems that might be caused by misnamed of the species. It is apparent that microbial identifications based only on morphological characterization have, until recently, failed to achieve the necessary requirements of prediction, stability and objectivity (Priest and Austin, 1993).

Therefore, the aims of this research is to identify of species based on microbiological and ecophysiological characterization supported by molecular techniques using 16S rDNA approach.

**Materials and Methods**

**Culture of algae**

Laboratory cultures were grown in 100 ml flasks. The microcosms consisted of 100 mL cultures in 4 erlenmeyer. All cultures were grown in a temperature of 30°C. For induction of β-carotene synthesis, cells were grown in a sulfate-depleted media (MgCl₂
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)

**Preparation of Media**

The medium Bold Basal Medium (BBM) used and modified from Stein (1998) in Bidwell, J.P. and Spotte S., 1983 consisted of KH$_2$PO$_4$ 17.5 g/l; CaCl$_2$.2H$_2$O 2.5 g/l; MgSO$_4$.7H$_2$O 7.5 g/l; NaNO$_3$ 25 g/l; K$_2$HPO$_4$ 7.5 g/l; NaCl 2.5 g/l; Na$_2$EDTA 10 g/l; KOH 6.2 g/l; FeSO$_4$.7H$_2$O 4.98 g/l; H$_2$SO$_4$ 1 ml/l; “Trace Metal” solution 1 ml/l (H$_3$BO$_3$ 2.86 g/l; MnCl$_2$.4H$_2$O 1.81 g/l; ZnSO$_4$.7H$_2$O 0.222 g/l; NaMoO$_4$.5H$_2$O 0.39 g/l; CuSO$_4$.5H$_2$O 0.079 g/l; Co(NO$_3$)$_2$.6H$_2$O 0.0494 g/l; H$_3$BO$_3$ 11.5 g/l. The ingredients were dissolved in 1 L of distilled water. The pH was adjusted to 6.8 with HCl or NaOH. Sterilization was done by autoclaving at 15 lb/in$^2$ (103 kPa and 120°C). The medium was using by adding 0.1 ml solution to each 10 mL of seawater.

**Microbiological and Ecophysiological Characterization**

Morphological and microbiological characterization was done according to Holt et al., (1994) and Logan (1994). Cultural characterization consist of colony shape, margin, elevation, surface appearance, opacity, texture, pigmentation and appearance of growth in broth. Morphological characters include cell shape, curvature, size and arrangements. Pleomorphisms, formation of daughter cell, cell division and reproduction, 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 and staining reactions such as Gram.

Ecophysiological characterization was conducted according to Borowitzka dan Borowitzka (1988) and Ben-Amotz (1993) including the maximum and minimum temperatures permitting sustained growth, reproducibility, temperature tolerance, atmospheric requirements such as aeration and illumination, also growth at different NaCl concentrations (0 – 30 %). Ecophysiological experiment was measured by cell count and cell density absorbancies at OD$_{600}$ nm. Illumination was observed at 660 $\mu$Einstein. m$^{-2}$.sec$^{-1}$ 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 phycobilins remains in the cell (Goodwin and Britton, 1988; Holt et al., 1994). Chlorophyll concentration were analyzed by extracting cell pellet with methanol until the pellet color is dissappeared. Concentration of chlorophyll was measured by OD$_{665}$ nm and OD$_{645}$ nm, then calculated with formulas (Harborne, 1984; Goodwin and Britton, 1988) :

\[
\text{Total chlorophyll} = 17.3 \times A_{645} + 7.18 \\
A_{663} \text{ mg/ml} = 12.21 \times A_{663} - 2.81 \\
A_{645} \text{ mg/ml} = 20.13 \times A_{645} - 5.03 \\
A_{663} \text{ mg/ml}
\]

**DNA Extraction**

Preparation of a green algae DNA isolate was carried out by modification of CTAB methods (Ausubel et al., 1995). Culture of algae about 15 ml were centrifugated 13,000 rpm for 3 minutes. Pellet was pulverized on cold mortar and pestled to a fine powder. One ml of warm CTAB extraction buffer [(2% (w/v) CTAB, 100 mM Tris-HCl pH 8; 20 mM EDTA pH 8; and 1.4 M NaCl, 1% (w/v) pre warmed on 65°C] was added to the pulverized algae and mixed to wet thoroughly. 25 µl Lisozyme enzyme with concentration of 25 mg/ml was added to suspension, homogenized and incubated in waterbath on 37°C for 1 hour with occasional mixing. 750 ml of SDS 10% was added,
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incubated again in waterbath on 37°C for 1 hour. The extraction was incubated in waterbath on 65°C for 1 hour with occasional mixing. The homogenate was extracted, with an equal volume of chloroform, and mixed well by inversion. The homogenate was centrifuged 5 min at 13,000 rpm in microcentrifuge. The top (aqueous) phase was recovered and the supernatant was removed. The nucleic acid was precipitated by adding 0.6 vol isopropanol and 1/10 vol Sodium asetat 3 M. The suspension was incubated over night on -20°C. The suspension was centrifugated 5 min at 13,000 rpm. The pellet was washed with 100 µl ethanol 70 %, air dried and resuspended in 50 µl TE buffer (10 mM Tris pH 8; 1 mM EDTA pH 8). DNA was purified with RNaseA and incubated in waterbath on 37°C for one hour. DNA was kept on -20°C or used directly for PCR.

**Amplification of 16S rDNA**

The 16S rDNA fragment was amplified by PCR using specific primers in 16S-23S ribosomal genes spacer region. Sequence of forward primer was 5'-AGAGTTTGATCMTGGCTCAG-3', reverse primer was 5'-TACGGYTACCTTGTTACGACTT-3' corresponding to base pairs 1541 respectively (Garcia-Martinez et al., 1999). PCR was carried out in mixture containing 50 ng of genomic DNA, 2,0 mM of MgCl₂, a 0,2 mM concentration of each deoxynucleoside triphosphate, 2,5 pmol of each primer, and 1,8 U of Taq Polymerase and ddH₂O until volume 25 µl. PCR conditions were performed with hot start for 2 minutes at 94°C, denaturation for 1 minute at 94°C, annealing for 1 minute at 50°C, polymerization for 1 minute at 72°C, extra extention at 72°C for 2 minutes, with 30 cycles of PCR reactions. In this PCR, a single DNA fragment of 1,5 kb was amplified.

**Sequencing and Phylogenetic Analysis**

The 16S rRNA fragment was sequenced on BPPT Jakarta. Sequencing process involves several steps. First step was purification of amplification product of PCR by Qiagen Purification Kit. The next step was cycle seq process. The reaction composition consists of DNA template, primers, buffer, ddH₂O and big dye (DNA polymerase enzyme, ddNTP, and dNTP). The last step was sequencing using ABI Prism 310 sequencer.

Sequence of 16S rDNA of a green algae isolate was used to search its homology, process of comparing a new sequence with all other known sequences in the database. Then attempting to infer the function of the new sequence by assessing the matches and their biological annotations as described in the database. Sequence analysis was analyzing by similarity (homology). Sequence data was submitted to GenBank website at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) and European Bioinformatics Services website at [www.ebi.ac.uk](http://www.ebi.ac.uk). Setting up database search was using BLASTN Program.

Database searches and phylogenetic analyses also performed for the DXS gene of several species. Homologous protein sequences were retrieved from public and proprietary genomic sequence databases. Preliminary sequence data were also obtained from GenBank. The nucleotides were aligned using the program of CLUSTALW version 1.7 with the BLOSUM62 similarity matrix and gap opening and extension penalties of 10.0 and 0.05, respectively. Phylogenetic trees were constructed by maximum-parsimony (MP) and neighbor-joining (NJ) methods for each set of alignments.

**RESULTS AND DISCUSSION**

**Microbiological and Morphological characterization**

According to microscopic view as illustrated in Fig 1. and Fig 2, morphological
characteristics of C1-isolate of green algae are unicellular, solitary, spherical or elongate in shape, widely oval before division and after division hemispherical; the cell length was 5 - 6.5 µm. Cells of C1-isolate of green algae are non motile cells and do not have flagella. They have two cell wall, the color of the cell is bright green and turn to greenish yellow on the sixth day of growth. Forming green colonies on solid media look form aggregates of the cell. Cells are always surrounded by narrow, fine, pinkish 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, 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. Cell of C1-isolate of green algae was stain gram negative, this characteristic is closely related to a typical feature of Cyanobacteria.

![Microscopic View of a C1-isolate of Green Algae 48 hours](image1)

**Fig 1.** Microscopic View of a C1-isolate of Green Algae 48 hours

![Microscopic View of a C1-isolate of Green Algae 120 hours](image2)

**Fig 2.** Microscopic View of a C1-isolate of Green Algae 120 hours
Table 1. Characteristic of a C1-Green Algae Isolate

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C1-Isolate</th>
<th>Cyanobacteria (Synechocystis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cellular organization</td>
<td>procaryotic</td>
<td>procaryotic</td>
</tr>
<tr>
<td>2. Growth temperature</td>
<td>-20°C – 30°C</td>
<td>26 – 39°C</td>
</tr>
<tr>
<td>3. salinity</td>
<td>0 – 30%</td>
<td>-</td>
</tr>
<tr>
<td>4. source of energy and carbon</td>
<td>photoheterotroph</td>
<td>Photoheterotroph, photoautotroph</td>
</tr>
<tr>
<td>5. habitat</td>
<td>Sea Waters</td>
<td>Tropical, fresh water</td>
</tr>
<tr>
<td>6. unicellular</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7. coccoid or spherical</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8. binary fission in 2 successive planes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9. Extracellular sheath</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10. Chlorophyll a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11. Chlorophyll b</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12. Phycobilin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13. filament</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14. thylakoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15. cell diameter</td>
<td>2 – 7 µm</td>
<td>5 – 6 µm</td>
</tr>
<tr>
<td>16. motility/movement</td>
<td>slow gliding</td>
<td>slow gliding</td>
</tr>
<tr>
<td>17. Cell</td>
<td>solitary</td>
<td>solitary</td>
</tr>
<tr>
<td>18. Colonies</td>
<td>Forming colonies</td>
<td>Never forming colonies</td>
</tr>
<tr>
<td>19. Cell color</td>
<td>Bright green</td>
<td>pale blue-green, olive-green, bright-green or pinkish chromatoplasma</td>
</tr>
<tr>
<td>20. Color of sheath</td>
<td>pink</td>
<td>Yellow pigment or red-blue pigment</td>
</tr>
<tr>
<td>21. Gram stain</td>
<td>Gram negative</td>
<td>Gram negative</td>
</tr>
<tr>
<td>22. Cell division</td>
<td>Binary fission</td>
<td>Binary fission</td>
</tr>
<tr>
<td>23. Reproduction</td>
<td>solitary cells</td>
<td>solitary cells</td>
</tr>
</tbody>
</table>

Reproduction by solitary cells. Comparison of cell morphology between the C1-isolate of green algae and literature from Cyanobacteria is shown in Fig. 2. showed that their cells were resembles to member of Cyanobacteria, *Synechocystis*, (confirmed also by 16S rDNA alignment). The comparison characteristic between these two organisms are presented in Table 1.

Ecophysiological characterization of C1-isolate of green algae

Ecophysiological characterization of the C1-isolate of green algae was carried out by growth and factor influencing growth including temperature, salinity and light. The C1-isolate exhibit ranges of tolerance for ecophysiological factors that determine their limits of growth. When the culture was tested in survival on the extreme temperature minus 20°C and without light, it was still able to live. Although low temperatures restrict the rates of growth and enzymatic activities of organisms, they did not kill these organisms. Therefore, when the culture is thawed, they can grow. Supporting the previous results, it can be explained that prokaryotic organization of green algae isolates cells similar to cyanobacteria which allows them to grow under extreme conditions, where they...
may thrive in the absence of competition from other algae.

Cyanobacteria could grow over a wide range of temperatures. If the C1-isolate was a member of cyanobacteria, it is possible that it might follow an alternative system of cyanobacteria photosynthesis in keeping its growth under anaerobic conditions (anoxygenic photosynthesis). In the absence of oxygen, some non-heterocystous cyanobacteria show nitrogenase activity in ordinary vegetative cells. They may fix nitrogen at night or in oxygen-depleted region. (Cohen et al., 1975 in Sze, 1993).

The growth of the C1-isolate as illustrated in Fig. 3. was seven days under illumination 600 – 1000 lux on the room temperature. High light intensity is generally detrimental to many algae. At a very intense illumination, the algal cells generally bleach. According to Orset and Young (2000), illumination will affect growth and had a marked effect both on growth of algae (that will be suppressed by low and high illumination) and on the accumulation of β-carotene. Research on D. bardawil showed that under stress condition such as high light intensity, the cells turn orange due to massive β-carotene formation under high light intensities (Rabbani et al., 1998). The β-carotene globules in Dunaliella will protect the cell against the high intensity irradiation to which it is exposed in the natural habit by absorbing energy in the blue region of the spectrum. Strains unable to accumulate β-carotene die when exposed to high irradiation, while the β-carotene-rich Dunaliella strains flourish (Shaish et al., 1991 in Ben-Amotz, 1993).

As shown in Fig 2. the cell of C1-isolate also turns their colour into yellow-brown starting on day fourth under the treatment of high light intensities when the growth decreased gradually. Analysis of total pigment production on C1-isolate also exhibit an increase pigment production as illustrated in Fig. 4.

**Fig 3.** Growth curve of C1-isolate on Walne media
The result of salinity treatment for the local isolate of green algae with a variety concentration of NaCl from 0% until 30% presented in Fig 5., shows that the optimal concentration of NaCl for growth was 5% and 10%. Many marine bacteria typically grow best at salt concentration of about 3%. The outer membranes of marine bacteria require at least 1,5% NaCl to maintain their integrity (Atlas, 1995). In all concentration of NaCl, there is no significant difference on the growth and colour of the culture after several days, as illustrated in Fig 5. This result clearly shows agreement with previous experiment which exhibits similarities of local isolate of green algae with cyanobacteria in toleration ability 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*. On salinity 0.5-2.0 M *D. salina* appeared yellow-green after less than one week of growth (Wong *et al.*, 2000). It has been observed that *Dunaliella* osmoregulates by varying the intracellular concentration of the photosynthetic glycerol in response 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.

### 3. Molecular characterization of C1-isolate

The result of 18S rDNA analysis as a typical methods to identify molecular eucaryotic species revealed negative result. Electroferogram of a C1-isolate 16S rDNA amplification was showed one clear band as illustrated by Fig. 6.
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Surprisingly, a control conducted by amplification and analysis of 16S rDNA fragments revealed close homology with 16S rDNA sequence from some member of Cyanobacteria especially with *Cyanobacterium* sp. MBIC1021 in 99% similarity (Fig. 7) and 95% similarity with *Synechocystis* PCC6308 (Fig. 8)
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Synechocystis PCC 6308 gene for 16S rRNA, partial sequence

Length=1435
Score = 682 bits (344), Expect = 0.0
Identities = 403/420 (95%), Gaps = 2/420 (0%), Strand=Plus/Minus

Query 434 CTTTCCGGTACCGGTACCTTGTTACGACTTCACCCAGTCACTAGTCCTTCGGCGC 493

Sbjct 1355 CCTTCCGGTACCGGTACCTTGTTACGACTTCACCCAGTCACTAGTCCTTCGGCGC 1414

Query 494 CTCCTCCATTA-CGGTTGAGATACGACTTCGGGCGTGACCAACTTCTAGCTGTGACG 552

Sbjct 1376 CTCCTCCATTA-CGGTTGAGATACGACTTCGGGCGTGACCAACTTCTAGCTGTGACG 1435

Query 555 GCCGTTGTTGATACAAGACCGCGGAAACGATTACCGCGAGATGCTGACTTAGACTGATACCAG 612

Sbjct 1316 GCCGTTGTTGATACAAGACCGCGGAAACGATTACCGCGAGATGCTGACTTAGACTGATACCAG 1375

Query 613 CGATTCCTCCTTATGCGGATTCGACTTCGGGGCTGACCAACTTCTAGCTGTGACG 672

Sbjct 1256 CGATTCCTCCTTATGCGGATTCGACTTCGGGGCTGACCAACTTCTAGCTGTGACG 1315

Query 673 GAGGATTGCTCCACCTGGCGGGTTCCGGACCCCTTGTGCCCAACATCTGAGTAGCTGTGT 732

Sbjct 1196 GAGGATTGCTCCACCTGGCGGGTTCCGGACCCCTTGTGCCCAACATCTGAGTAGCTGTGT 1255

Query 733 AGGCAGCACAGGAGGACATGCTGTACGCTTCACCATCCTCCGAGTTCTCCTCC 792

Sbjct 1136 AGGCAGCACAGGAGGACATGCTGTACGCTTCACCATCCTCCGAGTTCTCCTCC 1195

Query 793 CCGGGGCTTCCTCCATAGCTCCCAACCTTCAAGTTGTGGACGAGAGGTCTTGGCCTC 852

Sbjct 1076 CCGGGGCTTCCTCCATAGCTCCCAACCTTCAAGTTGTGGACGAGAGGTCTTGGCCTC 1135

Fig 8. The multiple alignment analysis result of 16S rDNA sequence of green algae isolate with Synechocystis PCC6803 (Query = 16S rDNA sequence of C1-isolate, Sbjct = 16S rDNA sequence of Synechocystis PCC6803)

However, ecophysiological, morphological and microbiological analysis supports the molecular analysis in showing more similarities with feature of Synechocystis instead of Cyanobacterium. Synechocystis as shown in Fig 9, has twenty four described species and three unclear taxa. Its taxonomic position with higher hierarchy was Cyanophyceae, Chroococcales, Merismopediaeae, Merismopedioideae. The common characteristic of this species is solitary cells, never forming colonies. The cell length S. salina was 10 µm, while S. minuscula 5 µm. Mainly it has thin, fine, colourless mucilaginous envelopes with homogeneous content or with several prominent granules, sometimes with visible chromatoplasma, pale blue-green, olive-green, bright-green or pinkish; different but stable pigment ratios in related species (and populations); cell division by binary fission. Several species are planktonic in fresh water reservoirs or in salinic or sea waters, others grow in metaphyton of pools, of thermal and mineral springs, of salinic (brackish) swamps or in moors (Lewin 1983). According to Holt et al. (1994) Synechocystis is unicellular, coccoid, or spherical, with binary fission central but in 2 or 3 successive planes at right angles to each other. Aggregates sometimes occur with amorphous slime holding together. Cells are 2-7 µm in diameter, most strain in the 2-4 µm range; thylakoid is present.
Despite the similarities between the local isolate of green algae and 
*Synechocystis*, there is a clear several different factors as we can see from Table 1, which has to be considered among the two species. Blue green algae has phycobilins as their principal accessory pigments. Extraction of the Cyanobacteria pellet with methanol or ethanol will cause blue to red residual color which corresponds to its pigment phycobilins. It has been proved in our experiment that the local isolate of green algae displays a green to yellow color of the residu. The result of this analysis shows that cell of green algae isolate only has chlorophylls a and b but lack phycobiliproteins. It shows that these characteristics are considered not corresponding to typical characteristic of Cyanobacteria especially *Synechocystis*. However, Lewin (1983) reported the discovery of a unicellular prokaryotic alga which was not a cyanobacterium. The alga, later named Prochloron, resembles the coccoid cyanobacterium *Synechocystis*, which its cells have chlorophylls a and b and lack phycobiliproteins. Prochloron resembles cyanobacteria in its cell structure and biochemistry (Lewin and Cheng, 1989 in Sze, 1993), but its photosynthetic pigments are similar to green algae. Table 2 shows different characteristics among these three species.

**Table 2. Comparison of Cyanophyta, Prochlorophyta and Green Algae (Sze, 1993)**

<table>
<thead>
<tr>
<th></th>
<th>Cyanophyta</th>
<th>Prochlorophyta</th>
<th>Chlorophyta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular organization</td>
<td>prokaryotic</td>
<td>prokariotyc</td>
<td>eukaryotic</td>
</tr>
<tr>
<td>Chlorophylls</td>
<td>a</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>Phycobiliproteins</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Thylakoids</td>
<td>solitary</td>
<td>paired</td>
<td>stacked (2-6)</td>
</tr>
</tbody>
</table>

Lewin (2002) mentioned that Prochlorophyta was assigned to a new algal sub-class in showing clearly photosynthetic prokaryotes like cyanophytes, but it contains no blue or red bilin pigment. However, since their possible phylogenetic relationships to ancestral green-plant chloroplasts have not received support from molecular biology, it now seems expedient to consider them as aberrant cyanophytes.
CONCLUSION

Characterization of C1-green algae isolate from Jepara Sea Waters based on ecophysiological, microbiological and molecular analysis clearly shows a prokaryotic characteristic. Based on the experiment results, it can be assumed that the species of green algae isolate was the member of Cyanobacteria, and it was not *Dunaliella*. Instead of showing similarities with *Synechocystis*, green algae isolate also provides some characters which resemble to Prochlorophyta. In conclusion, C1-isolate of green algae from Jepara Seawaters posses some characters similar to both of two species, Prochlorophyta and *Synechocystis*.

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Effect of Various Salinity Level to the Growth and Characterization of *Dunaliella* sp Isolated from Jepara Waters, in Laboratory Scale. *ILMU KELAUTAN*. 9(3) : 136 – 140


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