MOLECULAR DETERMINATION OF A GREEN ALGAE ISOLATE TO DETECTING 1-Deoxy-D-Xylulose-5-phosphate Synthase (*DXS)* GENE IN IMPROVEMENT OF CAROTENOID PRODUCTION

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Abstrak

Sintesis karotenoid alami belum pernah melebihi produk sintetik pada skala komersial. Kurangnya pemahaman mengenai aspek mikrobiologis dan ekofisiologis isolat penghasil karotenoid menyebabkan terjadinya kesalahan penamaan spesies. Satu isolat lokal alga hijau dari BBAP Jepara yang digunakan sebagai pakan alami sumber karotenoid hewan-hewan perikanan, pada mulanya dianggap sebagai *Dunaliella*. Namun pengembangan produksi karotenoid menggunakan teknologi rekayasa genetik dan rekayasa metabolit terhadap isolat alga hijau lebih lanjut memperlihatkan ketidaksesuaian hasil dengan penamaan yang ada.

Akumulasi karotenoid jalur non-MVA pada alga hijau ditentukan oleh enzim D-1-Deoksixilulosa 5-fosfat Sintase, yang disandi oleh gen *D-1-deoksixilulosa 5-fosfat sintase* (*DXS)*. Determinasi spesies secara molekuler menjadi penting dilakukan untuk menentukan spesies isolat dan jalur biosintesis karotenoid yang digunakan. Hasil determinasi digunakan untuk analisis keserupaan *putative partial fragment* gen *DXS* Isolat alga hijau yang telah berhasil diperoleh pada penelitian sebelumnya. Tujuan utama penelitian ini adalah menentukan spesies satu isolat lokal alga hijau secara molekuler menggunakan 23S rRNA untuk mendeteksi keberadaan gen *DXS* penyandi biosintesis karotenoid.

Hasil penelitian memperlihatkan bahwa Isolat alga hijau menunjukkan keserupaan yang tinggi dengan anggota-anggota Sianobakteria. Keserupaan tertinggi dimiliki dengan *Cyanobacterium* sp. MBIC 1021 sebesar 99 %, diikuti *Synechocystis* PCC6308 sebesar 95 %. Satu-satunya anggota Cyanobacteria yang memiliki gen *DXS* adalah *Synechocystis*. Hasil analisis keserupaan parsial gen *DXS* isolat alga hijau terhadap tujuh parsial gen DXS pada daerah lestari yang telah ditemukan, memperlihatkan bahwa *putative partial fragment* gen *DXS* Isolat lokal alga hijau juga memiliki keserupaan tertinggi dengan gen *DXS* Sianobakteria *Synechocystis*.

Abstract

Carotenoids production levels are not yet competitive with carotenoid levels presently produced by fermentation, synthesis and isolation. It needs application of metabolic engineering and genetic engineering techniques in improving their production. An attempt to optimize carotenoid production from local isolate of green algae from BBAP Jepara has faced several problems, primarily related to the microbiological and eco-physiological characteristic which affecting growth that have not sufficiently been understood. A misnamed of species also have arisen due to wrong characterization. One local isolate of an algal species from BBAP Jepara 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*. Previous studies to improve carotenoid production using molecular approach on have shown unagreement. Therefore, the present study aimed to determinate the species of green algae isolate from Jepara waters based on molecular techniques using 23S rRNA approach for detecting *DXS* gene.

Molecular analysis by 23S rRNA alignment showed the close relationship among isolate of green algae and most all of member of Cyanobacteria. Closest similarities was showed by *Cyanobacterium* sp. MBIC 1021 with 99 % similarity and *Synechocystis* PCC6308 with 95 % similarity. *Synechocystis* was the only member of Cyanobacteria which have DXS gene. Multiples aligment sequences of partial *DXS* gene on the conserve region among seven species confirmed this result. The *DXS* gene analysis also showed closest relationship between partial *DXS* gene of Cyanobacteria *Synechocystis* and a green algae isolate. The result of this analysis proven as valuable parameter for the interpretation of the relation among *DXS* gene of a green algae isolate and Cyanobacteria and increase the possibility in getting the complete *DXS* gene from local isolate of green algae by designing primers from *DXS* gene of *Synechocystis* as a member of Cyanobacteria.

Keywords : a green algae isolate, *Dunaliella*, *DXS* gene, 23S rRNA, Cyanobacteria, *Synechocystis*

INTRODUCTION

Carotenoids, some of which are provitamin A, have range of diverse biological function and actions, such as species specific 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 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).

For many years, it was accepted that carotenoid was synthesized through the well known acetate/mevalonate pathway. However, recent studies have demonstrated that the mevalonate-dependent pathway does not operate in all living organisms. More recently, photosynthetic organisms such as green algae, *Scenedesmus obliquus, Chlorella fusca, Chlamydomonas reinhardii* and higher plants use non mevalonate pathway known as deoxyxylulose 5-phosphate (DXP) pathway for their carotenoid biosynthesis. DXP Synthase gene (*DXS*) was catalyzes a limiting enzyme on DXP pathway. 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, suspected as representing a strain of *Dunaliella,* was found potentially useful as source of carotenoids in food additives or as food supplement in fish farming. This indigenous algae has been successfully isolated, purified and put into axenic culture. Thus, it was of great interest to know if this local isolate of algae would also follow the non-MVA pathway for carotenoid biosynthesis. It was therefore attempted to solve this problem through detection of a *DXS* gene, using a molecular approach. To achieve this, we subsequently used *Polymerase Chain Reaction* (PCR). Further research in detecting *DXS* gene from this "*Dunaliella*", has faced several problems that might be caused by misnamed of the species (Kusumaningrum *et al*., 2004; Kusumaningrum *et al*., 2006). It is apparent that microbial identifications based only on microbiological characterization have, until recently, failed to achieve the necessary requirements of prediction, stability and

objectivity (Priest and Austin, 1993). Therefore, it is important to examine identification of species based on molecular technique using 16S rRNA sequence, for supporting microbiological and eco-physiological characterization. The present study aimed to investigate the species determination of a green algae isolates from Jepara Waters based on 23S rRNA approach.

The genes for 23S rRNAs are particularly suitable as targets for identifying most organisms in delivering objective result. Molecules of RNA are valuable as indicators for identifications of species because the rRNA are essential elements in protein synthesis. Therefore, the rRNA present in all living organisms. The rRNA genes contain both highly conserved sequences and variable regions. The conserved functions of these molecules have changed very little during evolution. Thus, rRNAs from even the most taxonomically distant organisms, that share virtually no DNA sequence homology, will have rRNA sequences in common, and, therefore, relatedness can be assessed (Logan, 1994). Ribosomal RNA is probably unique amongst macromolecules in this respect. Some segments of rRNA evolve more rapidly than others and sequence variation occurs between closely related organisms allowing comparisons to be made at the spesies level. Phylogenetic lines of descent may be inferred from rRNA sequences. The 23S rRNA has been used extensively for comparative sequencing studies (Priest and Austin, 1993;).

MATERIAL AND METHODS

1. Culture Media

The medium artificial sea water (ASW) used was modified from Johnstons (1963) and Quraishi and Spencer (1971) in Bidwell, J.P. and Spotte S. 1983. . ASW media was enrichment solution for *Dunaliella primolecta*. 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 boiling for 10 min while adjusting the pH to 7.6 with HCl or NaOH, filtered and bring to 250 ml. Sterilization was done by autoclaving at 15 lb/in^2 (103 kPa and 120^oC). The medium was using by adding 0.1 ml solution to each 10 mL of seawater. For induction of β-carotene synthesis, cells was grown in a sulfate-free medium ($MgCl_2$ instead of $MgSO_4$). BBM (Bold basal Medium)

was consist of: KH_2PO_4 17.5 g/l; $CaCl_2.2H_2O$ 2.5 g/l; $MgSO_4.7H_2O$ 7.5 g/l; $NaNO_3$ 25 g/l; K_2HPO_4 7.5 g/l; NaCl 2.5 g/l; Na_2EDTA 10 g/l; KOH 6.2 g/l; $FeSO_4.7H_2O$ 4.98 g/l; H_2SO_4 1 ml/l; larutan "Trace Metal" 1 ml/l (H_3BO_3 2.86 g/l; $MnCl_2.4H_2O$ 1.81 g/l; $ZnSO_4.7H_2O$ 0.222 g/l; $NaMoO_4.5H_2O$ 0.39 g/l; $CuSO_4.5H_2O$ 0.079 g/l; $Co(NO_3)_2.6H_2O$ 0.0494 g/l; H_3BO_3 11.5 g/l; agar 1.5 %; pH 6.8.

2. Isolation and purification of an algae isolate

Mix culture of algae were came from BBAP (Balai Budidaya Air Payau Jepara). An isolate of green algae which was suspected as representing a strain of *Dunaliella* was a dominant species. "*Dunaliella*" were isolated and purified by growing in ASW agar media and BBM agar media under high light intensities (1000 lux) treatment using Halogen lamps in the dark room. Single cell colony of "*Dunaliella*" were picked up and grown in 250 ml flasks with 100 ml BBM media under agitation and illumination (Rabbani *et al.*, 1998).

3. DNA Extraction

Preparation of a green algae DNA isolate was carried out by modification of CTAB methods (Sambrook *et al.*, 1989; Ausubel *et al,* 1995). 15 ml Culture of algae were centrifugated 13.000 rpm for 3 minutes. Pellet were pulverized on cold mortar and pestled to a fine powder. 1 ml 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 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, 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 centrifugated 5 min at 13.000 rpm in microcentrifuge. The top (aquaeous) 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 etanol 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 1 hours. DNA was kept on -20 °C or used directly for PCR.

4. Amplification of 23S rRNA of a green algae isolate

The green algae 23S rRNA gene was amplified by PCR using spesific primers. Sequence of forward primer was 5'-CGTCCTTCATCGGCTCTT-3', reverse primer was 3'-CAAGGCATCCACCGT-5' corresponding to base pairs 2024 respectively (Widada, 2005 - pers.com.). PCR was carried out in Ready-to-Go PCR kit by Amersham Inc. containing 50 ng of genomic DNA of a green algae, 1.5 mM of $MgCl_2$, a 0.2 mM concentration of each deoxynucleoside triphosphate, 2.5 pmol of each primer, and 1.8 U of Taq Polymerase and ddH_2O until volume 25 µl.

PCR conditions were performed with *hot start* for 2 minutes at 94 °C, denaturation for 15 second at 94 °C, *annealing* for 15 second at 50 °, polimerization for 45 second at 72 °C, extra extention at 72°C for 2 minutes, with 30 cycles of PCR reactions. In this PCR, a single DNA fragmen of 1.8 kb was amplified.

5. Amplification of partial *DXS* gene on conserve region of a green algae isolate

The amplification of *DXS* gene from a green algae isolate was performed by Polymerase Chain Reaction (PCR) methodology using DNA isolate of green algae as a template. Two strategies were applied in getting *DXS* gene from local isolate of green algae. First, the *DXS* gene was amplified using primer wich designed from *dxs E.coli*. This primers will amplified the whole *DXS* gene about 1863 bp (Lois *et al*., 1998). The second strategy was using primers designed from partial *DXS* gene of green algae fromf six species *Arabidopsis thaliana, Mentha piperita, Synechocystis, Chlamydomonas reinhardtii, Escherichia coli, Streptomyces sp.* and codon usage of *Dunaliella* by CODEHOP Programs (Rose et al, 1997; Kuzuyama, 2000; Pramono 2005-pers.com). This primers will amplified the conserved region of *DXS* gene.

6. Sequencing and Phylogenetic Analysis

The amplification products were sequencing in BPPT and Atmajaya Jakarta. Sequenencing process involves several steps. Fist step was *cycle seq* with PCR methods. The reaction composition consist of DNA tempate, primers, buffer, ddH_2O and *big dye* (DNA polymerase enzyme, ddNTP, and dNTP). Purification was done by adding 5 µl EDTA to DNA template. The next step was addition of 60 µl etanol absolut followed by incubation for 15 minutes at room temperature. Suspension was centrifugated on 6000 rpm at 4 ^oC for 30 menit. Pelet was added with 60 µl etanol 70%then centrifuged on 4000 rpm for 15 menit at 4 ^oC. The pellet was washed with etanol, and air dried. Denaturation step was done with addition of 13 µl bufer *TSR,* vortexed and incubated on 95^oC for 2 menit then quickly chilled on ice. Suspension was runned using ABI Prism 310 *sequencer*.

Sequence of 23S rRNA of a green algae isolate was used to search its homology, process of comparing a new sequence with all other known sequences in the databases. Then attempting to infer the function of the new sequence by assessing the matches and their biological annotations as describe in the database. Sequence analysis was analyzing by similarity (homology). Sequence data was submitted to GenBank website at www.ncbi.nlm.nih.gov and European Bioinformatics Services website at 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 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.

RESULT AND DISCUSSION

a. Species determination of a green algae isolate by 23S rRNA

The result of 23S rRNA nucleic acid sequencing analysis of green algae with all of 23S rRNA in GenBank and European Bioinformatics shows similar result. Analysis by multiple alignment methods revealed a close relationship of a green algae isolate with some member of Cyanobacteria as illustrated from Treeview in Fig. 1.

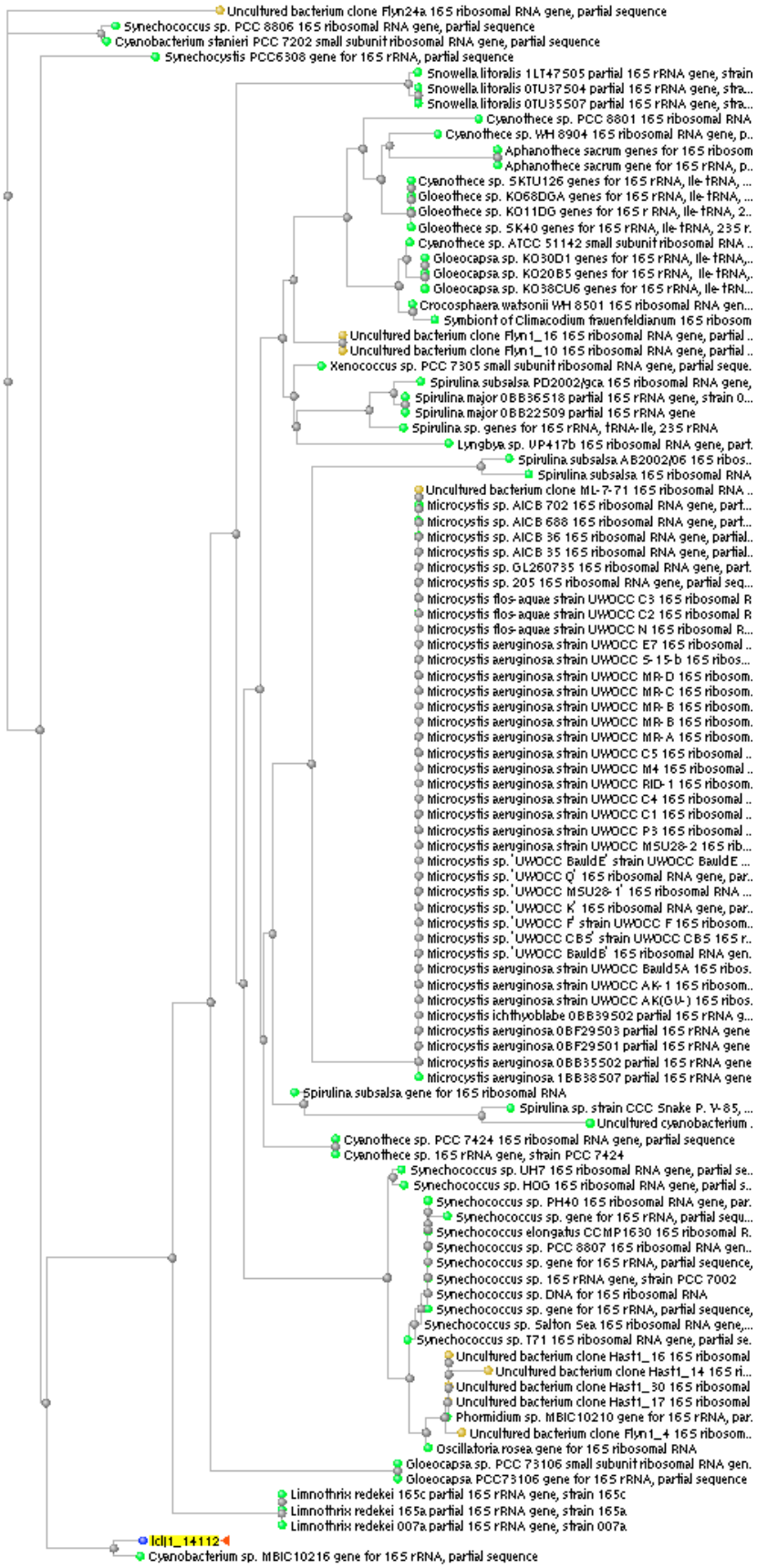

Figure 1. Phylogenetic Tree of a Green Algae Isolate and Cyanobacteria using Treeview Program (Idi1_14112 = a green algae isolate)

The closest similarities was achieved by *Cyanobacterium* sp. MBIC 120 (99%) and *Synechocystis* PCC6308 (95%) as described in Fig. 2 and 3.

```
>gi|24817732|dbj|AB058249.1|  Cyanobacterium sp. MBIC10216 gene for 16S rRNA,
partial sequence
Length=1252

 Score =  613 bits (309),  Expect = 4e-173,  Identities = 315/317 (99%), Gaps = 0/317
(0%) , Strand=Plus/Minus

Query  536   ACTTCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGGATTCACCGCAGTATGC  595
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1252  ACTTCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGGATTCACCGCAGTATGC  1193

Query  596   TGACCTGCGATTACTAGCGATTCCTCCTTCATGCAGGCGAGTTTCAGCCTGCAATCTGAA  655
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1192  TGACCTGCGATTACTAGCGATTCCTCCTTCATGCAGGCGAGTTTCAGCCTGCAATCTGAA  1133

Query  656   CTGTGGCTGGGTTTGATGAGATTCGCTCCACCTCGCGGTTTCGCACCCCTTTGTCCCAAC  715
             |||||||||||||||||||||||||||||||||||| ||||| ||||||||||||||||
Sbjct  1132  CTGTGGCTGGGTTTGATGAGATTCGCTCCACCTCGCGGCTTCGCTCCCCTTTGTCCCAAC  1073

Query  716   CATTGTAGTACGTGTGTAGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCAC  775
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072  CATTGTAGTACGTGTGTAGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCAC  1013

Query  776   CTTCCTCCGAGTTCTCCCCGGCGGTCTCCCTAGAGTCCCCAACTTAATGCTGGCAACTAA  835
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1012  CTTCCTCCGAGTTCTCCCCGGCGGTCTCCCTAGAGTCCCCAACTTAATGCTGGCAACTAA  953

Query  836   GGACGAGGGTTGCGCTC  852
             |||||||||||||||||
Sbjct  952   GGACGAGGGTTGCGCTC  936
```

Figure 2. The multiple alignment analysis result of 23S rRNA sequence of a green algae isolate, Query = a green Algae Isolate, Sbjct = *Cyanobacterium* MBIC10216 (www.ncbi.nlm.nih.gov)

```
>gi|14625357|dbj|AB039001.1|  Synechocystis PCC6308 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   CCTTCCGGTACGGCTACCTTGTTACGACTTCACCCCAGTCACTAGTCCCACCTTCGGCGC  493
             |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1435  CCTTCCGGTACG-CTACCTTGTTACGACTTCACCCCAGTCACTAGTCCCACCTTCGGCAT  1377

Query  494   CTCCCTCCATTA-CGGTTGAGATAACGACTTCGGGCGTGACCAACTTCCATGGTGTGACG  552
             | | |||| | | |||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  1376  CCCTCTCCGTAAACGGTTGAGGTAACGACTTCGGGCGTGACCAACTTCCATGGTGTGACG  1317

Query  553   GGCGGTGTGTACAAGACCCGGGAACGGATTCACCGCAGTATGCTGACCTGCGATTACTAG  612
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1316  GGCGGTGTGTACAAGACCCGGGAACGGATTCACCGCAGTATGCTGACCTGCGATTACTAG  1257

Query  613   CGATTCCTCCTTCATGCAGGCGAGTTTCAGCCTGCAATCTGAACTGTGGCTGGGTTTGAT  672
             |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1256  CGATTCCTCCTTCATGCAGGCGAGTTTCAGCCTGCAATCTGAACTGGGGCTGGGTTTGAC  1197

Query  673   GAGATTCGCTCCACCTCGCGGTTTCGCACCCCTTTGTCCCAACCATTGTAGTACGTGTGT  732
               ||||||||||| |||| |||||||  ||||||||||||||||||||||||||||||||
Sbjct  1196  AGGATTCGCTCCACTTCGCAGTTTCGCCTCCCTTTGTCCCAACCATTGTAGTACGTGTGT  1137

Query  733   AGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCACCTTCCTCCGAGTTCTCC  792
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1136  AGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCACCTTCCTCCGAGTTCTCC  1077

Query  793   CCGGCGGTCTCCCTAGAGTCCCCAACTTAATGCTGGCAACTAAGGACGAGGGTTGCGCTC  852
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1076  CCGGCGGTCTCCCTAGAGTCCCCAACTTAATGCTGGCAACTAAGGACGAGGGTTGCGCTC  1017
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Figure 3. The multiple alignment analysis result of 16SrRNA sequence of green algae isolate (Query = a Green Algae Isolate, Sbjct = *Synechocystis* PCC6803) www.ncbi.nlm.nih.gov

The result of this analysis was a valuable parameter for the interpretation of observation on the identiyng the species of green algae isolate from Jepara Waters. From this analysis, it can be assumed that the species of green algae isolate was the member of Cyanobacteria, and it was not *Dunaliella*. According to Kusumaningrum (1999) based on sequence pair distances among 50 species of genus *Bacillus* from Ribosomal Data Project and GenBank using ClustalW Programs, the distances of intraspecies was range between 91.5% and 99.1 %. The distances of interspecies was range between 66.4 % and 90.27 %. Although a green algae isolate was most similar to *Cyanobacterium* and *Synechocystis* but with similarity result about 95- 99% still need further examination. It is possible that a green alga isolate was different species but still one of the Cyanobacteria member. The microbiological experiment support this result in showing unique characteristics of a green alga isolate based on their major pigment composition (data not shown).

b. Detection of DXS gene based on 23S rRNA determination of a green algae isolate

The PCR result on detecting *DXS* gene of green algae isolate using primers designed from *DXS* gene of *E.coli* showed several bands detected on the gel but the sizes does not similar to *dxs* gene of *E.coli* (data not shown). Although this observation can not clearly demonstrated the spesific fragment, but the result of this experiment could be a valuable parameter in detection of *DXS* gene in an isolate of green algae. It can be assumed that by getting any band with *DXS* primers , there might be a strong possibility that isolate of green algae also contain *DXS* gene which means following non-MVA pathway.

Further experiment using second primer designed from conserved region of DXS gene from six spesies showing positive result. As shown in Fig.4, the result of PCR amplifications were a clear single bands with size that match the size of the partial fragments of *DXS* gene in six species that already submitted in GenBank (www.ncbi.nlm.nih.gov).

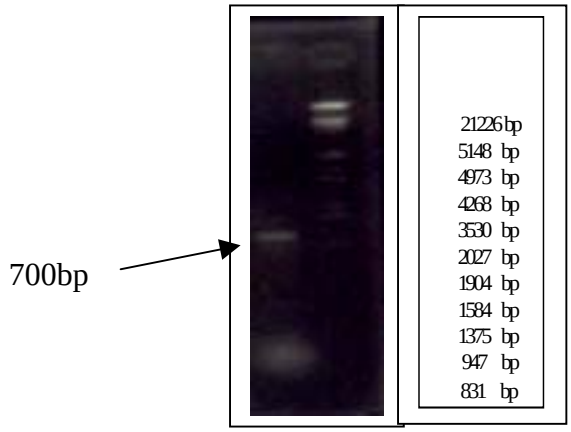

Figure 4. Electroferogram analysis of *DXS* gene from local isolate of green algae . This 700 bp fragment, amplified by PCR methods on conserved region, was loaded from 53 ng DNA on 2% gel agarose. DNA was visualized by staining the gel with ehidium bromide. Arrow on lane 1. shows a band that assumed as partial *DXS* gene on conserved region that present on the isolate of green algae, lane 2. marker λ*Hind*III*Eco*RI

Result of molecular determination using 23S rRNA on a green algae isolate showed close similarities with all of the member of Cyanobacteria. Assuming that a green algae isolate was the member of Cyanobacteria, therefore it was important to examine the position of a green algae isolate partial *DXS* gene in the published and well known *DXS* gene of several species. The *DXS* gene was found in several species including plants, bacteria, Chlorophyta and Cyanobacteria. Among Cyanobacteria, *DXS* gene was found only in *Synechocystis*. Fig 5. was illustrated phenogram tree of several *DXS* gene as a result of multiple alignment analysis by ClustalW Programs.

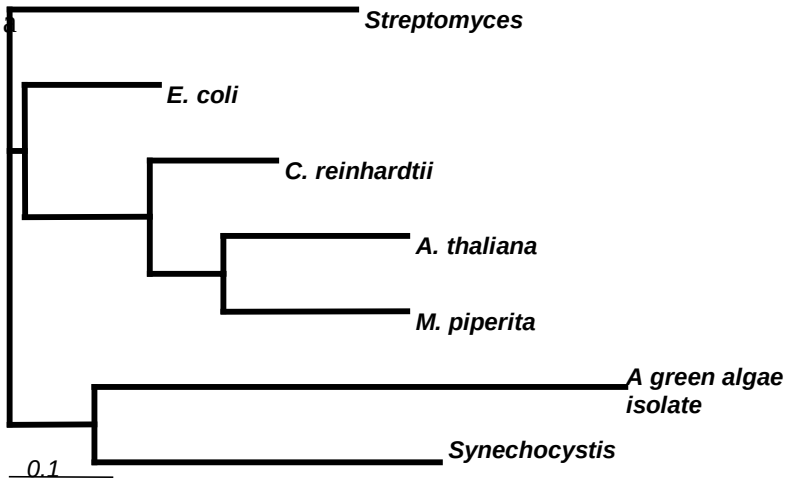

Figure 5. Phylogram of partial *DXS* gene of a green algae isolate among *DXS* gene of several species analyzed by ClustalW Programs

The result shows the closest similarities between partial *DXS* gene on conserved region from an isolate of green algae and partial of *DXS* gene of *Synechocystis*. The result of this experiment could be a valuable parameter in detection of complete *DXS* gene. The difficulty in detecting complete *DXS* gene from green algae isolate may be caused by the primers. The primer used to amplify was not specific for *DXS* gene of green algae isolate. If it is assumed that green algae isolate was true a member of Cyanobacteria, it is possible to detect the whole *DXS* gene of a green algae isolate by using *Synechocystis DXS* gene sequence to design the primer.

Further research will be done by designing new primer based on *Synechocystis DXS* gene, cloning or hibridization using partial *DXS* gene of green algae isolate as a probe on cDNA genomic library.

Conclusion

The results obtained through 23SrRNA based characterization indicated that an algal isolate possesses similarities to Cyanobacteria. The closest similarities was achieved by *Cyanobacterium* sp. MBIC 120 (99%) and *Synechocystis* PCC6308 (95%).

Sequence analysis of putative fragment of a gene encoding a highly conserved region in *DXS* in a variety of species also confirmed the result. High degree of similarities was showed between *DXS* gene of a green algae isolate and *Synechocystis*.

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