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ORIGINAL ARTICLE

Molecular Characterization of β - subunit (*accD* region) of Acetyl - CoA Carboxylase gene in Microalgae

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Abstract:

Acetyl – CoA Carboxylase enzyme is triggering the fatty acid biosynthesis pathway in both prokaryotic and eukaryotic cells. The enzyme consist of 4 subunits among thisBeta Subunit (accD) is the functional subunit of ACCase enzyme, which is present in plastid, transported into cytosol and assembles with other ACCase subunits such as AccA, AccBand AccC.In this study two fresh water microalgae Chlorella vulgaris, Scenedesmusobliquusand two marine microalgae Isochrysis galbana, Nannochloropsisgaditanawere taken for sequencing using designed accD primer based on already exist closest sequence of microalgae in database. Our results, suggests that the microalgae N. gaditana(738 bp)synthesize more fatty acids. It directly indicates the high level expression of accD gene due to faster transcriptional rate because of smaller gene size. The pairwise alignment showedC. vulgaris and N. gaditana had 69% similarity than other pairs.

KEYWORDS:

Acetyl – CoA Carboxylase, Beta Subunit, Primers, Microalgae, Sequence.

INTRODUCTION

The first important committing step in Lipid biosynthesis begins with acetyl-CoA carboxylase (ACC), which catalyzes the biotin-dependant carboxylation of acetyl-CoA to form malonyl-CoA. This enzyme molecular weight about 210 – 250 kDa, which composed of biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC) and Carboxyl transferase (CT) (Kannangara and Stumpf,1972).

Two physically distinct types of enzymes are present in nature. Heteromeric (Consist of four subunits found in prokaryotes) and Homomeric (Single large polypeptide present in eukaryotes). ACCase enzyme composed of four subunits, which are encoded by genes *accA,accB, accC* and *accD* that are located at different positions on the chromosome (Li and Cronan, 1993). Yoshio Kimura, (2000) characterized the carboxyl transferase gene of Myxococcus Xanthus. Acetyl - CoA Carboxylase gene information from different microalgae genera will help to identify various phenotypic changes of the enzymes and to determine their lipid production rate in a near future. Yuji Matsuda, (2005) reported sequence variation of rbcL and *accD* of Moraceae.

The two forms of ACCases play different roles in cellular homeostasis. They include cytosolic ACC1 produces malonyl-CoA for fatty acid synthesis. Second one is mitochondria-associated ACC2 is helped to regulate of fat β-oxidation pathway by inhibiting carnitinepalmitoyl transferase-1 (CPT-1) which controls the long chain fatty acyl-CoA transport into mitochondria. Hence, the two isoforms of ACCases play opposite roles in cellular energy homeostasis (Jan Podkowinski and AleksanderTworak, 2011).

Today, the focus on biofuel production from fatty acid of organisms has an interesting attention due to fuel demand. Cyanobacteria are the evolutionary ancestors of plant plastids, Therefore FAS

machineries of both are similar. Genetically modified cyanobacteria were made to produce desired free fatty acids (FFA) that are very similar to plant fatty acids (Liu *et al.*, 2011). Genetically altering or engineering the gene sequences of the ACCase for production of our product of interest such as desired carbon chain length and their chemical properties may be adjusted according to further needs. It is considered that the over expression of ACCase gene may enhance lipid production significantly. Several other metabolic engineering studies to improve TAG production in algae did not result in markedly better outcomes (Sheehan *et al.*, 1998). It is concluded that the molecular level database of the lipid producing genes are necessary to improve the genetic engineering methods for altering and produce more fatty acid for biodiesel production.

In this present study, we have designed primers for accD region of microalgalacc gene amplification. Indigenous microalgae strains *Chlorella vulgaris, Scenedesmus obliquus, Isochrysis galbana* and *Nannochloropsis gaditana* acc1 (Acetyl – CoA Carboxylase) genes were isolated and sequenced for characterizing the lipid productivity of above mentioned microalgae.

MATERIALS AND METHODS

Microalgae

Chlorella vulgaris, Scenedesmusobliquus, Isochrysisgalbana and Nannochloropsisgaditana strains were collected from fresh and brackish water lakes in Tamil Nadu coastal region, India. Species level identification were done by using 28LSU (D1-D2) region sequencing.

Primers and PCR Amplification

The Doyle & Doyle CTAB method (1990) was followed for isolation of DNA from microalgae cells. Polymerase chain reactions (PCR) were performed, the total reaction mixture consist of 10–50 ng/µl template DNA, 5.0 mM of each primer, 200 mMdNTPs (Promega), 0.5 µl Taq DNA polymerase and 10 µl 5M reaction buffer with 2.5 mM $MgCl_2$, the final volume should be 50 µl with sterile distilled water. The Primer sequences were designed using Primer3 software tool was used to design the primers and PCR conditions for each microalgae is represented in Table 1. About 800 – 1,200 bp for the region *accD*of acc1 gene was observed agarose gel electrophoresis (Fig. 1). Positive and negative control were included in the PCR.

Table 1. Lists of Primers and PCR Conditions

Sample Name	Primer Name	Primer Type	Primer Sequence	PCR Conditions
C. vulgaris **(SE002)**	f_cv	Forward	TACCCGTATAACAGGACCTCC	35 amplification cycles at 94°C for 60sec, 60°C for 60 sec, and 72°C for 120 sec
	r_cv	Reverse	CAGGTTTATTCTTCGAAGCC	
A. obliquus**(PRR02)**	f_ao	Forward	TAACGATACCTTTGAACCTA	35 amplification cycles at 94°C for 60sec, 62°C for 60 sec, and 72°C for 180 sec
	r_ao	Reverse	AACCAGCTTGACAATTAAGC	
I. galbana**(SEISO)**	f_ig	Forward	ATGTACGTGGATCTTCAGCTACA	35 amplification cycles at 94°C for 60sec, 62°C for 60 sec, and 72°C for 180 sec
	r_ig	Reverse	TCACTACGCTCAAGCCTACCG	
N.gaditana**(SE006)**	f_ng	Forward	TCCTAAGACGAATCATTCTGC	35 amplification cycles at 94°C for 60sec, 60°C for 60 sec, and 72°C for 90 sec
	r_ng	Reverse	TAGCGATAAGCAGGGTTAGG	

DNA Sequencing

Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM® BigDye TM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). Ethanol precipitation protocol was followed for purification of fluorescent-labelled fragments. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). The purified PCR products of approximately 800 - 1200 bp were sequenced by using primers mentioned in Table 1. Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). Table. 2 showed the genbank accession number of accD sequence of tested microalgal strains.

Table 2. Acetyl-CoA Carboxylase β-subunit size and accession Numbers

Microalgae	Size of DNA fragment	Accession Number (NCBI)
***C. vulgaris* (SE002)**	1146	KC584876
***A. obliquus*(PRR02)**	944	KC584877
***I. galbana*(SEISO)**	840	KC584875
***N. gaditana*(SE006)**	738	KC584874

Sequence Analysis

Similarity values obtained after pair wise alignment of the *accD* sequence of isolates using CLUSTAL Version 2.1 software tool(C. *vulgaris* SE002, A. *obliquss*PRR02, *I. galbana* SEISO, and *N. gaditana*SE006 were represented in (Fig 2). The results showed similarity between isolated genera due to the conserved nature of the gene.

RESULTS AND DISCUSSIONS

Isolated, screened unialgal microalgal cultures *accD* conserved regions of acc1 gene were amplified and sequenced. The expression of *accD* gene was studied in *C. vulgaris, A. obliquus, I. galbanaand N. gaditanaby* Real time PCR during stationary phase of the culture. Fig 1. Showed PCR amplification of *accD* from C. vulgaris, A. obliquus, I. galbana and N. gaditana . The length of accD region varied among these microalgae, *C. Vulgaris* had the sequence length of 1146bp, *A. obliquus* 944bp, *I. galbana*840bp and *N. gaditanashowed* 738bp. The expression and sequence pattern of *accD* region in three microalgae were compared with corresponding to lipid content. The *accD* sequence was confirmed using 'algaeblast' tool (www.genome.jgi.doe.gov)

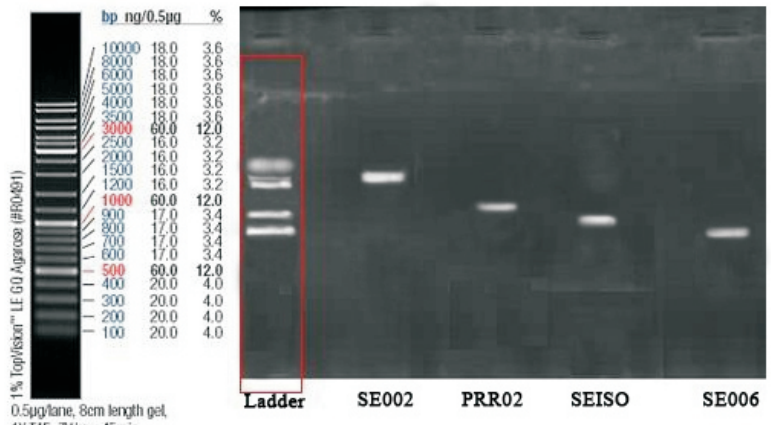

Figure 53: PCR amplification of *accD* from 1) DNA Ladder - 10kb 2) *C. vulgaris* showed large band size 1146bp band 3) *A. obliquus* showed 944bp band 4) *I. galbana* showed 840bp 5) *N. gaditana* showed 738bp band

Acetyl CoA Carboxylase is an enzyme that catalyzes the carboxylation of acetyl – CoA to malonyl – CoA. This is the first committed step of fatty acid synthesis in most of the organisms, and it acts as a universal precursor for various high value substances. In common unicellular eukaryotic microalgae the enzyme ACCase contain 4 subunit such as BCCP (Biotin Carboxyl Carrier Protein), BC (Biotin Carboxylase) and α- and β - subunit of CT (CaboxylTransferase). The expression of coding β-subunit of Carboxyltransferase in the chloroplast is crucial to the level of heteromeric ACCase (Nakkaew*et al*., 2008).

The pair wise alignment showed *C. vulgaris* and *A. obliquushad* similarity of 65% and *C. vulgaris* and *I. galbana* had 66% whereas *C. vulgaris* and *N. gaditana* had 69% but in the case of *A. obliquus* with *I. galbanashowed* 64% similarity, *A.obliquus* with *N. gaditana* had 65% and *I.gaditana* with *N. gaditanashowed* 57 % similarity (Fig. 2) These results suggested *C. vulgaris* and *N. gaditana* have close similarity in *accD* region sequence

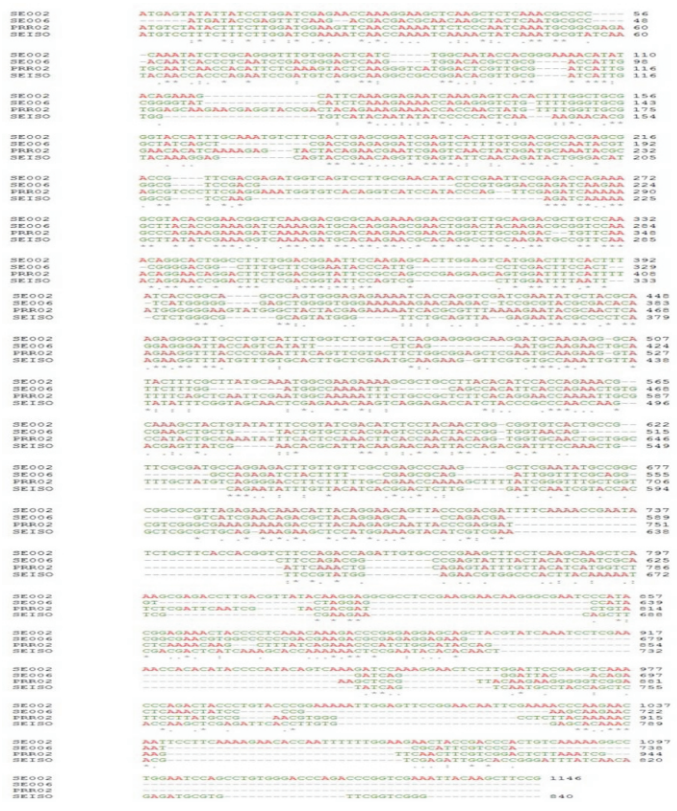

Figure 2. Alignment of *accD* sequences of *C.vulgaris* (SE002), *A.obliquus* (SE006), *I. galbana* (SEISO) and *N. gaditana* (PRR02). Asterisks (*), Period (.) and Colon (:) mark nucleic acids that are identical and conserved, respectively. The stop codon (TAA) and initiation codon (ATG) are underlined and double-underlined, respectively.

In our present work, *I. galbana* have higher level of *accD* gene expression, it reflected in lipid production in stationary phase. The length of *accD* region varied among these microalgae, *C. vulgaris* had the sequence length of 1146bp, *A. obliquus* 944bp, *I. galbana* 840bp and *N. gaditana* showed 738bp. The expression and sequence pattern of *accD* region in four microalgae were compared with corresponding to lipid content. Our results, suggests that the microalgae *N. gaditana* synthesize more fatty acids. It directly indicates the high level expression of *accD* gene. Beta Subunit (*accD*) is the functional subunit of ACCase enzyme, which is present in plastid, transported into cytosol and assembles with other ACCase subunits such as *accA, accC and accC.*

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