Research Paper

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Nucleotide Sequencing and Analysis of β – Subunit (accD region) of Acetyl-CoA carboxylase gene isolated from Nannochlropsis salina, Dunaliella tertiolecta and Tetraselmis suecica

Biology

KEYWORDS : acc gene, Acetyl CoA Carboxylase, Beta Subunit, Microalgae, Lipids, Sequence

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ABSTRACT

We have designed primers for β – Subunit (accD region) of Acetyl-CoA carboxylase gene isolated from indigenous strains of Nannochlropsis salina, Dunaliella tertiolecta and Tetraselmis suecica. The primers were designed based on closest microalgal sequences that exist in database. Three subunits of ACCase were encoded by a nuclear genome and accDregion is encoded by plastid genome. It is the functional and conserved region of acc (Acetyl-CoA Carboxylase) gene. In our studies we observed, Nannochloropsis salina had 866 bp of accD region which is smaller than Dunaliella tertiolecta and Tetraselmis suecica. Hence the transcriptional rate of accD gene in N. salina much faster than other microalgae; it directly influences the fatty acid anabolic pathway. The pairwise alignment showed N. salina and T. suecica has 60% than other pairs.

Introduction

Currently many laboratories have interest to develop renewable fuels from biological sources. Microalgae are the promising renewable fuel among others. Lipids are an essential component of all living organisms. Biochemical studies have suggested that acetyl-CoA carboxylase (ACCase), a biotin-containing enzyme that catalyzes an early step in fatty acid biosynthesis, involved in the control of this lipid accumulation process.

Carbons of all fatty acids are derived from the pool of acetyl-Co enzyme A (CoA) present in the plastid. The concentration of acetyl-CoA in chloroplasts in only 30 to 50 μM.In 1972, Kannangara and Stumpfirstly reported that the spinach ACCase resembles the *E. coli* enzyme. After that purification of the homomeric form of ACCase from rat and yeast were done by Tanabe *et al.,* 1975 and from plants (Harwood, 1988). Stump discovered two isomers of ACCase, a heteromeric form from plastids and homomeric form cytosol.

Acetyl-coenzyme A carboxylase (700 kDa) recognized as an essential enzyme for all Eukaryotes and Prokaryotes. Sequence data from all subunits of the prokaryotic type, plastidicACCase - biotin carboxylase, biotin carboxyl carrier protein, α-carboxyl transferase (CT), and β-CT are available from different algae as well as their biochemical characterizations. The corresponding gene sequence for Accase domains were named as *accA, accB, accC, accD*, that encodes carboxyl transferase α - subunit, BCCP, biotin carboxylase, and carboxyl transferase β – subunit respectively.

Comparison of the genomic nucleotide sequence to the sequences of cDNA clones has revealed the presence of two introns in the gene. It is considered that the over expression of ACCase gene may enhance lipid production significantly (Sheehan *et al.,* 1998). Therefore, it concluded that the molecular level database of the lipid producing genes is necessary to improve the genetic engineering methods to produce more fatty acids.

In our present study, *Nannochloropsis salina, Dunaliella tertiolecta* and *Tetraselmis suecica acc1* genes were isolated and sequenced the *accD* region (β – Subunit) of Acetyl-CoA carboxylase enzyme which triggers the fatty acid biosynthesis pathway. It could be most powerful molecular tool to choose the best lipid producing indigenous microalgae strain.

Materials and Methods

Microalgae Samples

Indigenous marine microalgae strains such as *Nannochloropsis salina* (JX415512), *Dunaliella tertiolecta* (KC415758) and *Tetraselmis suecica* (KC415759) were collected from Pulicat and Muttukadu brackish water lake, Chennai, Tamil Nadu coastal region, India and the strains were identified by LSU (D1-D2) region sequencing (Anna Godhe *et al.,* 2002).

Preparation of Template DNA

Pure Microalgae culture was taken at stationary phase of growth and suspended in 0.5 μl of sterile saline and centrifuged at 10,000 rpm for 10 min. The pellet is suspended in 2 % CTAB buffer. The Doyle & Doyle CTAB method (1990) was employed for isolation of DNA from microalgae.

Primers and PCR Amplification

Polymerase chain reactions (PCR) were performed in an FTS-1 Thermal Sequencer. The reaction mixture contained 10–50 ng/μl template DNA, 5.0 mM of each primer, 200 mM dNTPs (Promega), 0.5 μl Taq DNA polymerase and 10 μl 5M reaction buffer with 2.5 mM $MgCl_2$, made up to a final volume of 50 μl with sterile distilled water. The Primer sequences were designed using Prime 3 software tool and PCR conditions for each microalgae is represented in Table 1. DNA fragments are amplified about 800 – 1,200 bp for the region *accD* of *acc1* gene. Positive and negative control was included in the PCR.

Table. 1 Lists of Primers and PCR Conditions

Sample Name	Primer Name	Primer Type	Primer Sequence	PCR Conditions
N. salina PRR13	F_NS	Forward	TCCTAAGACGAATCATTCTGC	35 amplification cycles at 94°C for 60sec, 60°C for 60 sec, and 72°C for 90 sec
	R_NS	Reverse	TAGCGATAAGCAGGGTTAGG	
D.tertiolecta PL1	F_DT	Forward	TAGGAAGATGAGTTTACTCG	35 amplification cycles at 94°C for 60sec, 55°C for 60 sec, and 72°C for 180 sec
	R_DT	Reverse	TGGAATTGAAGGTGTAAGAG	
T. suecica MK1	F_TS	Forward	AACGGTGATGAACGGACCTA	35 amplification cycles at 94°C for 60sec, 55°C for 60 sec, and 72°C for 180 sec
	R_TS	Reverse	AGGCTTGTGTGACATTGATC	

DNA Sequencing

Single-pass sequencing was performed on each template using different *accD* primer. The fluorescent-labelled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The purified PCR products of approximately 800 - 1200 bp were sequenced by using 2 primers as described in Table 1. Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). The consensus sequences of *accD* region were obtained using BioEdit (Biological Sequence Alignment Editor) Version 7.9.1 (Hall, 1999) from DNA chromatogram files.

The conserved functional region *accD* (β - subunit) of Acetyl - CoA Carboxylase gene from screened native microalgal strains (*Nannochloropsis salina, Dunaliella tertiolecta* and *Tetraselmis suecica*) were amplified and sequenced. The gene sequences were submitted to GenBank and accession numbers were presented in Table 2

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

Microalgae	Size of DNA fragment	Accession Number (NCBI)
N. salina PRR13	866	KC572137
D. tertiolectaPL1	925	KC572136
T. suecica MK1	1150	KC572138

Data Alignment and Analysis

Similarity values obtained after pair wise alignment of the *accD* sequence of isolates using CLUSTAL Version 2.1 software tool(*N. salina* PRR13, *D. tertiolecta* PL1 and *T. suecica* MK1) were represented in Fig2.The result shows the similarity between isolated genera due to the conserved nature of the gene.

Results and Discussion

In the present work the isolated, screened unialgal cultures, *accD* domain of *acc1* gene were amplified and sequenced. The expression of *accD* gene was studied in *N. salina, D. tertiolecta*and *T. suecica* by Real time PCR during stationary phase of the culture. Fig 1 shows PCR amplification of *accD* from *N. salina, D. tertiolecta* and *T. suecica.* The length of *accD* region varied among these microalgae. *N. salina* had the sequence length of 866bp, *D. tertiolecta* 925bp and *T. suecica* showed 1150 bp.

The *accD* sequence was confirmed using 'NCBI BLAST' tool. It showed the amino acid sequence closely related to β - Subunit of *acc* gene of *Chlorella vulgaris* (AB001684), *C. variablis* (NC015359),*Chlorococcum humicola* (JF451126) and *Dunaliella salina* (EF363909). *N. salina* had 39% similarity with *C. vulgaris* and *D. Tertiolecta* matched 48% with *D. salina.* But *T. suecica* have similarity of 86% with *C. variablis.* The expression and sequence pattern of *accD* region in the three microalgae were compared which corresponding to lipid production. Beta subunit genes were sequenced and deposited in Genbank, the accession numbers were KC572137, KC572136 and KC572138 for *N. salina* PRR13, *D. tertiolecta* PL1 and *T. Suecica* MK1 respectively.

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 microalgal enzyme ACCase contains 4 subunit such as BCCP (Biotin Carboxyl Carrier Protein), BC (Biotin Carboxylase) and α- β - subunit of CT (Caboxyl Transferase). The expression of coding β-subunit of Carboxyltransferase in the chloroplast is crucial to the level of heteromeric ACCase (Nakkaew*et al.,* 2008).

The three subunits of ACCase were encoded by a nuclear genome and remaining one subunit is encoded by plastid genome (*accD*). Generally cells having 2 copies of a nuclear genome and more than 500 copies of a plastid genome. The copy number of *accD* gene in a cell is entirely different from that of the remaining 3 genes. The zinc finger motif $CX_2CX_{13\text{-}15}CX_2C$ is the conserved sequence in all the β-CT of the ACCase. Deletion of this motiff resulted in loss of activity which directly affected lipid synthesis pathway (Kozaki *et al.,* 2001). Therefore, the yields of TAGs are determined by*accD* active motiff. These conserved natures of sequences are also very much useful for phylogenic characterization of microalgae.

The Chloroplast genome of *Chlorella vulgaris* (JF451126), *Chlorella variablis* (NC015359) and *Chloroccocum humicola* are already sequenced for *accD* region. Our sequence results exactly matches with the above mentioned microalgae with varying percentages such as *N. salina* matches 39% with *C. vulgaris* (JF451126), *D. tertiolecta* 48% with *D. salina* (EF363909) and *T. suecica* has 86% similarity with *C. variablis* (NC015359).

Acetyl-CoA Carboxylase gene was upregulated during the stationary phase of microalgal growth. It denotes the expression of *accD* gene; it is the functional unit of ACCase enzyme. Compared with *D. tertiolecta* and *T. suecica, N. salina accD* gene expression level was higher, it reflected in lipid production during stationary phase. The substrate acetyl – CoA mainly involved in fatty acid synthesis will also act as a precursor for other biomolecule production,

The pair wise alignment showed *N. salina* and *D. tertiolecta* has similarity of 41%, *N. salina* and *T. suecica* has 60% whereas *D. tertiolecta* and *T. sueica* has 45%. These results suggestes *N. salina* and *T. suecica* have close similarity in*accD* region sequence (Fig 2).

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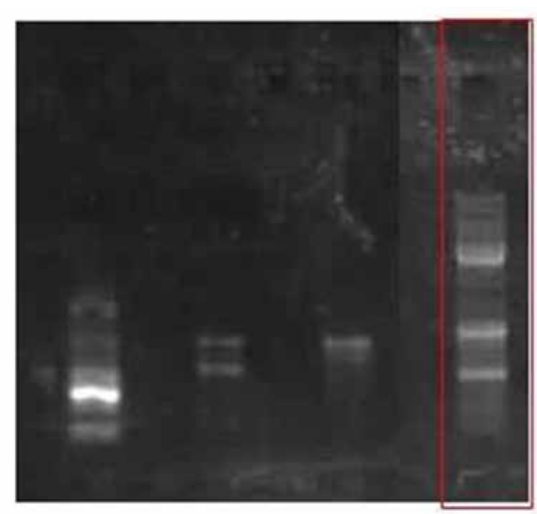

Fig 1.PCR amplification of *accD* from 1) *N. salina* showed 800bp band 2) *D. tertiolecta* touched nearly *900bp*band 3) *T. suecica* had large band size of 1200bp. 4) DNA Ladder - 10kb

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Fig 2. Alignment of accD sequences of N. salina(NS), D. tertiolecta(DT) and T. suecica(TS). Asterisks (*), Period (.) and Colon (:) mark nucleic acids that are identical and conserved, respectively.

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