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ISOLATION AND UTILIZATION OF ACETYL-CoA CARBOXYLASE FROM OIL PALM (*Elaeis guineensis*) MESOCARP

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ABSTRACT

*One of the targets of the MPOB oil palm genetic engineering programme is to synthesize biodegradable plastics. Biodegradable plastics were first discovered in bacterial systems. Polyhydroxybutyrate (PHB), the most common biodegradable plastic, is synthesized from acetyl-CoA by the sequential action of the following three enzymes: β-ketothiolase (*phbA *or* bktB*), acetoacetyl-CoA reductase (*phbB*) and PHB synthase (*phbC*). Acetyl-CoA is also the main substrate for fatty acid synthesis, where acetyl-CoA carboxylase (ACCase) catalyses the conversion of acetyl-CoA to malonyl-CoA, the building block for fatty acid synthesis. Down-regulating ACCase could divert the central metabolite acetyl-CoA to higher value products such as PHB. In this study, efforts were made to isolate both the multifunctional form of ACCase and biotin carboxylase (BC), a component protein of the multisubunit form of ACCase. Initially, reverse transcriptase polymerase chain reaction (RT-PCR) using degenerate primers designed based on the conserved region of plant biotin carboxylase gene was used to amplify a partial length of the oil palm cDNA. This was then used for further isolation of the full length cDNA by random amplification of cDNA ends (RACE), followed by end-to-end PCR. The RT-PCR was similary used to isolate a partial length multifunctional ACCase employing degenerate primers designed based on conserved regions of plant ACCase. After confirmation through sequencing and cross-reference with gene bank, the partial length cDNA of multifunctional ACCase was incorporated in an intervention strategy, where the cDNA was added in the antisense orientation into existing PHB and PHBV transformation vectors driven by an oil palm mesocarp specific (MSP1) promoter. It was envisaged that by down-regulating the activity of ACCase, fatty acid biosynthesis activity will be reduced and thus the acetyl-CoA pool diverted to production of PHB and PHBV. The resulting vectors were later transformed into oil palm embryogenic calli using the Biolistics™ approach. After selection on medium containing the herbicide Basta, resistant colonies were isolated and are currently undergoing regeneration into full plants.*

Keywords: acetyl-CoA carboxylase, gene isolation, biotin carboxylase, biodegradable plastics, oil palm.

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INTRODUCTION

Oil palm is an agroeconomically important crop valued for its ability to produce large amounts of oil in its fruit mesocarp and kernel. One of the research areas in the oil palm biotechnology programme at MPOB is to understand the carbon flow during lipid biosynthesis. This would enable re-channeling of the carbon source through genetic engineering for

production of higher value products. One potential class of products is polyhydroxyalkanoate (PHA), commonly known as bioplastics.

In bacteria, PHB, the most common PHA is synthesized from acetyl-CoA by the following three steps: condensation of two molecules of acetyl-CoA to yield acetoacetyl-CoA by β-ketothiolase (*phbA* or *bktB*), reduction of acetoacetyl CoA to (R)-3-hydroxybutyryl-CoA by acetoacetyl-CoA reductase (*phbB*) and polymerization of (R)-3-hydroxybutyryl-CoA to PHB by PHB synthase (*phbC*) (Anderson and Dawes, 1990). The co-polymer, polyhydroxybutyrate-co-valerate (PHBV), is a more economically useful PHA which also requires propionyl-CoA as substrate. The enzyme *threonine dehydratase* encoded by *tdcB* is required for production of propionyl-CoA. Acetyl-CoA is present in cytosol, plastids, mitochondria and peroxisomes of plants cells. Therefore, theoretically, PHA could be synthesized in any of these sub-cellular compartments (Poirier, 2002).

Acetyl-CoA carboxylase is one of the key enzymes that control the carbon flux into lipids in plants (Page *et al*., 1994). It catalyses the conversion of acetyl-CoA to malonyl-CoA, the first committed step of fatty acid synthesis. Malonyl-CoA is the source of two carbon addition units required both for fatty acid initiation and fatty acid elongation. Down-regulation of acetyl-CoA carboxylase in oil palm, coupled with the introduction of PHA biosynthetic genes could maximize the synthesis of PHA in oil palm tissues (Sambanthamurthi *et al*., 2002).

Acetyl-CoA carboxylase is considered the most important enzyme for primary enzyme regulation of fatty acid synthesis. There are several isoforms of acetyl-CoA carboxylase genes reported in the literature. Four different protein domains are present in the active acetyl-CoA carboxylase holoenzyme. These include biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP) and two transcarboxylase domains. These domains may be present in a single protein, in which case the enzyme is multifunctional. It is also possible for each domain to be present as a separate protein in the holoenzyme, *i.e.,* in the multisubunit form.

A previous study reported the use of radioassay to measure acetyl-CoA carboxylase activity in oil palm cell cultures (Ramli, 1999). Activity was also detected using slices of fresh oil palm mesocarp tissue (Sambanthamurthi, unpublished results). The results suggested that acetyl-CoA carboxylase activity in the mesocarp is highest at 20 weeks-after-anthesis (WAA).

This study aimed at isolating the genes encoding oil palm acetyl-CoA carboxylase. We utilized plant acetyl-CoA carboxylase gene sequences obtained from GenBank to identify highly conserved regions in the multifunctional and biotin carboxylase of the multisubunit enzyme. Degenerate oligonucleotide primers were designed based on these conserved regions. RT-PCR, was initially used to isolate partial fragments of acetyl-CoA carboxylase genes (multifunctional ACCase and biotin carboxylase) from oil palm mesocarp. Full length biotin carboxylase (BC) was then obtained by RACE followed by end-to-end PCR. The partial length multifunctional ACCase was later used in an intervention strategy, where the antisense gene was added into existing PHB and PHBV transformation vectors driven by an oil palm mesocarp specific (MSP1) promoter. This was carried out with the objective of down-regulating the activity of ACCase to reduce fatty acid biosynthesis activity and directing the acetyl-CoA to the production of PHB and PHBV. The new vectors were later transformed into oil palm embryogenic calli.

MATERIALS AND METHODS

Isolation and Sequence Analysis of cDNA Clones of Acetyl-CoA Carboxylase Genes

Primer design. Degenerate synthetic primers were designed based on the highly conserved regions of plant BC and ACC (multifunctional) available in the database. Primers used in this study are shown in *Tables 1a* and *1b*.

TABLE 1a. PRIMERS FOR RT-PCR REACTIONS FOR BIOTIN CARBOXYLASE (BC)

Gene code	Primer sequence	Peptide seq.
AccC (F)	5′-CA(CT)GG(AGCT)AT(ACT)AA(CT)TT(CT)AT(ACT)GG -3′	HGINFIG
AccC (R)	5′-GT(AG)TTCAT(CT)TCCAT(AG)AA(AG)TA -3′	YFMEMNT

TABLE 1b. PRIMERS FOR RT-PCR REACTIONS FOR MULTIFUNCTIONAL ACCase

Gene code	Primer sequence	Peptide seq.
ACC (F)	5′-CA(CT)CA(AG)AA(AG)AT(ACT)AT(ACT)AT(ACT)GA(GA) -3′	HQKIIEE
ACC (R)	5′-GT(AG)TTCAT(CT)TCCAT(AG)AA(AG)TA -3′	EVEVMKM

RT- PCR. Reverse-transcriptase PCR (RT-PCR) reactions were performed using RNA isolated from the mesocarp at 19 WAA. A single step RT-PCR reaction was carried out using GeneAmp® Gold RNA PCR Reagent Kit (PE Biosystems) according to the method recommended by the supplier. The cycle parameters were programmed at 42°C for 20 min, 95°C for 10 min, 94°C for 1 min, 43-45°C for 2 min, 72°C for 3 min and 72°C for 7 min. Steps 3-5 were repeated for 43 cycles. Amplification was carried out using a PTC-200 thermal cycler (MJ Research) in a 20 µl reaction mixture containing 0.3 µM primers and 1.5 µl (0.3 µg μl^{-1}) RNA with annealing temperature set at about 5°C below the primer T_m. PCR products were analysed by agarose gel electrophoresis. PCR products were cloned using TOPO TA cloning vector (INVITROGEN) and sequenced using M13 forward and reverse primers.

Rapid amplification of cDNA ends (RACE) experiments. Rapid amplification cDNA ends (RACE) was carried out to amplify the 3′ and 5′ end sequences of the biotin carboxylase gene using primers designed based on the PCR product amplified by RT-PCR. Gene-specific primers used in this experiment are shown in *Table 2*. RACE-ready cDNA template was prepared from 19 WAA oil palm mesocarp RNA using SMART RACE cDNA amplification kit (Clontech). PCR amplification was carried out on a PE 9600 PCR machine in a 25 µl reaction mixture containing 10 µM primers and RACE-ready first-strand cDNA with annealing temperature set at 3°C-5°C below the primer T_m.

Amplification of full length cDNA clone of biotin carboxylase from oil palm. End-to-end PCR was performed to amplify full length cDNA of BC from oil palm using primers designed based on the sequence information from the RACE experiments (*Table 3*). The PCR was programmed at 94°C for 5 s, 72°C for 3 min, 94°C for 5 s, 71°C for 10 s, 72°C for 3 min, 94°C for 5 s, 68°C for 10 s and 72°C for 3 min. Steps 1-5 were repeated for five cycles and steps 6-8 for 27 cycles. PCR products were cloned into pCR-TOPO 2.1 (TOPO TA Cloning® kit, Invitrogen) cloning vector for sequence analysis.

Cloning and sequence analysis. Plasmid DNA was digested using *Eco*RI for insert verification. Big Dye Terminator sequencing reagent and an ABI 377 DNA sequencer, Applied Biosystem, Foster City, CA (MIT Biopolymer Laboratory), were used to determine the nucleotide sequence of the fragment. Sequences were analysed using Blastx search, GenBank (www.ncbi.nlm.nih.gov), EditSeq and MegAlign (LaserGene).

DNA manipulation. Clones carrying the multifunctional ACCase gene used are listed in *Table 4*. DNA manipulations were carried out using standard protocols (Sambrook *et al*., 1989). Plasmid DNA isolation was carried out using the Plasmid

TABLE 2. GENE SPECIFIC PRIMERS FOR RAPID AMPLIFICATION OF cDNA ENDS (RACE)

Primer	Primer sequence
5′ end	5′ - CAC TTT CAG GTA CAG TCG GGA CGC CTG C – 3′
Acc3a	5′ - GAG CAT GGA ATC AAC TTT ATC GGG CCA A – 3′
Acc3b (nested for Acc3a)	5′ – GGG CCA AAT CCT AAC AGC ATT CGA GTC A – 3′

TABLE 3. GENE SPECIFIC PRIMERS FOR END-TO-END PCR

Primer	Primer sequence
Acc3 (F)	5′ – CAT CTC TTC TCC TCC CCT CTA ATC CTT CGA– 3′
Acc9 (R)	5′ – CAT AGC ACT TAT TTC ACG GGC TCG AAG TCA – 3′

TABLE 4. PLASMIDS USED IN THIS STUDY

Plasmid	Description	Reference or source
pWSI	Carrying coding region of oil palm *ACCase* gene	MPOB
pFC2	Cloning vector with multiple cloning sites of pBlueSkript SK- flanked by unique rare cutter enzymes sites for cloning useful genes	Lonsdale *et al*. (1995)
pMSP3	Carrying the *MSP1Tp-Nos* cassette	MPOB
pMS29	PHB expression vector driven by MSP1 promoter	MPOB
pMS31	PHBV expression vector driven by MSP1 promoter	MPOB

Mini Kit (QIAGEN). Fragments generated by restriction endonuclease digestions were separated on agarose gel and purified by QIAquick Gel Extraction Kit (QIAGEN). Sticky-end ligations were carried out using T4 DNA ligase from Research Biolabs. Blunt-end ligations were carried out by filling the cohesive ends in a reaction volume using 2.5 units of the Platinum *Pfx* DNA polymerase (Invitrogen), 1 mM dNTPs mixture, 1X *Pfx* amplification buffer, 1 mM $MgSO_4$ followed by incubation at 72°C for 30 min. *Escherichia coli* strain DH5α and strain STBL4 (Life Technologies) were used for cloning experiments.

PCR amplification for DNA manipulation. PCR was carried out in a 25 µl volume containing 100 ng plasmid DNA, 10 µM dNTPs mixture, 1.75 units High Fidelity PCR System (Roche). Buffer solution contained 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl (pH 8.75), 20 mM $MgSO_4$, 1% Triton X-100, 1000 µg BSA ml^{-1} supplemented with 1 µM respective primers. The primers were based on the sequence of the gene of interest and contained convenient cloning sites. Amplification reactions were carried out in a MJ Research Inc. Programmable Thermal Controller (PTC-100™ or PTC-200™). The amplified DNA fragments were later digested with the appropriate restriction endonucleases and cloned into pFC2 (Lonsdale *et al*., 1995) cloning vector for DNA sequencing and further cloning procedures.

Vector construction. The primers ACC1-F (5'-GCGGCGCGCCAAGATTATTGAGGAG-3') and ACC1-R (5'-GCGGCGCGCCGTTTGCAAGGTGTAT-3') were used to amplify the oil palm ACCase coding region flanked by *Asc*I (underlined sequences in primers ACC1-F and ACC1-R) using plasmid pWSI as template. The PCR product was digested with *Asc*I, gel purified and ligated overnight at 16°C into the *Asc*I site of pFC2. The positive clones designated as pACC were selected by screening the clones with *Asc*I. The *Asc*I DNA fragment from pACC was cloned into pMSP3 for addition of the MSP1 promoter at the 5' end, transit peptide and NOS terminator at the 3' end of the ACCase gene and designated as pACCaseMSP1. The pACCaseMSP1 was later inserted into PHB (pMS29) and PHBV (pMS31) transformation vectors for constructing new PHB (pMS32) and PHBV (pMS33) transformation vectors with an addition of antisense ACCase gene.

Transformation into Oil Palm Target Tissues

DNA-microcarrier preparation. DNA precipitation onto gold microcarriers was carried out according to manufacturer's instructions for the Biolistics PDS/He 1000 (Bio-Rad) device. Five µl pMS32 or pMS33 DNA solution (1 µg $µl^{-1}$), 50 µl $CaCl_2$ (2.5M) and 20 µl spermidine (0.1 M, free base form) were added sequentially to the 50 µl gold microcarrier suspension. The mixture was vortexed for 3 min, spun for 10 s in a microfuge and the supernatant discarded. The pellet was washed with 250 µl absolute ethanol. The final pellet was resuspended in 60 µl absolute ethanol. Six µl of the solution were loaded onto the centre of the macrocarrier and air dried.

Bombardment of embryogenic calli. Five µl PHB or PHBV plasmid DNA solution (1 µg $µl^{-1}$), 50 µl $CaCl_2$ (2.5 M) and 20 µl spermidine (0.1 M, free base form) were added sequentially to the 50 µl particle suspension. The mixture was vortexed for 3 min, spun for 10 s at 10 000 rpm and the supernatant discarded. The pellet was washed with 250 µl absolute ethanol. The final pellet was resuspended in 60 µl absolute ethanol. Six µl of the solution were loaded onto the centre of the macrocarrier and air dried. Bombardments were out once at the following conditions: 1100 psi rupture disc pressure; 6 mm rupture disc to macrocarrier distance; 11 mm macrocarrier to stopping plate distance, 75 mm stopping plate to target tissue distance and 67.5 mmHg vacuum pressure (Parveez, 1998).

Maintenance of embryogenic calli. Embryogenic calli were maintained on agar-solidified medium containing MS macro- and micronutrients supplemented with 2.2 mg $litre^{-1}$ 2,4-D and 30 g $litre^{-1}$ sucrose. The medium was adjusted to pH 5.7 with KOH prior to autoclaving. Embryogenic calli were cultured at 28°C in the dark, and subcultured every 30 days onto fresh medium (Parveez, 1998).

Selection and regeneration of transformed embryogenic calli. Minimal inhibitory concentrations of selection agents for oil palm have been determined previously (Parveez *et al.*, 1996). Bombarded embryogenic calli were exposed to medium containing 50 mg $litre^{-1}$ Basta herbicide at three to four weeks after bombardment. Tissues were subcultured on fresh medium under selection pressure at monthly intervals. Embryogenic cultures were transferred onto media containing MS macro and micronutrients, and Y_3 vitamins supplemented with 100 mg $litre^{-1}$ each of myo-inositol, L-glutamine, L-arginine and L-asparagine, 5 µM IBA, 0.7% agar and 30 g $litre^{-1}$ sucrose (pH 5.7) to form polyembryogenic cultures. Once the transgenic polyembryogenic calli are obtained, they will be regenerated into whole plants under selection pressure.

RESULTS AND DISCUSSION

Isolation of Biotin Carboxylase Gene

A PCR-based method was employed in our attempts to isolate the full length cDNA for biotin carboxylase from oil palm. Initially, degenerate synthetic primers were designed based on highly conserved regions of plant acetyl-CoA carboxylase available in GenBank database. Regions used for designing the primers are shown in *Figure 1*. The primers were subsequently used in the RT-PCR reactions using RNA prepared from 19 WAA oil palm mesocarp template. The first partial fragment of the oil palm ACCase (BC) gene was isolated by RT-PCR which resulted in a DNA fragment of approximately 580 bp in size (*Figure 2a*). Several putative clones were sent for sequence analysis and nucleotide homology searches carried out using BLASTx and BLASTn provided by NCBI. For the putative BC fragment, high homology was found with biotin carboxylase from other plants. Homology search indicated that the 580 bp partial length of BC had very significant homology to BC from other plants such as cotton, soyabean, rapeseed and *Arabidopsis*.

Efforts were then directed to isolate the full length BC gene by RACE. Gene-specific primers were designed based on the 580 bp nucleotide sequence (*Table 1a*). Both the 5′ and 3′ ends (*Figure 4*) were successfully amplified by RACE and sequencing analysis revealed high homology to BC from other plants. End-to-end PCR was then used to amplify the full length cDNA. As shown in *Figure 5*, a 2049 bp-cDNA fragment representing the full length BC was amplified. The full length sequence of the cDNA coding for oil palm BC was also determined (*Figure 6*). The open reading frame was predicted to encode a 534 aa protein and protein alignment carried out also showed high homology to plant BC genes.

Isolation of multifunctional ACCase gene. Isolation of partial cDNA clone of multifunctional ACCase from oil palm was conducted by RT-PCR. An approximately 1.2 kb PCR product was amplified using degenerate primers designed based on a highly conserved region of plant ACCase (*Figure 1*).

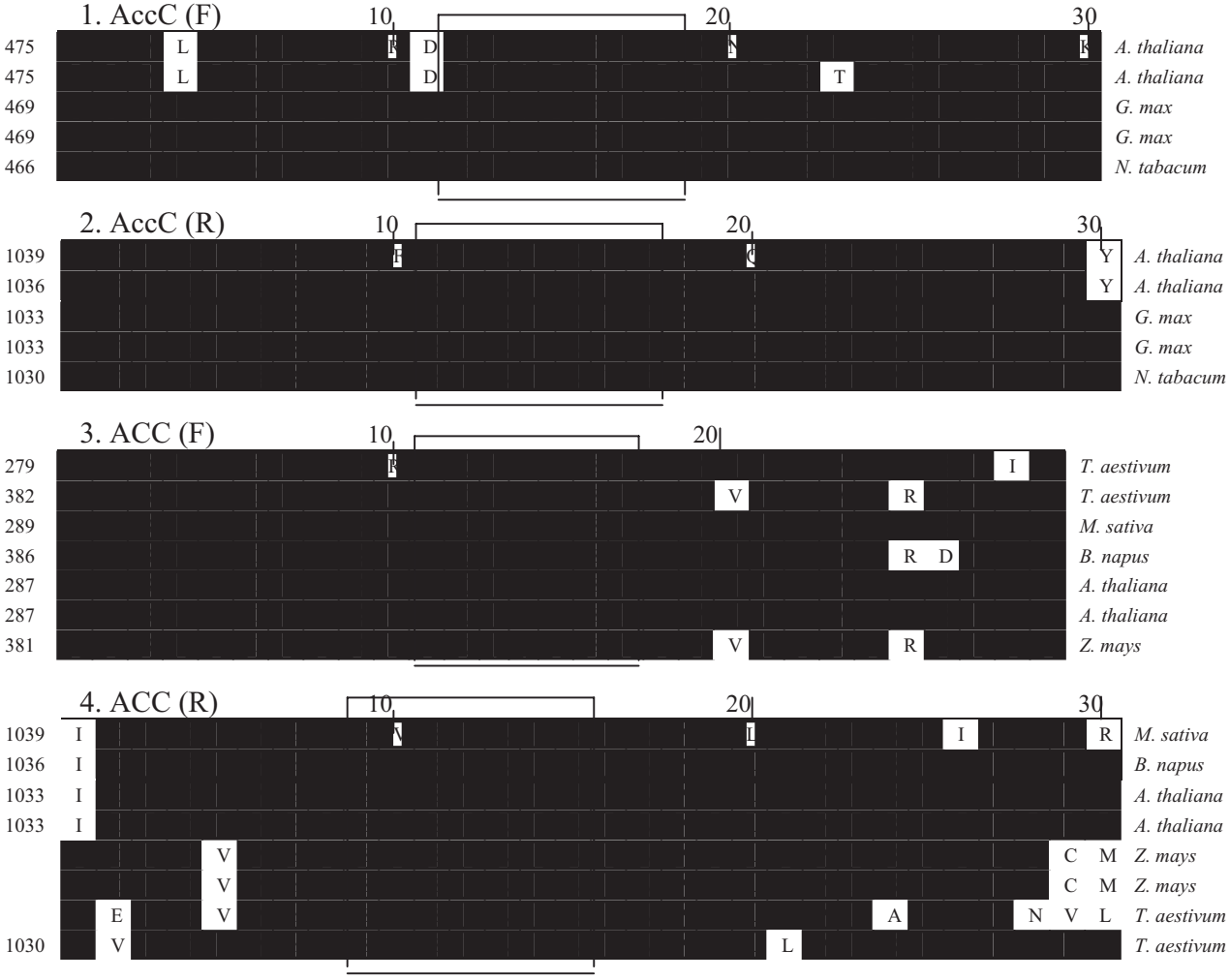

Figure 1. Protein primer sequence and regions used for design of primers for RT-PCR. Degenerate synthetic primers were designed based on highly conserved regions of plant acetyl-CoA carboxylase available in GenBank.

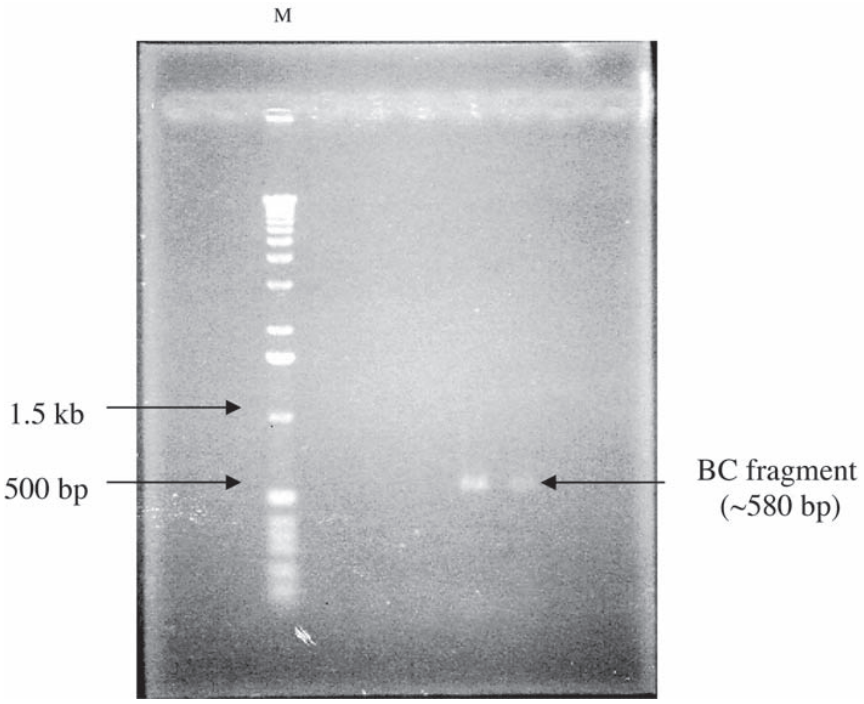

Figure 2a. 580 bp PCR product of BC obtained by RT-PCR.

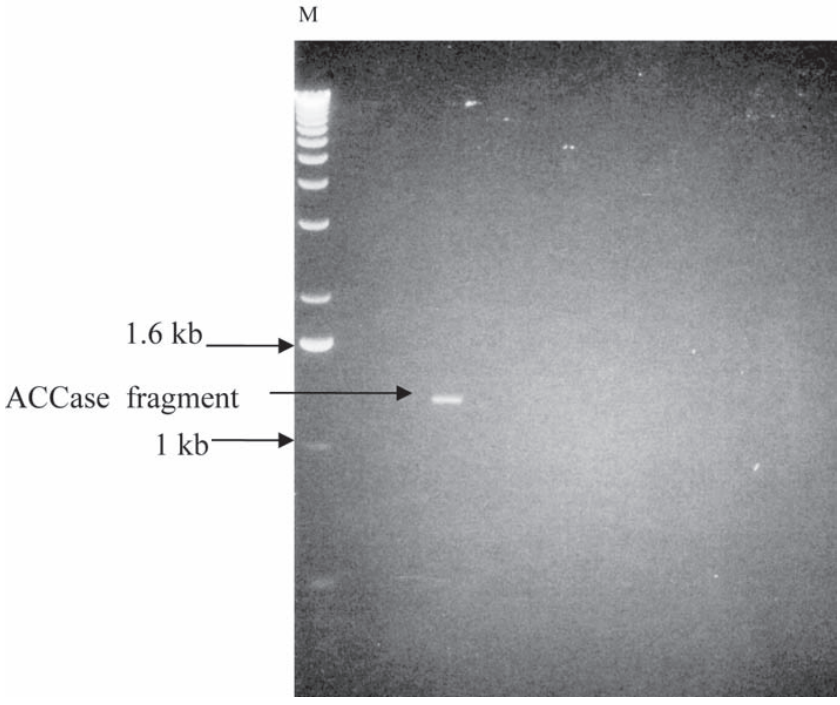

Figure 2b. A 1.2 kb fragment of multifunctional ACCase obtained by RT-PCR.

Figure 2b shows the PCR product while *Figure 3* shows the plasmid after digestion with *EcoR1* to release the expected insert of 1.2 kb for manipulation studies.

Construction of pMS32 and pMS33. An antisense sequence of the 1.2 kb multifunctional ACCase gene with transit peptide was cloned into pMS29 and pMS31 to create pMS32 and pMS33, respectively (*Figure 7*). The pMS32 and pMS33 were constructed using blunt-end ligation due to non-availability of suitable restriction sites. The plasmid DNA of pACC was digested with *Asc*I and the 1.2 kb multifunctional ACCase DNA fragment purified by agarose gel electrophoresis. This fragment was ligated to the pMSP3 cleaved by *Asc*I to create pACCaseMSP1, carrying multifunctional ACCase gene driven by MSP1 promoter, transit peptide and terminated by NOS sequences. The antisense orientation of the multifunctional ACCase sequence

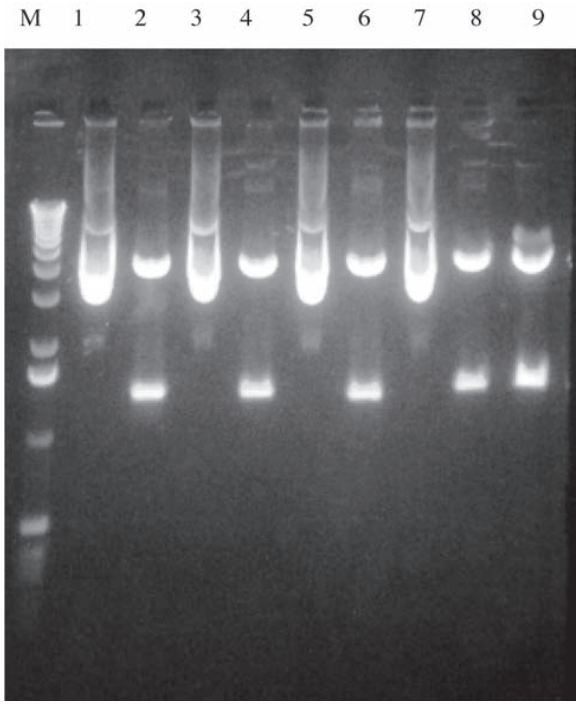

Figure 3. ACCase plasmid digested with Eco*R1. All clones examined had inserts of the expected size M: 1 kb marker (Gibco BRL). Lanes 1, 3, 5, 7 undigested plasmid, lanes 2, 4, 6, 8 and 9 digested plasmid with* Eco*R1.*

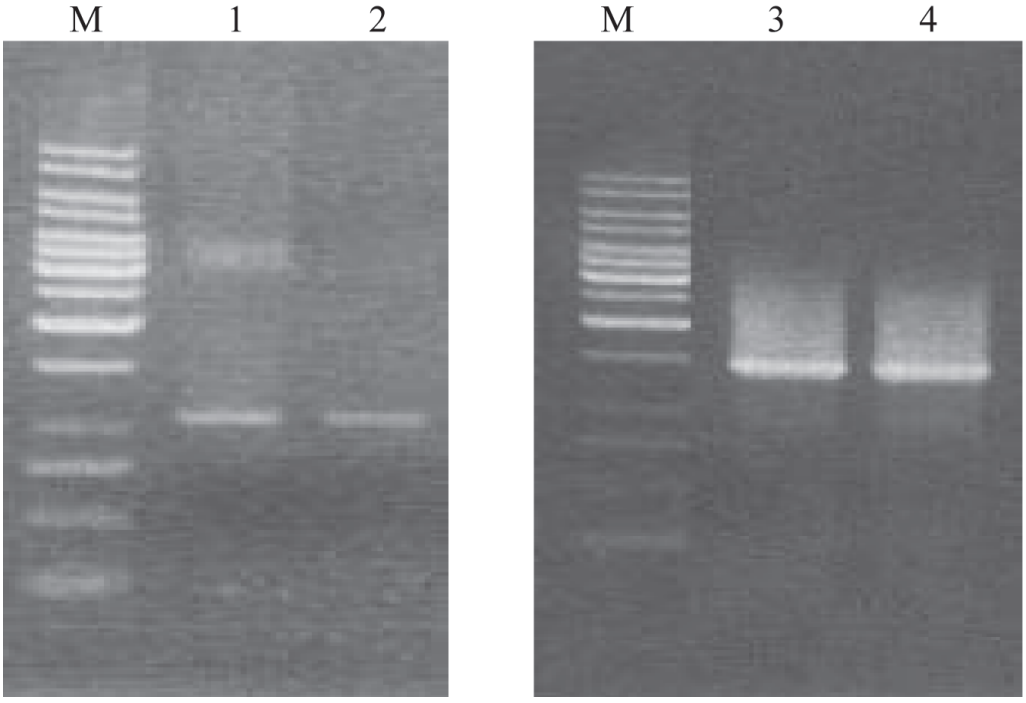

Figure 4. PCR products for 5′and 3′ ends.

Note: M: 1 kb DNA ladder, lanes 1 – 2 amplified product for 5′ ends. Lanes 3 – 4 PCR product for 3′ ends.

was confirmed by PCR analysis using primers ACC-F and NOS2-R. The pACCaseMSP1 was then digested with the restriction enzymes *Eco*RV and *Not*I, and the 2.6 kb *MSP1-ACCase-Nos* fragment rendered blunt by *Pfx* DNA polymerase. This resulting ACCaseMSP1 fragment was then ligated into the blunt-end *Hind*III site of pMS29 and pMS31 to produce clones designated pMS32 and pMS33, respectively. The presence of the inserted ACCase fragment was then confirmed by PCR analysis using a combination of ACC1-F and ACC1-R primers to result in a 1.2 kb PCR product (*Figure 8*).

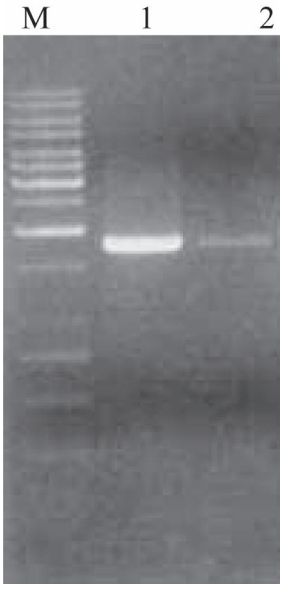

Figure 5. Full length BC by end to end PCR.

Note: M: 1 kb DNA ladder, lanes 1 – 2 amplified product for end-to-end PCR.

CGACTGGAGCACGGGACACTGACATGGACTGAAGGAGTAGAAAATCGATCTCCT
CCTCTTCGCTTCTCCTAATTTAACTCTCAACCCCTCCCCTCGGAACCCATCTCTTCT
CCTCCCCTCTAATCCTTCGATACCTCTCCTCCTCATCCCCGACGGCTACCCTTCTTT
CCAGATCCGCCCATCTCTCCCACCATTTCGCGGATTTGATAGCTGGAGTATCTTCT
TTCTCTATCCGAACCGAGATCGGTATTAATTTTTTTCGTTCTGTTTGAGGATTTGAT
CCGAAG**ATG**AACTCCATGATCACTTGCAAGTCGGCCTGCTCGCCTCTTGGGTTAG
TCATCGGACCAGCCAGAGGAATTAGGAGCTCTCAATGTACCTTTATGGTGGGAAA
TGCTCCAAACTTTCACAAGATAAGCTCTCCGAGGCAAAGAGCTGCTTGTGCAAAC
CGGAAATCAAAAAGGAGCGGAGGAGCTCTGCATGCAACTTGCCATGATGAGAGG
ATTCTTGTGGCAAATAGAGGGGAAATTGCAGTCAGGGTGATTCGAACTGCACATG
AGATGGGGATTCCCTGTGTTGCTGTGCACTCCACAATAGATGAGGATGCTCTTCAT
GTGCGACTCGCAGATGAGGCAGTGTGCATTGGTGAAGCACCAAGCAGTCAATCGT
ACTTGTTCATTCCAAATGTTTTATCTGCTGCTGTTAGCCGGGGGTGCACTATGTTG
CATCCTGGGTATGGTTTCCTAGCTGAGAATGCTGGCTTTGTTGATATATGCAAAGA
GCATGGAATCACTTTATTGGGCCAAATCCTGACAGCATTCGAGTCATGGGCGATA
AATCTACAGCCAGAGAACCAATGAAGAAAGCAGGCGTCCCGACTGTACCTGGAA
GTGATGGATTGTTACAGTCCACTGAAGAGGCTGTCAAGCTTGCACATGAAATTGG
TTTCCCAGTGATGATTAAGGCAACTGCTGGTGGTGGAGGGCGTGGAATGCGTCTA
GCTCATGAACCTGAAGAGTTTGTAAAGTTACTCCAGCAAGCTAAGAGTGAGGCGG
CAGCAGCATTTGGAAATGATGGGGTTTACCTGGAGAAATACATCCAGAATCCAAG
GCACATTGAGTTCCAGGTTCTTGCAGACAAGTATGGTAATGTTGTTCATTTTGGTG
AGCGTGATTGCAGTATACAGAGAAGGAATCAGAAGCTCCTAGAAGAAGCTCCAT
CCCCTGCATTGACGCCTGAGCTACGGAAAGCTATGGGTGATGCAGCTGTAGCTGC
TGCTGCATCTATAGGTTACATTGGTGTTGGAACTGTGGAATTTCTCTTGGATGAAA
GAGGTACCTTCTACTTCATGGAGATGAACACCAGGATTCAGGTAGAGCACCCTGT
CACTGAAATGATTTCTTCAACCGACTTGATTGAAGAACAAATTCGAGTTGCTCTTG
GAGAGAGGCTGACATACAAGCAGGAAGACATTGTTCTGAGAGGACATTCAATTG
AATGCCGGATTAATGCAGAAGATGCCTTCAAAGGATTTCGTCCAGGGCCTGGAAA
GATTACATCATACTTGCCATCTGGAGGTCCATTTGTGAGAATGGATAGTCATGTTT
ATCCAGGCTATGTGGTTCCCCCAAGCTATGACTCCTTGTTAGGAAAGCTTATTGTG
TGGGCACCAACCAGGGAAAAAGCGATTGAACGGATGAAAAGAGCTCTTGATGAC
ACGATAATAACAGGAATTCCTACAACCATTGAATACCATAAGCTAATTCTTGATA
TCGAGGATTTTAGAAATGGAAAGGTAGATACTGCCTTCATACCGAAGCATGAGAA
GGATTTGGCTGCACCCCAGAAACTAGTGCTGTTGACATTGGAGAAAGAGCTTGCC
GGG**TGA**AGGGTGATTGAAGCCAAGTTGCCAGAGATAAGCCAGGGAATAATTCCT
TGTAGCTGCATTTCATGATGCAGGTGGGTAATTGACTTCGAGCCCGTGAAATAAG
TGCTATGATTATTAGAAGCATTTCCAGATATTCTCAACTCTCTTCTTTTCTTAGATT
CAAAATTGTTTTTAGTCTTTCAGACACATTTGGCATCAATTGTTTGACAAACGCTG
CC

Figure 6. Full length cDNA sequence for the oil palm biotin carboxylase gene.

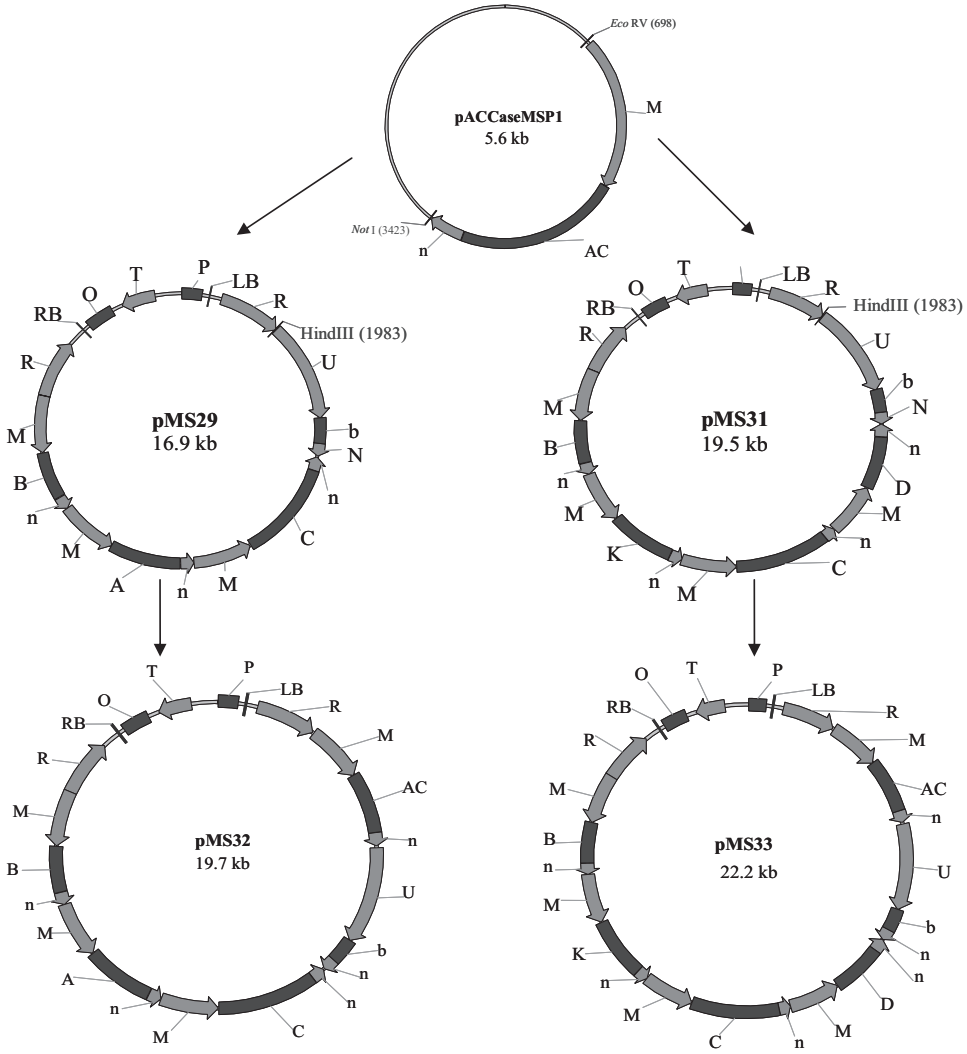

Figure 7. Construction of pMS32 and pMS33. The restriction sites and the numbers indicate the approximate positions of the restriction site in the vectors. A: TpphbA, B: TpphbB, C: TpphbC, D: TptdcB, K: TpbktB, M: MSP1, N and n: Nos, O: CoIE1 ori: P: pSa Ori: R: RB7MAR, T: npt1, U: UbiPro,b:bar, AC: antisense ACCase with transit peptide, LB: left border, RB: right border.

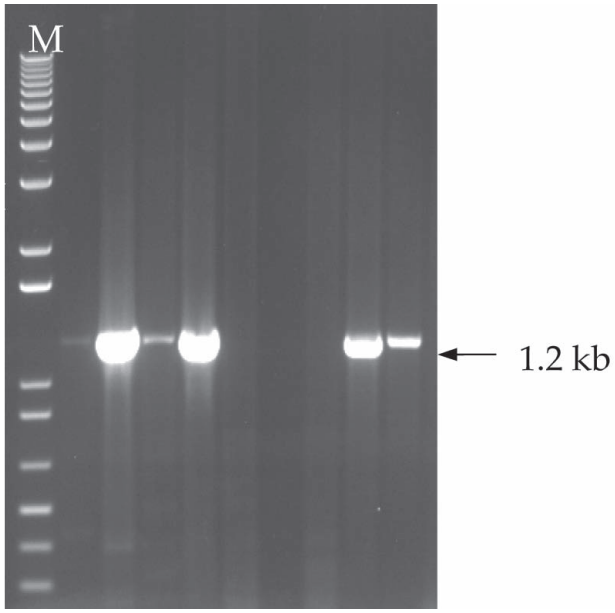

Figure 8. Selection of pMS32 and pMS33 (Arrow indicates the PCR products of ~1.2 kb TpACCase).

Transformation of oil palm. The optimized conditions for DNA delivery into oil palm embryogenic calli, via Biolistics™, have been determined earlier (Parveez *et al*., 1997; 1998). Using the optimized conditions, oil palm embryogenic calli were bombarded with the two newly constructed transformation vectors, pMS32 and pMS33, which also carried the *bar* gene (conferring resistance to the herbicide Basta). Simultaneously, embryogenic calli were also bombarded without any DNA as controls. Bombarded tissues were first cultured on embryogenic medium in the absence of selection agent for three to four weeks. Upon transfer to fresh medium containing selection agent, untransformed embryogenic calli began to die, allowing only resistant embryogenic callus to proliferate. The transformed embryogenic calli were subcultured onto fresh medium containing selection agent once a month. Basta-resistant embryogenic calli colonies normally appear after five to six months on selection medium. The Basta-resistant embryogenic calli are currently undergoing regeneration to produce whole transgenic oil palm plants.

In this study, we transformed the antisense copy of partial length multifunctional ACCase into the oil palm fruit mesocarp, targeted to the plastid to down-regulate the activity of ACCase and subsequently reduce the fatty acid biosynthesis activity. This will direct the unutilized large pool of acetyl-CoA to the production of PHB and PHBV. The multifunctional enzyme form, also designated as a homomeric- or eukaryotic-type enzyme in the literature, is found in the cytoplasm of many eukaryotic organisms including plants (Gornicki *et al*., 1994; Anderson *et al*., 1995). On the other hand, the multisubunit type of ACCase, also known as the heteromeric or prokaryotic form in the literature, has been described in plant chloroplasts (Kannangara and Stumpf, 1972; Sasaki *et al*., 1993). The malonyl CoA produced in cytosol by the multifunctional ACCase is used for fatty acid elongation and biosynthesis of phytoalexins and flavonoids (Ebel *et al*., 1984), and the multisubunit enzyme in chloroplast used for *de novo* fatty acid biosynthesis (Sasaki *et al*., 1993, Kinoshi *et al*., 1996). However, the chloroplasts of rape seem to contain both types of ACCase (Elborough *et al*., 1996; Shulte *et al*., 1997). Even though the multifunctional ACCase is found in the cytoplasm (cytosol) and involved in flavonoids biosynthesis, targeting the multifunctional ACCase gene into the plastid may function to down regulate the multisubunit ACCase presence in the plastid. The homology between the domains on the multifunctional and multisubunit may allow the multifunctional ACCase to down regulate the multisubunit ACCase in the plastid. The effectiveness of this multifunctional ACCase can only be evaluated once the plants start bearing fruit.

CONCLUSION

BC and a partial fragment of the oil palm multifunctional ACCase were isolated. The partial ACCase fragment was transformed into oil palm to down regulate fatty acid synthesis in an effort to channel acetyl-CoA to PHA production. The embryogenic cultures carrying the ACCase and PHA genes are currently being regenerated into whole plants.

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