Table 1
Comparison of relative levels of gene expression using microarray and Northern-blot analysis

All values are relative to BKR on a per mol basis. G3P was estimated by quantitative Northern-blot analysis of BKR and G3P using the same blots and after sequential probing. ACP was estimated by dual probing of Northern blots with BKR. ACCase, acetyl-CoA carboxylase; BCCP, biotin carboxylase carrier protein type I; delta 9, 69-stearoyl-ACP desaturase; DH, 3-ketoacyl-ACP dehydratase; ER, enoyl-ACP reductase; MCAT, malonyl-CoA:ACP transacylase; TE, acyl-ACP thioesterase.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Microarray</th>
<th>Northern blot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 DAF</td>
<td>23 DAF</td>
</tr>
<tr>
<td>ACCase</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>BCCP</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>MCAT</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>BKR</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>DH</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>ER</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>TE</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>G3P</td>
<td>2.4</td>
<td>2.1, 2.4, 2.4</td>
</tr>
<tr>
<td>Delta 9</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>ACP</td>
<td>8.2</td>
<td>6.6, 6.9, 7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0, 4.4, 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4, 4.4, 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.1</td>
</tr>
</tbody>
</table>

'electronic Northerns' because of differences in species, tissue type and stage of development. Therefore the results suggest that core fatty acid synthase components are transcribed at constant ratios. Further evidence for this came from both the quantitative Northern-blot analysis, which indicated that for G3P high levels of expression are maintained between 23 and 42 DAF, and from dual probing of Northern blots with ACP and BKR, which indicated only a small decrease in relative expression from 32 DAF onwards (Table 1).

References

Received 26 June 2000

Cloning of a palmitoyl-acyl carrier protein thioesterase from oil palm
A. Othman1, C. Lazarus, T. Fraser and K. Stobart
School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, U.K.

Abstract
A palmitoyl-acyl carrier protein (ACP) thioesterase cDNA clone was isolated from an oil palm cDNA library. The cDNA was expressed in Escherichia coli as a glutathione S-transferase fusion protein and a crude bacterial extract was assayed for acyl-CoA-hydrolysing activity. The recombinant enzyme was able to hydrolyse medium- and long-chain acyl-CoAs. Northern-blot analysis showed a high level of gene expression in leaf, flower and 15-, 17- and 18-week mesocarp tissues. Low-level gene expression was detected in germinated seedlings and 8- and 12-week mesocarp tissues, but no transcript was detected in any kernel tissues. Southern-blot analysis indicated the presence of a single gene and we have also isolated a genomic clone using the cDNA as a probe. Two genomic fragments were subcloned and a 7 kb contiguous stretch of the oil palm genome was sequenced. Comparison of this
sequence with the cDNA sequence identified a putative 93 amino acid transit peptide, most of which is missing from the cDNA. The coding region of the gene consisted of seven exons and six introns.

Introduction

Most vegetable oils contain 10–20% saturated fatty acids but palmitic acid (C16,16) accounts for about 44% of the total fatty acid in oil palm. Acyl-acyl carrier protein (ACP) thioesterase (EC 3.1.2.14) has been implicated as one of the factors responsible for the high level of palmitic acid in the oil palm mesocarp [1].

Plant acyl-ACP thioesterases play an important role in the control of seed-oil composition. They terminate chain growth by cleaving the growing fatty acid moiety from ACP for eventual export from the plastid [2]. During transport from the plastid the fatty acid is reactivated to a usable form by ligation to CoA on the plastid envelope. Acyl-CoAs are then used for further biosynthetic reactions, such as chain elongation and/or incorporation into membrane lipids or triacylglycerol for storage in lipid bodies during the development of oilseeds [3].

Characterization of acyl-ACP thioesterase expression in the oil palm is in progress, with the aim of understanding its role in fatty acid biosynthesis. While this study contributes to the further study of plant fatty acid synthase systems in general, it is also relevant to efforts to modify oil palm lipid composition.

Materials and methods

Recombinant protein production

A 50 ml overnight starter culture was used to inoculate 500 ml of fresh Luria–Bertani broth (containing 100 µg·ml⁻¹ ampicillin), and the culture was incubated in an orbital shaker at 37 °C for 1.5 h. Then, 1 ml of the ‘uninduced’ sample was removed, centrifuged and resuspended in 100 µl of 2 x sample loading buffer [125 mM Tris/HCl, pH 6.8/10% (v/v) glycerol/10% (w/v) SDS/10% (v/v) 2-mercaptoethanol/Bromophenol Blue]. Isopropyl β-D-thiogalactoside (IPTG; 1 mM) was added to the remaining culture, which was incubated for a further 3 h. A 1 ml sample from the ‘induced’ cells was removed, centrifuged and resuspended in 100 µl of 2 x sample loading buffer. The rest was centrifuged at 14000 g for 10 min, and the pellet resuspended in 5 ml of thioesterase assay buffer.

Figure 1

SDS/PAGE of expression products of the pGEX-THIO construct in E. coli BL21

Samples were taken from the cultures before induction and after 1 and 3 h of induction with 1 mM IPTG. Expression of a 66 kDa GST-fusion protein (A) was obtained. pGEX, control sample expressed using a non-recombinant vector showing expression of the 26 kDa GST protein in induced tracks (B). M, Rainbow™ molecular-mass markers (Amersham) with the sizes of the bands (in kDa) indicated. I, induced culture; U, uninduced culture.
(0.1 M potassium phosphate, pH 7.5). The resulting suspension was sonicated with three bursts of 30 s and centrifuged at 10000 g for 20 min. A sample of the supernatant was analysed by SDS/PAGE to verify expression and the rest stored at -20 °C for enzyme activity assay.

**Screening an oil palm genomic library**

An amplified oil palm genomic library in λEMBL3 (Stratagene, La Jolla, CA, U.S.A.) was provided by Siti Nor Akmar Abdullah (Malaysian Palm Oil Board, Bandar, Baru Bangi, Selangor, Malaysia). Approx. 25 000 plaque-forming units were mixed with 3 ml of *Escherichia coli* KW251 plating cells and plated on a 24 x 24-cm plate. The plaques were transferred to a nitrocellulose filter and prehybridized for 15 min at 65 °C in 7 % (w/v) SDS/0.25 M sodium phosphate buffer, pH 7.2. Subsequent hybridization with the 1450 bp insert from clone pHA-3 was carried out for 16 h under the same conditions. The membrane was washed under medium-stringency conditions: twice for 15 min at 65 °C in 2 x SSC/0.1 % (w/v) SDS (where 1 x SSC is 0.15 M NaCl/0.015 M sodium citrate). The membrane was exposed to Hyperfilm™-MP X-ray film overnight.

**Results and discussion**

**Expression of an oil palm thioesterase in *E. coli***

A 1450 bp cDNA (designated pHA-3) encoding an acyl-ACP thioesterase was isolated from an oil palm 15 week mesocarp cDNA library [4]. The incomplete cDNA encodes the whole mature protein, but lacks most of the associated transit peptide. The cDNA sequence corresponding to the mature protein is 66 % identical with *Cuphea hookeriana* Ch FatB1 and 35 % identical with *Garcinia mangostana* Garm FatA1, indicating that this clone belongs to the FatB type of acyl-ACP thioesterases. The 5' region of pHA-3, which contains an *SpeI* site, was amplified by PCR using primers AEP-1 (5'-GCCAGGATCCATGCTGCTGAGCGTGC-3') and AEP-2 (5'-AAGCCATCGCCCTCGAGCCCAGCACT-3'). The DNA product was digested with *BamHI* and *XhoI* and ligated into the corresponding sites of the modified pGEX vector, pGEX-BHEX (gift from Dr S. E. Screen, University of Bristol, Bristol, UK). The cloned DNA was checked for PCR-induced mutations, then the rest of the coding region was inserted as an *SpeI*-XhoI fragment. The final plasmid, designated pGEX-THIO, was transferred to *E. coli* BL21 (DE3).

Proteins produced in induced and uninduced cultures were compared by SDS/PAGE. A new protein band was observed in the IPTG-induced culture relative to the pGEX-BHEX control. Figure 1 shows the expression of the thioesterase cDNA as a glutathione S-transferase (GST) fusion protein of the predicted size (66 kDa). Western immunoblot analysis of total proteins from BL21 (DE3)/pGEX-THIO and pGEX-BHEX with anti-GST antibodies showed one
strong signal in the 1- and 3-h induction fractions (results not shown). This strong signal corresponds to the position of the pGEX-THIO fusion protein (66 kDa).

**Enzymic characterization of the recombinant thioesterase**

The ability of extracts from *E. coli* cultures expressing GST-THIO to hydrolyse a range of acyl-CoA substrates was tested. Crude lysates of transformed *E. coli* strains expressing pGEX-THIO were assayed for *in vitro* acyl-CoA hydrolytic activity. The acyl-CoA thioesterase activity observed in *E. coli* cells transformed with pGEX-THIO was 34-fold higher than in control cultures. The lysates showed the highest activity with C16-CoA, followed by C14-CoA, C16-CoA, C18-CoA and C12-CoA (Figure 2). Acyl-ACP thioesterases in oil palm mesocarp were found to have a similar activity trend for both acyl-ACP and acyl-CoA esters, except that the activity was 10 times lower for CoA esters [1].

**Characterization of the acyl-ACP thioesterase gene and its expression**

A palmitoyl-ACP thioesterase gene was isolated by screening a genomic library with pHA-3 cDNA as a hybridization probe. Comparison with the cDNA sequence revealed the presence of six introns, the longest of 1.8 kb, extending over 6 kb of the coding region. The genomic sequence also complements that of the incomplete cDNA by indicating the presence of a 93 amino acid transit peptide. Southern-blot analysis indicated that the gene is probably unique. A single hybridizing band was detected in EcoRI-digested genomic DNA, where as four bands hybridizing with various intensities were detected in a HindIII digest.

Expression of the gene was assayed by Northern analysis (results not shown). Transcript accumulated to high levels in leaf, flower and 15-, 17- and 18-week mesocarp tissues. Lower levels of expression were detected in germinated seedlings and 8- and 12-week mesocarp tissues; no transcript was detected in any kernel tissues. The pattern observed from this analysis suggests that thioesterase is important for oil deposition in mesocarp.

A.O. thanks Siti Nor Akmar and Zaini Abdullah for assistance with Northern analysis and Malaysian Palm Oil Board for the provision of a studentship.

**References**


Received 30 June 2000