

List of figures

Series No	Name of figure	Page No.
Figure 1.1	Structure of fats	1
Figure 1.2	<i>cis</i> and <i>trans</i> conformation of oleic acid.	3
Figure 1.3	Structures of n-3 and n-6 PUFAs.	4
Figure 1.4	Biosynthesis of the n-6 and n-3 PUFA.	21
Figure 1.5	Chromosomal localization of the Fads cluster.	22
Figure 1.6	Crystal Structure of Δ -9 desaturase.	25
Figure 1.7	Current topological model for membrane-bound fatty acid desaturases.	25
Figure 3.1	Colony and morphological characterization of yeast isolates.	65
Figure 3.2	Comparison of the deduced amino-acid sequences of previously reported <i>fad-3</i> genes from several fungi, plants, algae and cyanobacteria.	67
Figure 3.3	PCR based screening for <i>fad-3</i> gene (~600 bp PCR).	68
Figure 3.4	Semi-nested PCR for the confirmation of <i>fad-3</i> gene using 600 bp amplicon as template.	69
Figure 3.5	Dot blot analysis.	70
Figure 3.6	Southern hybridization analysis of yeast isolates.	70
Figure 3.7	Yeast morphology on HiCHROMagar	72
Figure 3.8	Evolutionary relationships of the identified <i>fad-3</i> positive yeast isolates.	74
Figure 3.9	Combined maximum likelihood analysis of six genes (ACT1, EF2, RPB1, RPB2, 18S rDNA and 26S rDNA).	75
Figure 3.11	Representative GC chromatogram of FAMES derived from yeast total fatty acids extracts.	78
Figure 3.12	Representative GC chromatogram of FAMES derived from yeast total fatty acids extracts (A-9) showing production of LA only.	78
Figure 3.13	Comparative analysis of screening for FAD-3 activity.	79

Figure 4.1	Schematic representations of Ct- <i>fad-3</i> gene isolation and cloning.	89
Figure 4.2	Cloning of N- and C-terminal fragments of Ct- <i>fad-3</i> .	90
Figure 4.3	Generation of complete Ct- <i>fad-3</i> gene in pBS- <i>fad-3</i> .	90
Figure 4.4	Construction of pSP- <i>fad-3</i> .	91
Figure 4.5	Hydropathy plot of Ct-FAD-3.	92
Figure 4.6	Schematic representation of predicted trans-membrane regions of Ct-FAD-3.	93
Figure 4.7	Molecular Phylogenetic analysis of deduced Ct-FAD-3 protein sequence.	94
Figure 4.8	Analysis of effect of FAD-3 expression on growth of yeast cells.	95
Figure 4.9	Reverse transcriptase PCR of Ct- <i>fad-3</i> .	96
Figure 4.10	GC chromatograms of FAMES derived from yeast total fatty acids extracts.	97
Figure 4.11	Analysis of culture supernatant proteins.	98
Figure 4.12	Analysis of whole cell proteins by SDS-PAGE.	98
Figure 4.13	Analysis of galactose-induced <i>S. cerevisiae</i> W9100 total cell proteins by SDS-PAGE.	101
Figure 4.14	Cloning of Ct- <i>fad-3</i> in pET-28c(+) expression vector.	102
Figure 4.15	SDS-PAGE analysis of Ct-FAD-3 expression in <i>E. coli</i> BL21(DE3)	103
Figure 4.16	Induction of FAD-3 under different conditions in <i>E. coli</i> BL21(DE3).	103
Figure 4.17	Purification of Ct-FAD-3 from <i>E. coli</i> BL21(DE3).	104
Figure 4.18	Isoelectric focusing and 2-D analysis of Purified FAD-3 protein	105
Figure 4.19	Purification and determination of optimal titer of anti-FAD-3 antibodies.	106
Figure 4.20	Analysis of FAD-3 protein expression by Indirect ELISA.	106
Figure 4.21	Western blot analysis of <i>E. coli</i> BL21(DE3) total proteins.	107
Figure 4.22	Western blot analysis of total cell proteins from yeast cultures expressing Ct- <i>fad-3</i> gene constructs.	108
Figure 5.1	GC analysis of total fatty acids extracted from <i>E. coli</i> BL21(DE3) grown in LB medium supplemented with 1 mM LA.	109

Figure 5.2 A	Mass spectra of LA (C18:2: n-6) peak.	111
Figure 5.2 B	Mass spectra of ALA (C18:3: n-3) peak.	112
Figure 5.3	Figure 5.3 Substrate specificity of Ct-FAD-3 in <i>S. cerevisiae</i> W-9100 (pSP- <i>fad-3</i>). A:	113
Figure 5.4	Biotransformation of sunflower oil in <i>S. cerevisiae</i> W-9100.	114
Figure 5.5	Time course analysis of the FAD-3 Activity.	115
Figure II.A.1	pBlueScript KS (+)	136
Figure II.A.2	pET-28c(+)	136
Figure II.A.3	pGAL-MF	137
Figure II.B.1	pBSCtN10 with 1007 bp N-terminal Ct- <i>fad-3</i> fragment	137
Figure II.B.2	pBSCtC4 with 410 bp C-terminal Ct- <i>fad-3</i> fragment	138
Figure II.B.3	pBS- <i>fad-3</i> with complete Ct- <i>fad-3</i> gene	138
Figure II.B.4	pSP- <i>fad-3</i> with Complete Ct- <i>fad-3</i> gene	139
Figure II.B.5	pETSP28c(+) with complete Ct- <i>fad-3</i> gene	139
Figure 3.2	Comparison of the deduced amino-acid sequence of Ct-FAD-3 with previously reported FAD-3 from several fungi.	144