Appendix

APPENDIX-I

I.A Polyacrylamide gel electrophoresis reagents

SDS-PAGE was performed using a 5% stacking gel and a 8% separating gel as described by Sambrook *et al.*, 2001. Protein samples in SDS loading buffer were boiled for 4-5 min before being separated by SDS-PAGE. Afterwards, proteins were visualized by silver staining or by Coomassie Brilliant Blue R250 staining. The molecular weight standard used was from Bangalore genei and had the following size markers 205 kDa, 97.4 kDa, 66 kDa, 43 kDa and 29 kDa. About 2-20 µg of protein was applied to the gels.

30% Acrylamide stock solution: 23.2 g acrylamide + 0.8 g bisacrylamide (29:1) was dissolved in warm RO water and the volume made upto 80 ml. The solution was then filtered through Whatman filter paper and stored at 4 °C in the dark.

Tris buffers: The Tris buffers for resolving (1.5 M Tris pH 8.8) and stacking gels (1 M Tris pH 6.8) were prepared by dissolving Tris base in RO water and adjusting the pH with conc. HCl. The solutions were then autoclaved at 15 lbs for 15 min and stored at 4 °C.

10% SDS (Sodium Dodecyl Sulphate): 2 g of electrophoretic grade SDS was dissolved in 20 ml autoclaved RO water and stored at RT.

10% APS (Ammonium Per Sulphate): 0.1 g of APS was dissolved in 1 ml of autoclaved RO water and prepared freshly each time.

TEMED (N,N,N',N'-tetramethylethylenediamine): was a readymade solution stored in a dark bottle at 4 °C.

5 X Tris glycine tank buffer: 7.55 g Tris and 47 g glycine was dissolved in 500 ml RO water. The solution was autoclaved at 15 lbs for 15 min and stored at 4 °C.

2 X gel-loading buffer: 100 mM Tris-Cl (pH 6.8) + 20% (v/v) glycerol, 0.2% (w/v) bromophenol blue. SDS was added to 4% (v/v) for SDS-PAGE. β-mercaptoethanol was freshly added to 5% (v/v) at the time of sample preparation

I.B Dialysis buffer

80 ml of 1 M Tris pH 7 + 0.68 g of NaCl + 0.353 g of $CaCl_2.2H_2O$ were dissolved in RO water to make 1.6 L of solution which was autoclaved at 15 lbs for 15 min. The final concentration of each ingredient in the dialysis buffer was 50 mM Tris + 7.26 mM NaCl + 1.5 mM $CaCl_2.2H_2O$.

Preparation of dialysis tubing: Dialysis tubing (cut off 12 kDa) may contain significant amount of sulphur compounds and heavy metal compounds. These were removed by boiling the dialysis tubing in 2% sodium bicarbonate (w/v) + 0.05% EDTA (w/v) for about 15 min and then washed with autoclaved reverse osmosis (RO) water. This was followed by boiling again twice with RO water for 15 min periods. The prepared tubing was stored in water/dialysis buffer at 4 °C.

I.C Hydropathy index

The hydropathy index of an amino acid is a number representing the hydrophobic or hydrophilic properties of its side-chain. It was proposed in 1982 by Jack Kyte and Russell Doolittle. The larger the number is, the more hydrophobic the amino acid. The most hydrophobic amino acids are isoleucine (4.5) and valine (4.2). The most hydrophilic ones are arginine (-4.5) and lysine (-3.9). This is very important in determining the tertiary structure of a protein structure; hydrophobic amino acids tend to be internal; while hydrophilic amino acids are more commonly found towards the protein surface. Overall hydropathy plot of a polypeptide can give us an idea about the localization and topology of the protein.

Amino acids sorted by increasing hydropathy index

R	K	N	D	Q	E	H	P	Y	W	S	T	G	A	M	C	F	L	V	I
-4.5	-3.9	-3.5	-3.5	-3.5	-3.5	-3.2	-1.6	-1.3	-0.9	-0.8	-0.7	-0.4	1.8	1.9	2.5	2.8	3.8	4.2	4.5

Appendix II

A. Maps of vector plasmids used in present study

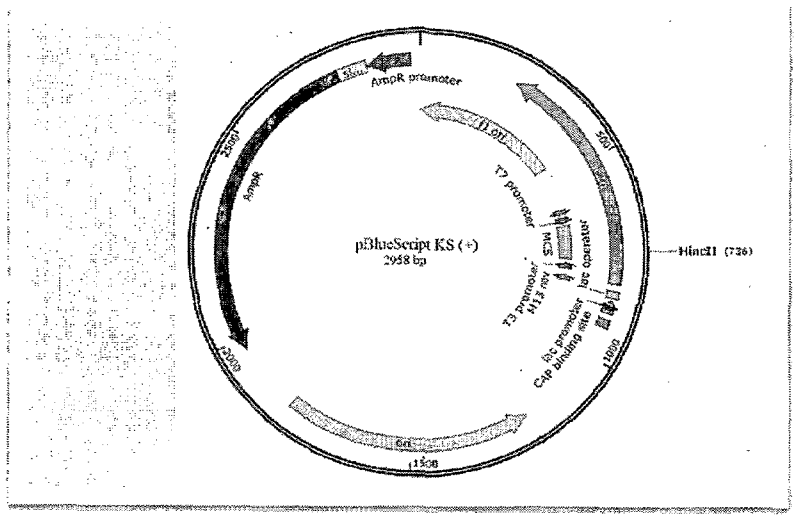


Figure II.A.1 pBlueScript KS (+)

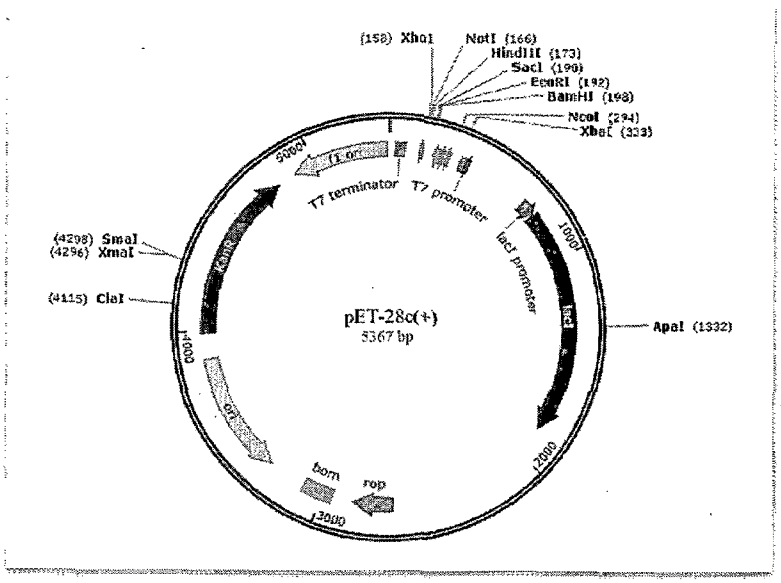


Figure II.A.2 pET-28c(+)

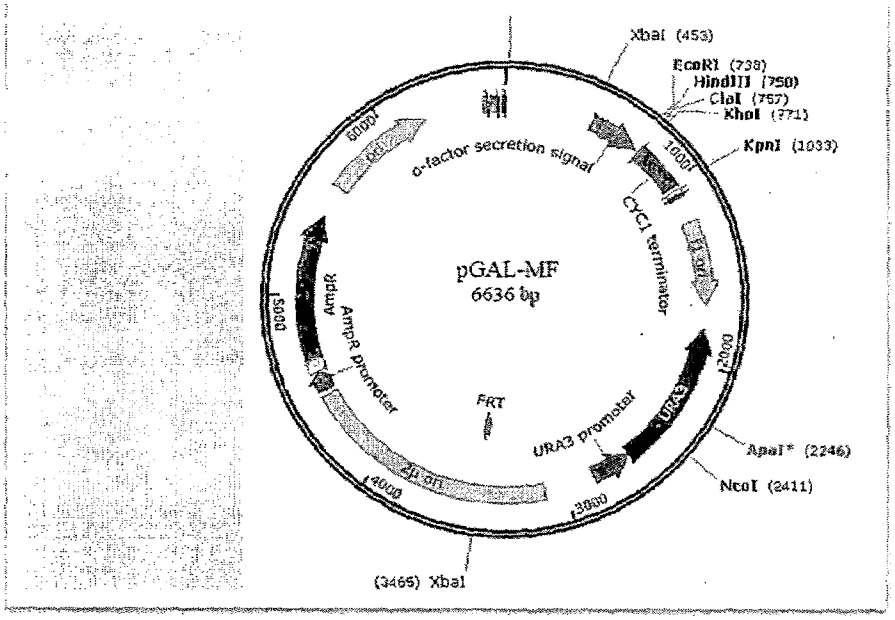
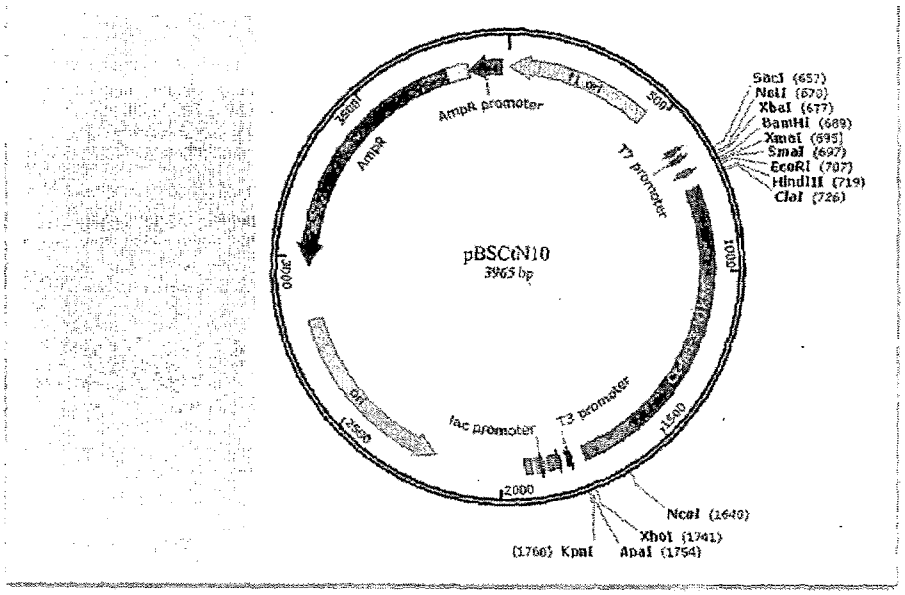
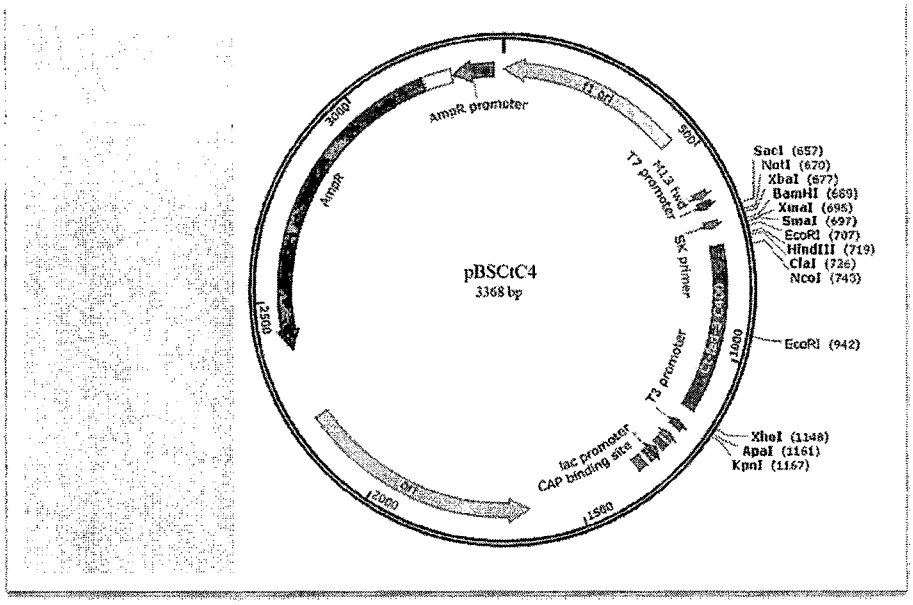


Figure II.A.3 pGAL-MF

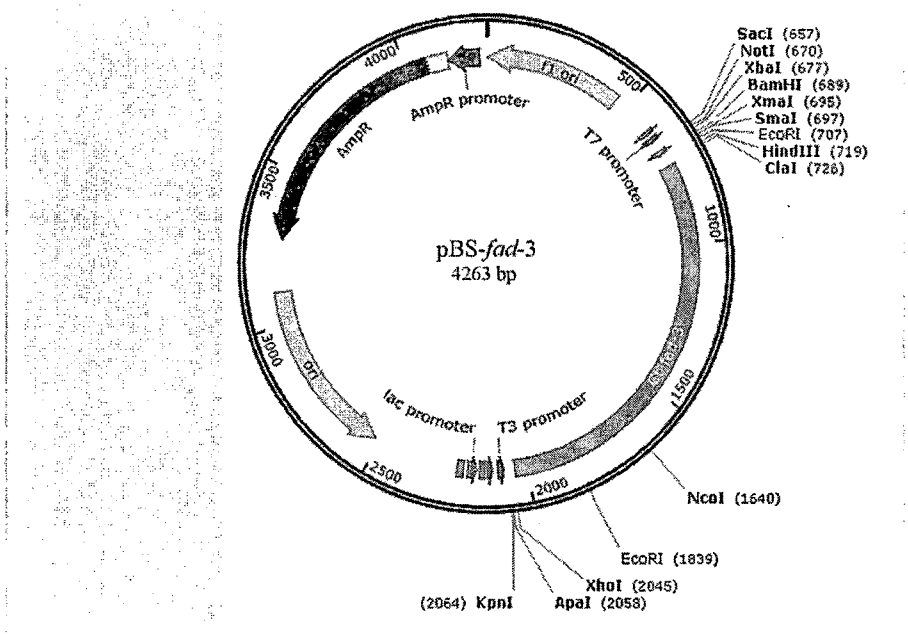
B. Maps of the recombinant construct generated in present study



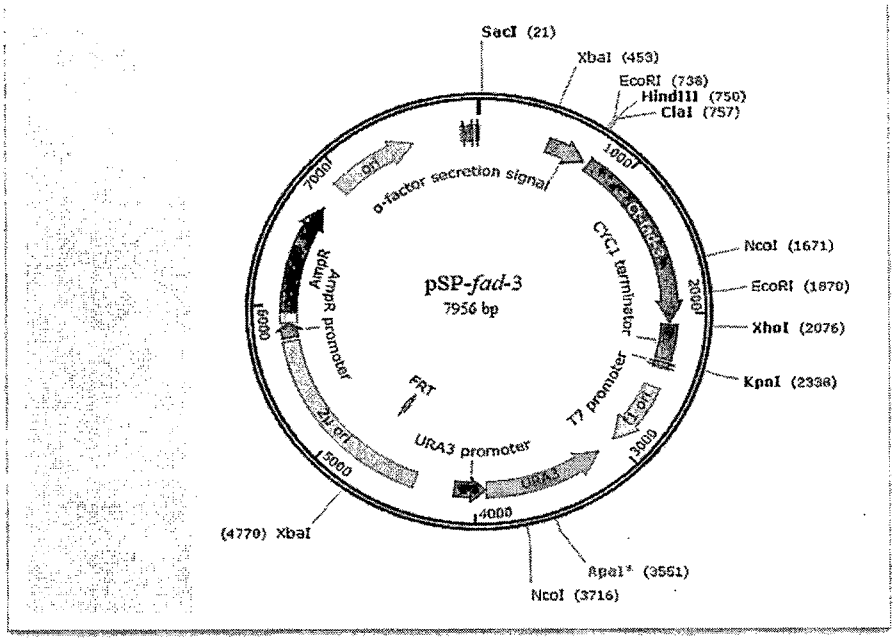
II.B.1 pBSCtN10 with 1007 bp N-terminal Ct-*fad*-3 fragment



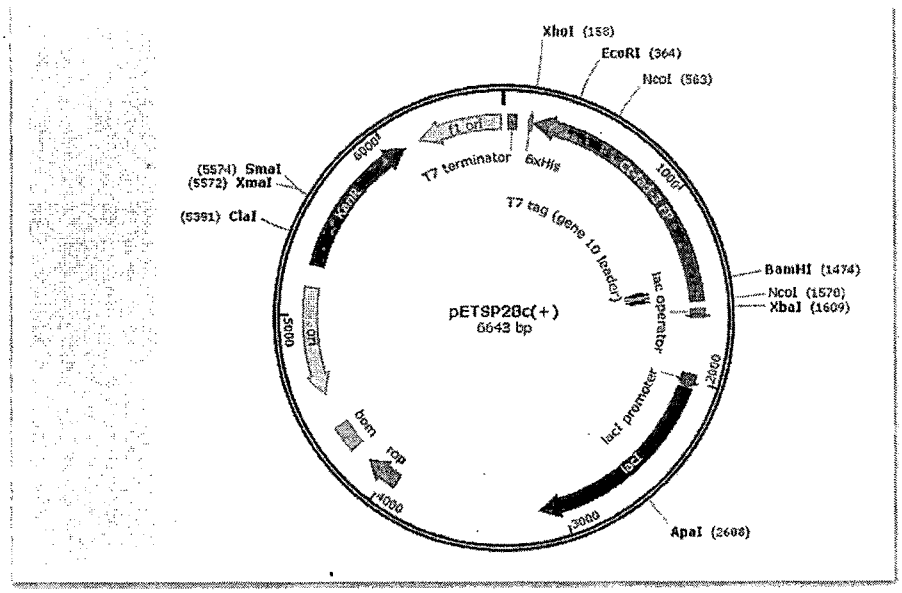
II.B.2 pBSCtC4 with 410 bp C-terminal Ct-*fad*-3 fragment



II.B.3 pBS-*fad*-3 with complete Ct-*fad*-3 gene



II.B.4 pSP-*fad*-3 w th Complete Ct-*fad*-3 gene



II.B.5 pETSP28c(+) with complete Ct-*fad*-3 gene

C. Primer series used in this study

Primer	Sequence (5'⟶ 3')
OF1F	
OF1F1	GGTTTTTGGATTTTGGCTCAYGAITGYGG
OF1F2	GGTATATGGATTTTGGCTCAYGANTGYGGNCA
OF1F3	TGGATTTTGGSTCATGANTGYGGNCATTC
OF1F4	SACAYGAATGYGGICATTC
OF1F5K *Kodemia* bias	CCAYGAATGYGGYCACTC
OF1F5C *Candida* bias	GCYCAYGAATGYGGYCATGG
OF1F5S *S. klyuvery* bias	GCTCAYGAATGYGGYCACTC
OF2R	
OF2R1	ATAGTACAAGCAGCACCTTTTTGAWAIKTCC
OF2R2	AGTACAAGCTTCACCTTTTTACAWARWCCAYTC
OF2R3	TAGCAGCAGCACCTTTCRCAAARKTCCAYTC
OF2R4	CCTTTGGCAAARGTCCATTC
OF2R5KC	CCWYKRGCAAAAGTCCATTC
OF2R5SP	WYKRGCAAAAGTCCATTC
OF3R	
OF3R1	ATCTTTAGTCATATGACCAGTABYYTTRTGRTG
OF3R2	AAAWTAYCATATCTTTAGTCATATGACCAG
OF3R3	ATGGCCWGTWGCTTTATGRTGYTT

Primer	Sequence (5'⟶ 3')
Primers used in Amplification of Ct-*fad*-3 gene sequence	
OF5CtF	*ATG*AGYGTWGTTGARGCATCWTC
OF2CtR	CCWYKRGCAAAAGTCCATTC
OF6CtF	TGGTTTRTTCCATGGTTRTGG
OF7CtR	*CTA*ATCTYTWGGTTTAACWGGWCC
OF5CtFN	AGC*CATATG*AGTGTTGTTGAAGCATCT
OF7rCtX	CA*CTCGAGCTA*ATCCTTTGGTTTGACAGG
Primers used in 18S rDNA characterization	
P108	ACCTGGTTGATCCTGCCAGT
M2130	CAATAAATCCAAGAATTTCACC
P1190	CAATTGGAGGGCAAGTCTGG
M3490	TCAGTGTAGCGCGCGTGCGG
D) Primers used in -ITS1-5.8S- ITS2- region amplification	
ITS1	TCCGTAGGTGAACCTGCGG
ITS4	TCCTCCGCTTATTGATATGC

II.D.1 Ct-*fad*-3 ORF

```
   1 atgagtgttgttgaggcatcttcaagttctattgctaatgactct
     M  S  V  V  E  A  S  S  S  S  I  A  N  D  S
  46 actggtaacggtagtagtaacgttgttcaaagaggaaatatttct
     T  G  N  G  S  S  N  V  V  Q  R  G  N  I  S
  91 tcatttgcatcaactactgctactacaaatttaacaactattgat
     S  F  A  S  T  T  A  T  T  N  L  T  T  I  D
 136 acaaacggtaatgtttttaaagttccagattattccattaaagat
     T  N  G  N  V  F  K  V  P  D  Y  S  I  K  D
 181 attttacaagctattccaaaacattgttatgaaagatctttgatt
     I  L  Q  A  I  P  K  H  C  Y  E  R  S  L  I
 226 agatctttgggttatgttgttagagatatcaccatgatggtttta
     R  S  L  G  Y  V  V  R  D  I  T  M  M  V  L
 271 attagttatgttggacattcttttattccattggttgatattgaa
     I  S  Y  V  G  H  S  F  I  P  L  V  D  I  E
 316 aaccatgaaactttaagtactgttgttagaggttctttatggatg
     N  H  E  T  L  S  T  V  V  R  G  S  L  W  M
 361 gtccattcttacttaattgggttatttggttttggtttatggatt
     V  H  S  Y  L  I  G  L  F  G  F  G  L  W  I
 406 ttagctcatgaatgtggtcatggtgcattttcagattatcaaaat
     L  A  H  E  C  G  H  G  A  F  S  D  Y  Q  N
 451 ttaaatgatctaattggttgggttatacattcttatttgatggtt
     L  N  D  L  I  G  W  V  I  H  S  Y  L  M  V
 496 ccttacttttcatggaaattttctcatgctaaacatcataaagca
     P  Y  F  S  W  K  F  S  H  A  K  H  H  K  A
 541 actggtcatttaactaaagatatggttttcattccatatactaaa
     T  G  H  L  T  K  D  M  V  F  I  P  Y  T  K
 586 gaagaatatttagaaaagaataaagttgaaaaagtttctgaattg
     E  E  Y  L  E  K  N  K  V  E  K  V  S  E  L
 631 gttgaagaatctccaatttattccctttttagttttaatttttcaa
     V  E  E  S  P  I  Y  S  L  L  V  L  I  F  Q
 676 caattgggtggtttacaattatatttagctaataatgcaactggt
     Q  L  G  G  L  Q  L  Y  L  A  N  N  A  T  G
 721 caagtttatcctggtgtttcatggtatgcaagatctcattattct
     Q  V  Y  P  G  V  S  W  Y  A  R  S  H  Y  S
 766 ccaatttctccagtttttgataaaaatcaatattggttcattgtt
     P  I  S  P  V  F  D  K  N  Q  Y  W  F  I  V
 811 ttatctgatattggtattatttcaactttaactgtggtttatcaa
     L  S  D  I  G  I  I  S  T  L  T  V  V  Y  Q
 856 tggtataaaaactttggtttatttaatatgatgatcaattggttt
     W  Y  K  N  F  G  L  F  N  M  M  I  N  W  F
 901 gttccatggttatgggttaatcattggttagtttttgttacattt
     V  P  W  L  W  V  N  H  W  L  V  F  V  T  F
 946 ttacaacatactgatccaacaatgcctcattatgctgctaacgaa
     L  Q  H  T  D  P  T  M  P  H  Y  A  A  N  E
 991 tggacttttgctcgtggtgctgctgctacaattgatagaaatttt
     W  T  F  A  R  G  A  A  A  T  I  D  R  N  F
1036 ggatttgttggtcaacacatcttccatgatattattgaaactcat
     G  F  V  G  Q  H  I  F  H  D  I  I  E  T  H
1081 gttttacatcattatgtttcaagaattccattttataatgccaga
     V  L  H  H  Y  V  S  R  I  P  F  Y  N  A  R
1126 gaagctactgaagctattaaaaaagttatgggtgaacattatagа
     E  A  T  E  A  I  K  K  V  M  G  E  H  Y  R
1171 tatgaaggtgaaaatatgtggttttctttatggaaatgtgttaga
     Y  E  G  E  N  M  W  F  S  L  W  K  C  V  R
```

```
1216 atgtgtcaatttgttgatgatgataaagaagatgccaaaggtgtt
     M  C  Q  F  V  D  D  D  K  E  D  A  K  G  V
1261 ttaatgtttagaaatgttaatggtcctgttaaaccaaaagattag 1305
     L  M  F  R  N  V  N  G  P  V  K  P  K  D  *
```

II.D.2 Ct-*fad*-3 gene sequence

ATGAGTGTTGTTGAGGCATCTTCAAGTTCTATTGCTAATGACTCTACTGGTAACGGTAG
TAGTAACGTTGTTCAAAGAGGAAATATTTCTTCATTTGCATCAACTACTGCTACTACAA
ATTTAACAACTATTGATACAAACGGTAATGTTTTTAAAGTTCCAGATTATTCCATTAAA
GATATTTTACAAGCTATTCCAAAACATTGTTATGAAAGATCTTTGATTAGATCTTTGGG
TTATGTTGTTAGAGATATCACCATGATGGTTTTAATTAGTTATGTTGGACATTCTTTTA
TTCCATTGGTTGATATTGAAAACCATGAAACTTTAAGTACTGTTGTTAGAGGTTCTTTA
TGGATGGTCCATTCTTACTTAATTGGGTTATTTGGTTTTGGTTTATGGATTTTAGCTCA
TGAATGTGGTCATGGTGCATTTTCAGATTATCAAAATTTAAATGATCTAATTGGTTGGG
TTATACATTCTTATTTGATGGTTCCTTACTTTTCATGGAAATTTTCTCATGCTAAACAT
CATAAAGCAACTGGTCATTTAACTAAAGATATGGTTTTCATTCCATATACTAAAGAAGA
ATATTTAGAAAAGAATAAAGTTGAAAAAGTTTCTGAATTGGTTGAAGAATCTCCAATTT
ATTCCCTTTTAGTTTTAATTTTTCAACAATTGGGTGGTTTACAATTATATTTAGCTAAT
AATGCAACTGGTCAAGTTTATCCTGGTGTTTCATGGTATGCAAGATCTCATTATTCTCC
AATTTCTCCAGTTTTTGATAAAAATCAATATTGGTTCATTGTTTTATCTGATATTGGTA
TTATTTCAACTTTAACTGTGGTTTATCAATGGTATAAAAACTTTGGTTTATTTAATATG
ATGATCAATTGGTTTGTTCCATGGTTATGGGTTAATCATTGGTTAGTTTTTGTTACATT
TTTACAACATACTGATCCAACAATGCCTCATTATGCTGCTAACGAATGGACTTTTGCTC
GTGGTGCTGCTGCTACAATTGATAGAAATTTTGGATTTGTTGGTCAACACATCTTCCAT
GATATTATTGAAACTCATGTTTTACATCATTATGTTTCAAGAATTCCATTTTATAATGC
CAGAGAAGCTACTGAAGCTATTAAAAAAGTTATGGGTGAACATTATAGATATGAAGGTG
AAAATATGTGGTTTTCTTTATGGAAATGTGTTAGAATGTGTCAATTTGTTGATGATGAT
AAAGAAGATGCCAAAGGTGTTTTAATGTTTAGAAATGTTAATGGTCCTGTTAAACCAAA
AGATTAG

II.D.3Amino acid sequence of Ct-FAD-3

MSVVEASSSSIANDSTGNGSSNVVQRGNISSFASTTATTNLTTIDTNGNVFKVPDYSIK
DILQAIPKHCYERSLIRSLGYVVRDITMMVLISYVGHSFIPLVDIENHETLSTVVRGSL
WMVHSYLIGLFGFGLWILAHECGHGAFSDYQNLNDLIGWVIHSYLMVPYFSWKFSHAKH
HKATGHLTKDMVFIPYTKEEYLEKNKVEKVSELVEESPIYSLLVLIFQQLGGLQLYLAN
NATGQVYPGVSWYARSHYSPISPVFDKNQYWFIVLSDIGIISTLTVVYQWYKNFGLFNM
MINWFVPWLWVNHWLVFVTFLQHTDPTMPHYAANEWTFARGAAATIDRNFGFVGQHIFH
DIIETHVLHHYVSRIPFYNAREATEAIKKVMGEHYRYEGENMWFSLWKCVRMCQFVDDD
KEDAKGVLMFRNVNGPVKPKD

434 amino acid polypeptide with 49850 Da molecular weight and pI 6.4

II.ECLUSTAL O(1.2.0) multiple sequence alignment

```
Pp_FAD_3      MSKVTVSGSEILE-G----STKTVRRSGNV-----ASFKQQKTAIDTFGNVFKVPDYTIK
Ct_FAD-3      MSVVEASSSS-IANDSTGNGSSNVVQRGNISSFASTTATTNLTTIDTNGNVFKVPDYSIK
Ca_FAD_3      MSVVEASSSS-VVEDST---ASNVVQRGNISSFASTTASSNLTTIDTNGKVFKVPDYSIK
Sk_FAD_3      MSIETVGSSSGVAINS-----------KAVSSTATTVVQPKTAIDTNGNVFKVPDYTIK
Kl_FAD_3      -----MSKSTGVEHHISGVAT-------TETATETVTVPPAKTAIDTHGNIFKVPDYTIK
                    . *  :                .      .:     *:*** *::******:**

Pp_FAD_3      DILDAIPKHCYERSLVKSMSYVVRDIVAISAIAYVGLTYIPLLPN-------EFLRFAAW
Ct_FAD-3      DILQAIPKHCYERSLIRSLGYVVRDITMMVLISYVGHSFIPLVDIENHETLSTVVRGSLW
Ca_FAD_3      DILQAIPKHCYERSLIRSLGYVVRDITMMVIIGYVGHTFIPMVQIPEYPSLAYGLRGALW
Sk_FAD_3      DILSAIPKECYKRDTLWSLHYVVRDIAAILVIGYLGTNYIPVLFP-----NSALLRGIAY
Kl_FAD_3      DILGAIPKECYKRDTLWSLHYVVRDIIAICIIGYVGTNYIPVWFP-----NSGLLRFVAY
              *** ****.**:*. : *: ****** :  *.*:* .:**:            :*   :

Pp_FAD_3      SAYVFSISCFGFGIWILGHECGHSAFSNYGWVNDTVGWVLHSLVMVPYFSWKFSHAKHHK
Ct_FAD-3      MVHSYLIGLFGFGLWILAHECGHGAFSDYQNLNDLIGWVIHSYLMVPYFSWKFSHAKHHK
Ca_FAD_3      MVQSYCIGLFGFGLWILAHECGHGAFSDYQNINDFIGWVLHSYLIVPYFSWKFSHAKHHK
Sk_FAD_3      AIQSYLIGLFGFGLWILAHECGHSAFSESNAVNDTVGWVLHSWWMVPYFPWKFSHSKHHK
Kl_FAD_3      MVQSYLIGLFGFGLWILAHECGHGAFSDSRLINDTVGWVLHSWWMVPYFSWKFSHSKHHK
                  : *. ****:***.*****.***:   :** :***:**  :**** *****:****

Pp_FAD_3      ATGHMTRDMVFVPYTAEEFKEKHQVTSLHDIAEETPIYSVFALLFQQLGGLSLYLATNAT
Ct_FAD-3      ATGHLTKDMVFIPYTKEEYLEKNKVEKVSELVEESPIYSLLVLIFQQLGGLQLYLANNAT
Ca_FAD_3      ATGHLTKDMVFIPYTKEEYLEKNKVEKVADLMEESPIYSFLVLVFQQLGGLQLYLATNAT
Sk_FAD_3      ATGHMTRDMVFIPYTKDEFITMKKKSKFAEITEEAPVMTLFNLIAQQVGGLQLYLATNAT
Kl_FAD_3      ATGHLTRDMVFVPYTKKEYLEMKGKSKLREITEEAPIVTLLTLIGQQIGGLQLYLATNAT
              ****:*:****:*** .*:   .   .. :: **:*: :.: *: **:***.****.***

Pp_FAD_3      GQPYPGVSKFFRSHYWPSSPVFDKKDYWYIVLSDLGILATLTSVYTAYKVFGFWPTFITW
Ct_FAD-3      GQVYPGVSWYARSHYSPISPVFDKNQYWFIVLSDIGIISTLTVVYQWYKNFGLFNMMINW
Ca_FAD_3      GQVYPGYSKIAKSHYTPTSPVFDKHQYWYIVLSDIGIILAFTTVYQWYKNFGLFNMMINW
Sk_FAD_3      GQPYPGVKKFFKSHYWPTSPVFDAKDFWWIIMSDIGIVSTLLINYLWYRAYGAHVVLINW
Kl_FAD_3      GQSYPGVPKFFKSHYWPTSPVFDTKDFWYIILSDIGIISTLTINYLWAKTYGSHVMLINW
              ** ***     :*** * ***** .::*:*::**:**: ::   *   : :*    :*.*

Pp_FAD_3      FCPWILVNHWLVFVTFLQHTDSSMPHYDAQEWTFAKGAAATIDREFGILG-IIFHDIIET
Ct_FAD-3      FVPWLWVNHWLVFVTFLQHTDPTMPHYAANEWTFARGAAATIDRNFGFVGQHIFHDIIET
Ca_FAD_3      FVPWLWVNHWLVFVTFLQHTDPTMPHYTSKEWTFARGAAATIDRNFGFVGQHIFHDIIET
Sk_FAD_3      FIPWLWVNHWLVFVTFLQHTDPTMPHYDAEEWTFAKGAAATIDRNFGFVGQHIFHDIIET
Kl_FAD_3      FVPWLWVNHWLVFVTFLQHTDPTMPHYEASEWTFAKGAAATIDRNFGFVGQHIFHDIIET
              * **: *************** :**** :.*****:********:**::*  ********

Pp_FAD_3      HVLHHYVSRIPFYHAREATECIKKVMGEHYRHTDENMWVSLWKTWRSCQFVENH----DG
Ct_FAD-3      HVLHHYVSRIPFYNAREATEAIKKVMGEHYRYEGENMWFSLWKCVRMCQFVDDDKEDAKG
Ca_FAD_3      HVLHHYVSRIPFYNAREATDAIRKVMGEHYRYEGESMWYSLWKCMRMCQFVDDDKEDAKG
Sk_FAD_3      HVLHHYCSRIPFYNARKATSAIKEVMGQHYRYEGENMWKSLWKVARSCQYVEGD----NG
Kl_FAD_3      HVLHHYCSRIPFYNARVATEAIKKVMGEHYRYEGENMWQSLWKVARSCQFVDGD----NG
              ****** ******.** **..*::***:***:  *.** ****  * **:*: .    .*

Pp_FAD_3      VYMFRNCNNVGVKPKDT---
Ct_FAD-3      VLMFRNVNG--PVKPKD---
Ca_FAD_3      VMMFRNVNGWGPVKPKD---
Sk_FAD_3      VRMFRNTNGVGVKPEDGSSQ
Kl_FAD_3      VLMFRNTNGVGAPCQE----
              * **** *          .
```

Figure 3.2 Comparison of the deduced amino-acid sequence of Ct-FAD-3 with previously reported FAD-3from several fungi. The amino-acid sequences of omega-3 desaturases from*Pichia pastoris* (Pp_FAD_3), *Candida tropicalis* (Ct FAD 3), *Candida albicans* (Ca FAD 3), *Saccharomyces kluyveri* (Sk FAD 3), *Kluyveromyces lactis* (Kl_FAD_3) are used. Sequences were aligned using the **Clustal omega** algorithm. The three conserved 'Histidine' boxes are shaded with light grey.Candida specific conserved amino acid stretch is shaded with green color.

descending, radial or two dimensional. The choice of technique depends upon the nature of substance to be separated.

2) Choice of filter paper : The paper with maximum degree of clarity of separation and with excellent rate of movement of solvent are commonly used. Whatmann filter papers which contain 98-99 % α-cellulose are more convenient. The choice of paper depends on the thickness, flow rate and purity. Characteristics of whatmann chromatographic papers are summarized in Table : 21.1

Table 21.1

Paper	Rate of Flow		
	Fast	**Medium**	**Slow**
Thin Papers	No. 4 No. 54 No. 540	No. 7 No. 1	No. 2 No. 20
Thick papers	No. 31 No. 17	No. 3 No. 3 mm	-

The paper may be impregnated with buffer solution before use or chemically modified by acetylation. For separation of lipids and similar hydrophobic molecules, silica impregnated papers are available commercially.

3) Preparation of paper : After selection of proper paper, it is to be cut in desired size and shape, on the basis of type of chromatographic technique like ascending, descending, radial or bidimensional. Square and rectangular shapes are in most common use.

4) Sample preparation and application : A weighed amount of substance is dissolved in a volatile solvent and by careful means the minimum volume of concentrated solution is applied on the paper. This is done simply to avoid diffusion through papers. The sample volume of 2 to 20 lit (10-20 micrograms of the substance) is the ideal quantity for spotting. Application is done with special commercial micropipettes, microsyringes or platinum loop or simply capillaries made from glass tubes.

5) Choice of proper developing solvent : The solvent that gives different Rf values for different constituents in a mixture should be selected. Both polar and non polar solvents (water miscible and immiscible) are used in paper chromatography. Most common solvents used are listed in Table 21.2 in order of their increasing polarity.

Table : 21.2 Solvents used in paper and thin layer chromatography

Non- polar slovent	n- hexane
	Cyclohexane
	Carbon hydrochloride
	Benzene
	Toluene
	Trichloroethane
	Diethylether
	Chloroform
	Ethy acetate
	n-butanol
	n-propanol
	Acetone
	Ethanol
	Methanol
Polar solvents	Water
	Acetic acid

If a pure solvent is not satisfactory, mixtures of different solvents in various proportions can be used. Some of such solvent systems are as follows

Isopropanol - ammonia - water (9: 1: 2)

n - butanol- acetic acid - water (4: 1: 5)

6) Drying of chromatogram : After development chromatog is dried in a special drying cabinets or in air.

THIN LAYER CHROMATOGRAPHY

The technique of thin layer chromatography was first introduced by Ismailoff and Schraiber in 1938. It is simple, quick method and allows a large number of samples to be studied concurrently. It can be used for analytical and preparative purposes.

Principle : The technique is based on the principle of adsorption as well as partition chromatography which depends upon the material chosen for the preparation of thin layer. Table 21.3 shows the materials for thin layer preparation, separation and components to be separated in thin layer chromatography.